Original Research Article

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

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.

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 shellfishis 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]. 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. MATERIAL AND METHODS

2.1 Study Design

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

2.2Study 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 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.

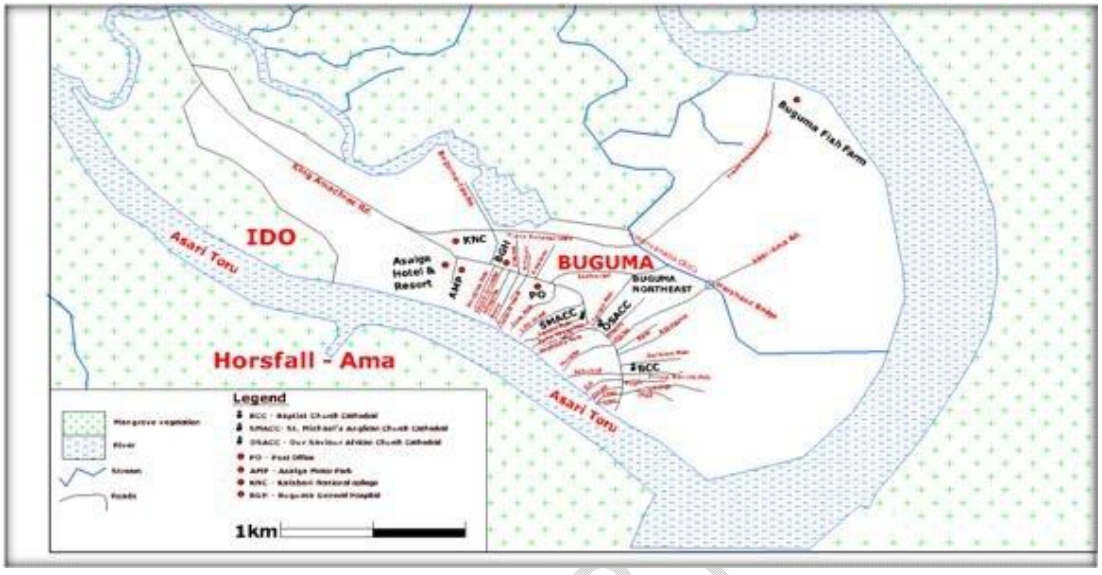

Fig. 1. Map of Buguma source: Ikiriko T. D, 2018

2.3Study 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.

2.4Questionnaire 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.

2.5Stool 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.

2.6Water 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.

2.7Biochemical 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 wereInoculated 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 wereadded 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. 5drops of methyl red reagent was added per 5 mL of broth and was observe for the color change in the broth medium.

2.7.4VP 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% α-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.

2.7.5Citrate 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

2.8Microbial 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. [20], other biochemical tests such as oxidase and catalase tests were also carried out.

2.9Data 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.

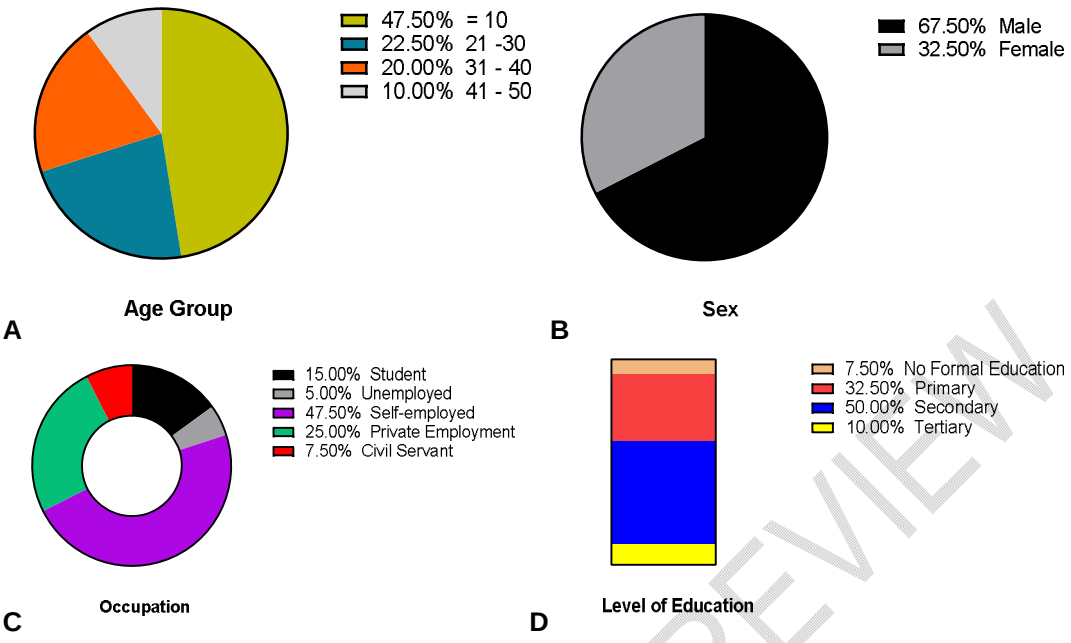

Fig. 2. Sociodemographic Information of the Participants.
A.) Age group B.) Sex C.) Occupation D.) Level of Education

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 = 09744)

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).

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

Variable	N	Positive (%)	Negative (%)	X^2	Df	*p*-value
Age						

≤ 10	19	2 (10.5%)	17			
21 – 30	9	1 (11.1%)	8	1.393	3	.2288
31 – 40	8	0	8			
41 – 50	4	0	4			
Total	**40**	**3**	**37**			
Gender						
Male	27	2 (7.4%)	25			
Female	13	1 (7.6%)	12	0.001027	1	.9744
Total	**40**	**3**	**37**			
Level of Education						
Informal	3	0	3			
Primary	13	2 (15.3%)	11	1.913	3	.5907
Secondary	20	1 (20%)	19			
Tertiary	4	0	4			
Total	**40**	**3**	**37**			

Df = Degree of freedom
X^2 = Chi-square

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 below

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

Variable	N	Positive (%)	X^2	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
Total	40	3			
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
Total	**40**				
Shrimp	Yes (38) No (2)	3 (7.8)	0.1707	1	.6795
Total	**40**				
Crab	Yes (32) No (8)	2 (6.2)	0.4276	1	.5132
Total	40				
Shellfish	Yes (33) No (7)	1 (3.0)	5.430	1	.0198*
Total	**40**				
Pork	Yes (23) No (17)	1 (4.3)	0.7751	1	.3786
Total	**40**				
Beef	Yes (39) No (1)	3 (7.6)	0.08316	1	.7731
Total	**40**				
Chicken	Yes (39) No (1)	3 (7.6)	0.08316	1	.7731
Total	**40**				
Duck	Yes (13) No (27)	0 (0)	1.562	1	.2114
Total	**40**				

*****Statistical significance *P*< .05; Values in parenthesis = percentages
Df = Degree of freedom
X^2= Chi-square

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 0f 40 (35%) of water samples. The isolates were confirmed by conventional microbiology methods in the laboratory as seen in Table 3.

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

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

Df = Degree of freedom
X^2 = Chi-square

3. DISCUSSION

Numerous bacteria, including *Salmonella spp*., *Clostridium botulinum, Yersinia spp.,* and *Listeria monocytogenes*, can be found in fish [21]. 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 [22]. Nonetheless, *Vibrio* species is a well-known bacterium that poses a serious threat to public health and can be found in fish [21]. 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. [23, 24] 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 [25]. 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.,* in2021 in their study noted a prevalence rate of vibrio species to be 77.8% amongst 11-15years, 80% prevalence in age group 16- 20years and recorded no positive in 6-10 years and 26 30years [23]. 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) [26], 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.* [23]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%) [27] 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 [25]. 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.

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.

Disclaimer (Artificial intelligence)
Option 1:
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 the writing or editing of this manuscript.
Option 2:
Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology
Details of the AI usage are given below:
1.
2.
3.

REFERENCES

1. Wang R, Huang J, Zhang W, Lin G, Lian J, Jiang L, Lin H, Wang S, Wang S. Detection and identification of Vibrio parahaemolyticus by multiplex PCR and DNA–DNA hybridization on a microarray. Journal of Genetics and Genomics. 2011 Mar 20;38(3):129-35.
2. Narracci M, Acquaviva MI, Cavallo RA. Mar Piccolo of Taranto: Vibrio biodiversity in ecotoxicology approach. Environmental Science and Pollution Research. 2014 Feb;21(3):2378-85.
3. Castillo D, D'Alvise P, Kalatzis PG, Kokkari C, Middelboe M, Gram L, Liu S, Katharios P. Draft genome sequences of Vibrio alginolyticus strains V1 and V2, opportunistic marine pathogens. Genome Announcements. 2015 Aug 27;3(4):10-128.

4. Brahim B, Mohammed H, Fatima H, Aicha AA, Mohammed B, Asma L, et al. Treated Wastewater Reuse inIrrigation:Detection and Characterization of Potentially Pathogenic Vibrio Spp. Amer J Inn Res Appl Sci. 2020;11(5):108-113
5. Håkonsholm F, Lunestad BT, Aguirre Sánchez JR, Martinez-Urtaza J, Marathe NP, Svanevik CS. Vibrios from the Norwegian marine environment: Characterization of associated antibiotic resistance and virulence genes. Microbiologyopen. 2020 Sep;9(9):e1093.
6. Monsreal JF, Serralta-Peraza LE, Abuxapqui JJ. Species of the genus Vibrio of clinical-epidemiological importance. MOJ Biol Med. 2021;6(3):116-25.
7. Takahashi E, Ochi S, Mizuno T, Morita D, Morita M, Ohnishi M, Koley H, Dutta M, Chowdhury G, Mukhopadhyay AK, Dutta S. Virulence of cholera toxin gene-positive Vibrio cholerae non-O1/non-O139 strains isolated from environmental water in Kolkata, India. Frontiers in Microbiology. 2021 Aug 20;12:726273.
8. Baker-Austin C, Lake I, Archer E, Hartnell R, Trinanes J, Martinez-Urtaza J. Stemming the rising tide of Vibrio disease. The Lancet Planetary Health. 2024 Jul 1;8(7):e515-20.
9. Percival S, Chalmers R, Embrey M, Hunter P, Sellwood J, Wyn-Jones P. Microbiology of waterborne diseases. 2004 Aug 4.
10. Pascual M, Rodó X, Ellner SP, Colwell R, Bouma MJ. Cholera dynamics and El Nino-southern oscillation. Science. 2000 Sep 8;289(5485):1766-9.
11. Martinez-Urtaza J, Bowers JC, Trinanes J, DePaola A. Climate anomalies and the increasing risk of Vibrio parahaemolyticus and Vibrio vulnificus illnesses. Food Research International. 2010 Aug 1;43(7):1780-90.
12. Frank C, Littman M, Alpers K, Hallauer J. Vibrio vulnificus wound infections after contact with the Baltic Sea, Germany. Weekly releases (1997–2007). 2006 Aug 17;11(33):3024.
13. Schets FM, Van den Berg HH, Demeulmeester AA, Van Dijk E, Rutjes SA, Van Hooijdonk HJ, de Roda Husman AM. Vibrio alginolyticus infections in the Netherlands after swimming in the North Sea. Weekly releases (1997–2007). 2006 Nov 9;11(45):3077.
14. Semenza JC, Herbst S, Rechenburg A, Suk JE, Höser C, Schreiber C, Kistemann T. Climate change impact assessment of food-and waterborne diseases. Critical reviews in environmental science and technology. 2012 Apr 15;42(8):857-90.
15. Naseer U, Blystad H, Angeloff L, Nygård K, Vold L, Macdonald E. Cluster of septicaemia and necrotizing fasciitis following exposure to high seawater temperatures in southeast Norway, June to August 2018. International Journal of Infectious Diseases. 2019 Feb 1;79:28.
16. Ukwuoma CI. Prevalence of Vibrio species in Sea Foods and Water Sources in Cross River State.
17. Kirschner AK, Schauer S, Steinberger B, Wilhartitz I, Grim CJ, Huq A, Colwell RR, Herzig A, Sommer R. Interaction of Vibrio cholerae non-O1/non-O139 with copepods, cladocerans and competing bacteria in the large alkaline lake Neusiedler See, Austria. Microbial ecology. 2011 Apr;61:496-506.
18. Canellas AL, Lopes IR, Mello MP, Paranhos R, de Oliveira BF, Laport MS. Vibrio species in an urban tropical estuary: Antimicrobial susceptibility, interaction with environmental parameters, and possible public health outcomes. Microorganisms. 2021 May 7;9(5):1007.
19. Teklehaimanot GZ, Genthe B, Kamika I, Momba MN. Prevalence of enteropathogenic bacteria in treated effluents and receiving water bodies and their potential health risks. Science of the Total Environment. 2015 Jun 15;518:441-9.
20. Levine ND. Buchanan, RE & Gibbons, NE, eds. 1974. Bergey's Manual of Determinative Bacteriology. Williams & Wilkins Co., Baltimore, Md. 21202. xxvi+ 1246 pp. $45.00.

21. Novoslavskij A, Terentjeva M, Eizenberga I, Valciņa O, Bartkevičs V, Bērziņš A. Major foodborne pathogens in fish and fish products: a review. Annals of microbiology. 2016 Mar;66:1-5.
22. Lou X, Zhai D, Yang H. Changes of metabolite profiles of fish models inoculated with Shewanellabaltica during spoilage. Food Control. 2021 May 1;123:107697.
23. Azike CA, Agi VN, Nwokah EG, Wachukwu CK, Chukwuemeka DT. Prevalence Rate of Vibrio cholerae and other Vibrio Species Isolated from Stool Samples in Andoni Community of Rivers State. Asian Journal of Research in Infectious Diseases. 2021 Jul 2;7(2):16-24.
24. Utsalo SJ, Eko FO, Antia-Obong OE. Cholera and Vibrio parahaemolyticus diarrhoea endemicity in Calabar, Nigeria. West African Journal of Medicine. 1991 Apr 1;10(2):175-80.
25. Baker-Austin C, Oliver JD, Alam M, Ali A, Waldor MK, Qadri F, Martinez-Urtaza J. Vibrio spp. infections. Nature Reviews Disease Primers. 2018 Dec;4(1):1-9.
26. World Health Organization. Selection and application of methods for the detection and enumeration of human-pathogenic halophilic vibrio spp. in seafood: guidance. World Health Organization; 2016.
27. Okogeri EI, Amala SE, Nwokah EG, Monsi TP. Cross-sectional study of extended spectrum β-lactamase genes (SHV, TEM, CTX-M and OXA) in Klebsiella species from clinical specimens in Port Hacourt, Nigeria. Am. J. Med. Sci. Med. 2020;8:180-6.
28. Blanch AR, Hispano C, Bultó P, Ballesté E, González-López JJ, Vilanova X. Comparison of Vibrio spp. populations found in seawater, in exhibition aquaria, in fish intestine and in fish feed. Journal of applied microbiology. 2009 Jan 1;106(1):57-65.
29. Hsiao, A., & Zhu, J. (2020). Pathogenicity and virulence regulation of Vibrio cholerae at the interface of host-gut microbiome interactions. *Virulence*, *11*(1), 1582-1599.
30. Teklehaimanot GZ, Genthe B, Kamika I, Momba MN. Prevalence of enteropathogenic bacteria in treated effluents and receiving water bodies and their potential health risks. Science of the Total Environment. 2015 Jun 15;518:441-9.
31. Tlusty M, Bengtson D, Halvorson HO, Oktay S, Pearce J, Rheault Jr RB. Marine Aquaculture and the Environment.
32. Abd-Elghany SM, Sallam KI. Occurrence and molecular identification of Vibrio parahaemolyticus in retail shellfish in Mansoura, Egypt. Food control. 2013 Oct 1;33(2):399-405.
33. Youssef AI, Farag AL, Helal IM. Molecular characterization of Vibrio parahaemolyticus isolated from shellfish and their harvesting water from Suez Canal area, Egypt. International Food Research Journal. 2018 Nov 1;25(6):2375-81.
34. Al-Mouqati S, Azad IS, Al-Baijan D, Benhaji A. Vibrio detection in market seafood samples of Kuwait by biochemical (API 20E) strips and its evaluation against 16s rDNA-based molecular methods. Res. J. Biotechnol. 2012 Aug 1;7:63-9.
35. de Moura Neves-Gonçalves T, Pinto E, Viegas O, Braga AR, de Souza Mesquita LM, Oliveira IM, Garcia-Jares C, De Rosso VV, Domene SM. Evaluation of the potential of annatto seed powder to reduce the formation of heterocyclic amines in charcoal-grilled and pan-fried beef patties. Food Chemistry. 2024 Aug 26:141015.