



# Prevalence of *Vibrio* Species Isolated from Water Bodies in Buguma Community in Rivers State, Nigeria

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

**Aim:** To assess the prevalence of *Vibrio* species isolated from water bodies and stool samples in Buguma community in Rivers State, Nigeria.

**Study Design:** This was a cross-sectional study with simple randomized sampling technique.

**Methodology:** The study used a total of 80 samples comprising of 40 stool and 40 water samples from different water bodies where public toilets were collected in Buguma community in Rivers State, Nigeria. The *Vibrio* species were isolated from the samples using Thiosulphate Citrate Bile Salt agar (TCBS), and blood agar as a confirmatory after which biochemical tests were carried out for further identification. Data collected was analyzed with Graph pad prism version 8. The prevalence and distribution of *Vibrio* were presented in frequencies and percentages with all analysis done at a 95% confidence interval and *P*-values less than .05 were considered significant.

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**Results:** From chi-square analysis there was a 35% prevalence rate in water bodies samples in comparison to stool samples that had a 7.5% prevalence ( $P$  value = .56). A higher prevalence was also reported in the female subjects (7.6%) compared to the male subjects (7.4%). There was a statistically significant difference in consumption of shellfish which poses to be a risk factor ( $P$  value = .01). There was also a statistically significant difference in prevalence of *Vibrio* species in water bodies and *Vibrio* species isolated from human stool sample ( $P$  value = .0026).

**Conclusion:** Higher prevalence of *Vibrio* species was found in water bodies compared to stool. The consumption of shellfish is potential a risk factor for Vibriosis in Buguma community in Rivers State of Nigeria.

**Keywords:** *Vibrio* species; rivers state; epidemiology; vibriosis.

## 1. INTRODUCTION

Worldwide, the autochthonous colonization of marine, surface, and brackish waters has been virtually documented for *Vibrio* species [1-6]. Because these *Vibrio* species have been found to be highly endowed with so many survival strategies and traits, their geographical distribution has not been related with the location and/or environment. Because of this, they are able to grow and thrive wherever they are.

Depending on whether the isolated strain of *Vibrio cholerae* (*V. cholerae*) is of type O1 or O139 and is producing the cholera toxin or not, illnesses are categorized as either cholera or non-cholera. *Vibrio cholerae* non-O1 non-O139 (NOVC) and other *Vibrio* spp. can induce cholera, but they can also seriously harm humans. Vibriosis, a noncholera disease, has been reported to cause either acute, watery diarrhea (cholera disease), a severe and potentially fatal infection [7], or vibriosis, which can present as a self-limiting gastroenteritis or a severe and potentially fatal septicemia with necrotizing fasciitis, wounds, and ear infections [6]. Individuals with impaired immune systems, those with underlying medical conditions like diabetes, cancer, liver disease, or those who have recently had stomach surgery are more susceptible to severe vibriosis. An estimated 80,000 illnesses and 100 fatalities are attributed to non-cholerae vibriosis in the US each year [8]. Most *Vibrio* species, including non-toxigenic *V. cholerae*, are found in aquatic habitats and are generally not harmful. Vibrios can withstand a wide variety of salinities, grow across a large temperature range (20 °C to >40 °C), and prefer an alkaline environment, however most species can tolerate pH levels between 6.5 and 9.0 [9]. NOVC has been linked to skin and soft tissue infections, including wound infections, cellulitis and necrotizing fasciitis, peritonitis, cholecystitis, endophthalmitis, ear infections, urinary tract infections, and meningitis, as well as extra-

intestinal infections like bacteremia and sepsis [2,3,4,5,6,7,10,11].

Globally, the prevalence of *Vibrio*-related illnesses has been steadily increasing [10, 11]. Some of these illnesses are contracted by swimming or bathing in coastal waters [12–15], eating seafood and vegetables from irrigated farms [16], and living in unsanitary and densely populated coastal areas. *Vibrio* species infections are turning into a major threat to public health worldwide. The two species that are most frequently implicated in infections in humans are *Vibrio parahaemolyticus* (*V. parahaemolyticus*) and *Vibrio cholerae* (*V. cholerae*) [17, 18].

The presence of these *Vibrio* species in environmental water bodies is frequently linked to the inappropriate management of waste from nearby towns and rural settlements, which causes surface runoff, streams, rivers, wells, ponds, and seawater to become contaminated with excrement [19,20]. If it is discovered that these possible pathogens are present in the environmental water bodies, it makes them unsafe for use at home or for recreational purposes. This study is aimed at assessing the prevalence of *Vibrio* species from water bodies in Buguma community and from stool sample from suspected *Vibrio* infested diarrhea patients.

## 2. MATERIALS AND METHODS

### 2.1 Study Design

The investigations were carried out using a cross-sectional study design with simple randomized sampling technique.

### 2.2 Study Area

Buguma shown in Fig. 1 (4°45 N, 6°53 E) is chosen for this study because the populace are predominately fishers. It is the headquarters of



**Fig. 1. Map of Buguma**  
(source: Numbere, 2020)

Asari-Toru local government area and base of Kalabari kingdom in Rivers State. Buguma is an island that is surrounded by water and a host to a large supply of mangrove forest. The Kalabari are a distinct part of the Ijaw-speaking peoples of the Niger Delta and live in thirty-three villages and three major towns in the mangrove swamps of the eastern part of the region. The three major cities are Abonnema, Bakana, and Buguma, which were settled in 1882, 1881 and 1884, respectively. According to the King of Kalabari Kingdom, the Kalabari formerly lived in Elem-Ama, or Old Calabar, from where they later migrated to their various towns in different parts of the eastern Niger Delta.

### 2.3 Study Population

The study comprised patients who visited the Buguma Health Center between May and August 2022 who had suspected diarrhoea. Individuals who are suspected of having diarrhoea may exhibit a unique set of symptoms associated with food poisoning, including fatigue, generalized body weakness, pain or cramping in the abdomen, and frequent stools.

### 2.4 Questionnaire Sampling

In order to gather data on sociodemographic characteristics and risk factors related to *Vibrio* infection, participants were given standardized questionnaires and conducted interviews. The authors created the both open-ended and

closed-ended questionnaire, which was then put through face and content validity testing. The questionnaire comprised items divided into two sections: risk factors and sociodemographic data. The first section of the questionnaire evaluated the respondent's age, sex, marital status, education level, and occupation. The second section evaluated the respondent's risk exposure to *Vibrio* infection, which includes contact with saltwater, clinical symptoms and outcome, and previous weeks' worth of travel. The type, amount, and frequency of consuming meat, fish, and vegetables, as well as the location of preparation and consumption, were all recorded together with the dietary history for the previous three days. To protect the privacy of the subjects, the names of the participants were not included in the questionnaire; instead, serial numbers were utilized to track respondents and outcomes. To guarantee that assay results and replies were matched to the appropriate subjects, the serial number on each questionnaire was duplicated on each stool sample vial.

### 2.5 Stool Sample Collection

40 stool samples were taken from both inpatients and outpatients who exhibited diarrheal infection signs and symptoms. All faeces samples were collected aseptically from patients and placed into universal wide-neck containers. These stool samples were collected, and they were sent right away for culture. Samples were kept in a refrigerator at 4°C when delays were anticipated.

The normal bacteriological procedures were applied to all obtained samples, and the morphological features of culture medium and biochemical tests were utilized to identify isolates.

## 2.6 Water Sample Collection

Using a sterile universal bottle, water samples were taken from every water body in all compounds in Buguma community where public toilets are situated. The samples were immediately sent to the laboratory for analysis. Samples were kept in a refrigerator at 4°C when delays were anticipated. The normal bacteriological procedures were applied to all obtained samples, and the morphological features of culture medium and biochemical tests were utilized to identify isolates.

## 2.7 Biochemical Test

### 2.7.1 Oxidase test

For the oxidase test, bacterial colonies were picked with a platinum wire and streaked on filter paper saturated with 0.5% tetramethyl-*p*-phenylenediamine hydrochloride (BBL Co.). Rapid appearance of a dark purple color was considered a positive reaction

### 2.7.2 Indole test

Pure culture of the isolate were Inoculated in a tubes of tryptone broth and Incubated at 35°C (+/- 2°C) for 24 to 48 hours. To test for indole production, 5 drops of Kovács reagent were added directly to each tube. The isolates were positive for indole test by the formation of a pink to red color ("cherry-red ring") in the reagent layer on top of the medium within seconds of adding the reagent

### 2.7.3 MR Test

A pure culture of the isolates were Inoculated in MR broth and Incubated at 35°-37°C for 48 hours in ambient air. 5 drops of methyl red reagent was added per 5 mL of broth and was observe for the color change in the broth medium.

### 2.7.4 VP Test

Using a sterile inoculating loop, isolate was inoculated in the broth and Incubated aerobically for 18 to 24 hours at 35°C. Following incubation, 2 mL of broth was transferred to a clean test tube

and 6 drops of Reagent A (5%  $\alpha$ -naphthol solution) and 2 drops of Reagent B (40% KOH solution) were added and mix properly by shaking and was observed for the formation of red-pink color at the surface of the medium within 30 minutes.

### 2.7.5 Citrate Utilization Test

Following 24 hours incubation of pure isolate in Simmons citrate medium bacterial growth and color change in the slant portion was observed. Negative result is indicated presumptive vibrio isolate.

### 2.7.6 Urease Test

isolated colonies were inoculated on the surface of a urea agar slant and tube incubated at 37°C in ambient air for 48 hours and was examined for the development of a pink color.

## 2.8 Microbial Analyses

According to a prior description (WHO, 2016), a loop full of surface growth was streaked on a pre-made thiosulfate-citrate-bile salt-sucrose (TCBS) agar plate and incubated at 37°C for 18–24 hours. Following incubation, bacterial colonies' morphology—including their size, shape, and color led to a provisional identification as *Vibrio* spp. For additional purification, a single, distinct colony (yellow or green in color) was streaked over recently made TCBS and blood agar plates. To assess the morphology of isolates believed to be *Vibrio* spp. Colonies. Gram staining was performed as indicated earlier by Bergey et al. [21], other biochemical tests such as oxidase and catalase tests were also carried out.

## 2.9 Data Analyses

The data generated from this study was represented as frequency and percentages, and inferential statistics were carried out using chi-square with the aid of GraphPad Prism Software Version 9. Statistical significance was defined as a *p*-value of less than 0.05 at a 95% confidence interval.

## 3. RESULTS

### 3.1 Sociodemographic Data of the Subjects

As shown in Fig. 2, a total of 40 subjects aged between 3 to 50 years participated in the study

with an average age of 27 years. Most of the subjects i.e., 19 (47.5%) were between the ages of 0-10 years, and subjects aged 40 and above made up the least group of subjects with a frequency of 4 (10%). Male subjects predominated the study with a frequency of 27 (67.5%) while females were 13 (32.5%).

There were only 3 (7.5%) subjects that had no formal education, with the highest number of subjects having at least secondary education with a frequency of 20 (50%). Those with primary and tertiary levels of education were 13 (32.5%) and 4 (10%) respectively. Most of the subjects were self-employed with a frequency of 19 (47.5%), 2, (5%) of the subjects were unemployed, 10 (25%) were private employed, 3 (7.5%) were civil servants and 6 (15%) were students.

### 3.2 Prevalence of *Vibrio species* and Sociodemographic Characteristics

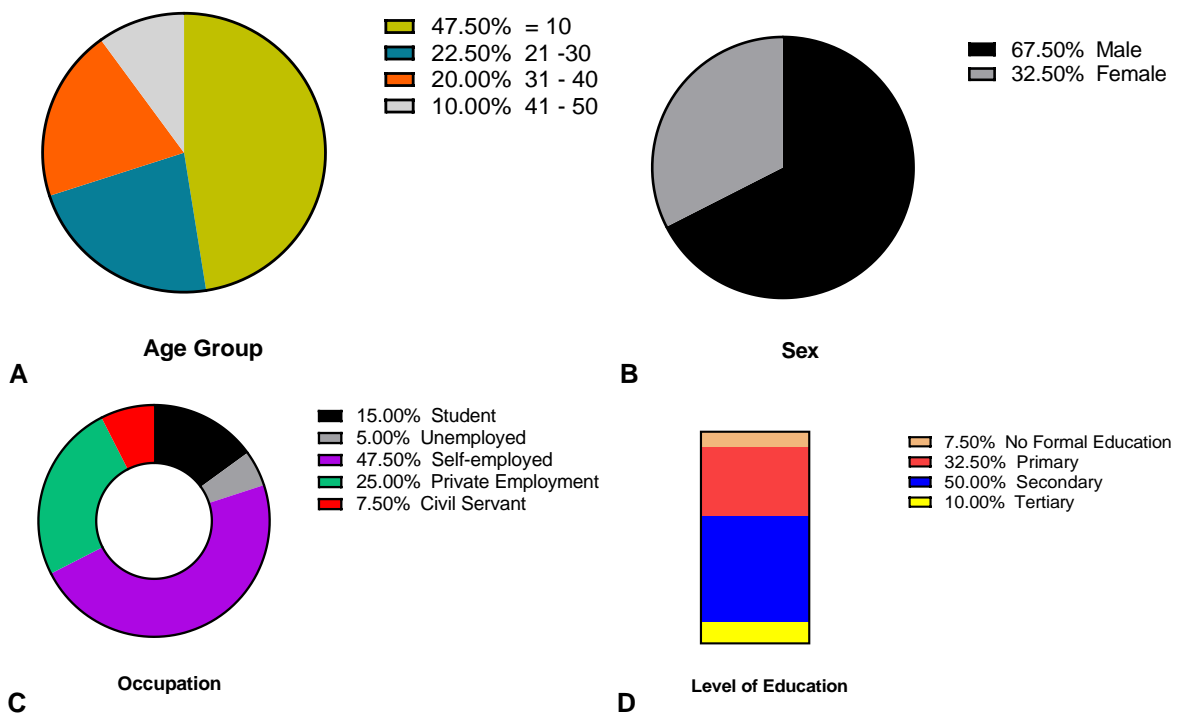
Samples obtained from the subjects were analyzed for the presence of *Vibrio species*. The overall prevalence of *Vibrio* infection was 7.5% as 3 subjects yielded positive *Vibrio* isolates out

of 40 subjects sampled in the study. This prevalence was further categorized according to the sociodemographic characteristics of the subjects.

As regards the age of the subjects, a prevalence of 2 (10.5%) was recorded amongst subjects aged 10 years or younger, a prevalence of 1 (11.1%) was reported amongst subjects aged 21 – 30 years. There were no positive samples (0 prevalence) from other age groups. The distribution was not found to be statistically significant ( $p = 0.2288$ )

A prevalence of 2 (7.4%) was observed among the male subjects. The females on the other hand recorded a prevalence of 1 (7.6%). The observed distribution was statistically insignificant ( $p = 0.9744$ )

As regards the level of education of the subjects, those with primary education have a prevalence of 2 (15.3%), while 1 (20%) prevalence was reported amongst secondary school leavers. The observed distribution based on the level of education of the subjects was not found to be significant ( $p = 0.5907$ ).



**Fig. 2. Sociodemographic Information of the Participants.**  
A.) Age group B.) Sex C.) Occupation D.) Level of Education

### 3.3 Risk Factors for *Vibrio species*

The subjects were questioned on practices and meal consumption that may serve as risk factors for infection with *Vibrio species*. with the following outcome. Most of the subjects occasionally eat raw (undercooked) Seafood with a frequency of 27 (67.5%), those who do not Eating raw (undercooked) Seafood were 13 (32.5%) while those who Eat raw (undercooked) Meat reported at a frequency of 33 (82.5%) and subjects found not to eat raw (undercooked) Meat among the study population were reported at a frequency of 7 (17.5%). As regards rationale on meal consumption, 38 (22.3%) eat freshwater fish, all 40 subjects eat sea water fish and

shrimps (100%), 32 of 40 subjects consume crab (80) and 33 (82.5) subjects eat shellfish. As regards meat, 23 (57.5) of the subjects were pork eaters, while 39 (97.5) of the subject population eats beef and chicken while 13 (42.5) of the entire population eats duck as seen in Table 2.

### 3.4 Prevalence of *Vibrio species*

A total of 80 samples (40 stool and 40 water) were collected and tested for the presence of *Vibrio specie*. *Vibrio* was isolated in 3 of 40 (7.5%) samples from stool and 14 Of 40 (35%) of water samples. The isolates were confirmed by conventional microbiology methods in the laboratory as seen in Table 3.

**Table 1. Prevalence of *Vibrio species* and Sociodemographic Characteristics**

Variable	N	Positive (%)	Negative (%)	X <sup>2</sup>	Df	p-value
Age						
≤ 10	19	2 (10.5%)	17	1.393	3	.2288
21 – 30	9	1 (11.1%)	8			
31 – 40	8	0	8			
41 – 50	4	0	4			
Total	40	3	37			
Gender						
Male	27	2 (7.4%)	25	0.001027	1	.9744
Female	13	1 (7.6%)	12			
Total	40	3	37			
Level of Education						
Informal	3	0	3	1.913	3	.5907
Primary	13	2 (15.3%)	11			
Secondary	20	1 (20%)	19			
Tertiary	4	0	4			
<b>Total</b>	<b>40</b>	<b>3</b>	<b>37</b>			

Df = Degree of freedom  
X<sup>2</sup> = Chi-square

**Table 2. Prevalence of *Vibrio species* and Risk Factors for Infection**

Variable	N	Positive (%)	X <sup>2</sup>	Df	p-value
Eating raw Seafood	Yes (27) No (13)	2 (7.4)	0.001027	1	.9744
Total	40				
Eating raw Meat	Yes (33) No (7)	2 (6.0)	0.5632	1	.4530
<b>Total</b>	<b>40</b>	<b>3</b>			
Fresh water fish	Yes (38) No (2)	3 (7.8)	0.1707	1	.6795
Total	40	3			
Sea water fish	Yes (38) No (2)	3 (7.8)	0.1707	1	.6795
<b>Total</b>	<b>40</b>				
Shrimp	Yes (38)	3 (7.8)	0.1707	1	.6795

Variable	N	Positive (%)	X <sup>2</sup>	Df	p-value
	No (2)				
<b>Total</b>	<b>40</b>				
Crab	Yes (32)	2 (6.2)	0.4276	1	.5132
	No (8)				
<b>Total</b>	<b>40</b>				
Shellfish	Yes (33)	1 (3.0)	5.430	1	.0198*
	No (7)				
<b>Total</b>	<b>40</b>				
Pork	Yes (23)	1 (4.3)	0.7751	1	.3786
	No (17)				
<b>Total</b>	<b>40</b>				
Beef	Yes (39)	3 (7.6)	0.08316	1	.7731
	No (1)				
<b>Total</b>	<b>40</b>				
Chicken	Yes (39)	3 (7.6)	0.08316	1	.7731
	No (1)				
<b>Total</b>	<b>40</b>				
Duck	Yes (13)	0 (0)	1.562	1	.2114
	No (27)				
<b>Total</b>	<b>40</b>				

\*Statistical significance  $P < .05$ ; Values in parenthesis = percentages  
Df = Degree of freedom  
X<sup>2</sup> = Chi-square

**Table 3. Prevalence of *Vibrio* in human and water sample**

Variable	Positive (%)	Negative (%)	Total(%)	X <sup>2</sup>	Df	p-value
Sample						
Human	3 (7.5)	37 (92.5)	40 (100)			
Water	14 (35)	26 (65)	40 (100)	9.038	1	.0026*
<b>Total</b>	<b>17 (21.3)</b>	<b>63 (78.7)</b>	<b>80 (100)</b>			

Df = Degree of freedom  
X<sup>2</sup> = Chi-square

### 3. DISCUSSION

Numerous bacteria, including *Salmonella spp.*, *Clostridium botulinum*, *Yersinia spp.*, and *Listeria monocytogenes*, can be found in fish [22]. This was consistent with our study as consumption of shellfish posed to be a risk factor to vibrio infection as it was statistically significantly as a risk factor for vibrio infection in our study at  $p$ -value 0.0198. It can be utilized by fish microbes as a feeding medium, and they can hydrolyze proteins to produce biogenic amines [23]. Nonetheless, *Vibrio* species is a well-known bacterium that poses a serious threat to public health and can be found in fish [22]. In this study forty (40) stool samples were collected from residents in the study area (Buguma community). Of the 40 samples analysed for the research, 3 were positive for *Vibrio* species infection which gave a prevalence rate of 7.5% in stool sampled, this finding differs from the findings of this study which Azike et al. [24, 25] who recorded

prevalences of 30% in Andoni local government and 30.6% in Calabar respectively. The disparity in the result may be due to continuous health awareness on vibrio infection and may be attributed also to difference in sample size in these studies.

Even in developed nations like the US, the number of cases of vibriosis is rising [26]. The *Vibrio* species isolated in this study responded favorably to the biochemical tests (oxidase, catalase, indole, and motility test) that were performed, according to the biochemical characterization and identification of the isolates that were reported. Additionally, the isolates were determined to be gram negative organisms using the Gram staining technique. The colonies showed up as green colonies and yellow colonies with a diameter of roughly 2-4 mm on Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar at 37C for a full day.

The investigation examined water samples and stool samples from residents in the study area who showed symptoms of diarrhea, which was statistically significant, with  $p$ -value of 0.0026 and  $P$  set at  $P > 0.05$ . *Vibrio* species infestations contaminated excrement, which occasionally is excreted straight into these waterways by the local populace surrounding them, in addition to a portion of the surface is removed by humans. activities that flow into the seas and rivers could act as the primary causes of pollution both directly from the water sources and indirectly, the marine life that inhabits them. The greater the consequences of these actions are focused on a specific the greater the pollution of the location sources, explaining the variations as a result *Vibrio* numbers found in this study showed research

As regards demographics, Azike *et al.*, in 2021 in their study noted a prevalence rate of *vibrio* species to be 77.8% amongst 11-15 years, 80% prevalence in age group 16- 20 years and recorded no positive in 6-10 years and 26 30 years [24]. This was contrary with the results obtained in this study as this study recorded the most prevalence amongst age 21-30 years with a prevalence of 11.1% and recorded a prevalence of 10.5 amongst age 0-10. The inconsistency in these studies might be as a result of difference in sample size. Also, the World Health Organization (WHO) has identified *Vibrio cholerae* as the most significant of the *Vibrio* genus. In their findings they noted that children in Bangladesh are often affected by diarrhea, which is a worldwide issue because of the country's location. According to the World Health Organization (WHO) [27], diarrheal sickness is the second most common cause of mortality in children under the age of five, accounting for 760,000 deaths yearly. Of these deaths, 10% occur in low- and middle-income nations, including Bangladesh (WHO, 2017). This result is consistent with our study, since the age group with the largest percentage was 0–10 years old. Azike *et al.* [24] in their study also discovered that among the sexes, 78.2% of the females tested were positive and 64.4% of males tested were positive. This is in line with another study that demonstrated the prevalence of pathogenic bacteria to be higher in female (73%) than male (27%) [28] although this is nearly 9-fold higher than the prevalences found in our study. In our study, a prevalence of (7.4%) was observed among the male subjects. The females on the other hand recorded a prevalence of (7.6%). There result was in tandem with the current study as most prevalence was

found in the female subjects, the lower in prevalence might be attributed to fewer subject participants.

The results obtained from this study show that out of 40 water bodies sampled 14, (35%) samples were positive for *vibrio*. The high prevalence of *Vibrio* spp. may be caused by salinity, temperature, or ecological pollution from the feed [26]. However, Since *Vibrio* spp. are naturally occurring in marine environments, the prevalence rates found in this study may not necessarily be cause for alarm [29]. Additionally, not all *Vibrio* species are regarded as pathogenic. The prevalence of these *Vibrio* species in environmental water bodies is frequently linked to poor waste management practices in rural and local populations, which contaminate surface runoff, streams, rivers, wells, ponds, and seas with human waste [30] this is true with what is observed in the study population where populace defecate directly into the water bodies and also the water bodies serve as waste dump site. Furthermore, some of the surface runoff from human activity that enters the rivers and seas, along with contaminated feces that the local population occasionally dumps directly into these bodies of water, may contribute directly to the contamination of the water sources themselves and indirectly to the seafood that inhabits them.

The comparative statistically significance of *vibrio* infection in shellfish than fish is Because actively growing clear shellfish particles from water at rates ranging from 1 to 4 L/h, the relative greater occurrence in shellfish than fish makes sense [31]. Thus, it's probable that the *Vibrio* spp. found in the water body are kept inside the mussel body during this filtration stage [31]. A study in on risk factors of *vibrio* specie in Egypt had a 9.3–16.7 percent shellfish content [32, 33]. According to Al-Mouqati *et al.* [34], there was an even greater level of contamination in Kuwait, where 78% of the seafood was contaminated with *Vibrio* spp. These findings were in corroboration with the results in this study where shellfish was statistically significant as a risk factor.

Furthermore, fish is typically eaten after undergoing a heat treatment, which is meant to eradicate any pathogenic *Vibrio* species [35]. For a clearer understanding, more research on the frequency of virulent strains is required. furthermore, Health sensitization on the danger of poor sanitation, eating undercooked sea food, use of antibiotics, washing of hands immediately



after using the toilet and eating of properly cooked foods and their association as risk factor in *Vibrio* infection needs to be carried out in the community.

#### 4. CONCLUSION

The present study shows that the prevalence of *Vibrio* species from water bodies in Buguma was 35%, and from human stool sample was 7.5%. it also demonstrates a statistical significance between *Vibrio* species from water and *Vibrio* species from stool at  $P < .05$ . There was also a statistical associated significance between eating shellfish and *Vibrio* infection which makes Shellfish consumption a risk factor for *Vibrio* infection. Health campaigns should be carried out to enlighten the populace about the risks involved in the prevalence of this infection so as to reduce its burden.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

#### CONSENT

The subjects were enlightened about the study and questionnaires were given to each of them. Written informed consent were also obtained from all subjects before samples were collected.

#### ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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