ISOLATION AND IDENTIFICATION OF *Vibrio.sp* FROM MARINE FISHES OF MUMBAI, MAHARASHTRA.

ABSTRACT: *Vibrio* is a common bacterium found in marine fishes. *Vibrio* species are pathogenic to humans and cause various gastrointestinal diseases. Fish markets, fish harvesting areas, vectors like flies, seawater and sometimes fresh water bodies are~~is~~ the source of spread of this bacterium. Improper handling or pathogen contamination during transit has an impact not only on human health but also on the population of marine fish. *Vibrio* is known to be a human pathogen, the onset and spread of this bacterium causes severe diarrhoea. In this study, the presence of *Vibrio* species in marine water fishes was determined from ~~the marine~~ fishes collected from various fish markets in Mumbai, Maharashtra. Various b~~B~~iochemical t~~T~~ests were performed to isolate and identify the *Vibrio* species. In the family *Vibrio*naceae three species such as *Vibrio cholera*, *Vibrio parahaemolyticus*~~,~~ and *Vibrio vulnificus* were identified. Additionally, a survey was conducted using google forms to understand the consumption rate of fish by people and also to gauge the awareness among ~~the~~ public~~individuals~~ about the bacterial disease caused by consumption of fishes. It was observed that a large number~~amount~~ of individuals consumed various types of fishes including marine and freshwater fishes on a regular basis. People also consume raw fish delicacies. However, very few individuals ~~a~~were aware of the bacteria and its related diseases, most of the participants were unaware of the presence of bacteria like *Vibrio cholera* and its associated diseases.

Keywords: *Vibrio*, Biochemical, Isolation, Identification, Gastrointestinal, Contamination.

1. INTRODUCTION

Mumbai, a metropolitan city situated along the seacoast, heavily relies on fish as a staple food. Fishing is not only an occupation but also a source of livelihood for many fishermen. However, fish is prone to bacterial contamination, which can have harmful effects on both the fish and those who consume it. Bacteria like *E. coli*, *Aromonas hydropilus*, and *Vibrio* can be found in fish, with *Vibrio* being the most dangerous as it

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can cause gastrointestinal diseases in humans. The consumption of various fish delicacies, such as dried fish and raw fish like sushi, especially if the fish is undercooked, increases the risk of bacterial infections. The main sources of bacterial contamination in seafood are the transit from the shore, landing centres, fish harvesting areas, and fish markets. *Vibrio* species, which are halophilic and commonly found in marine waters, can contaminate seafood and lead to illnesses like *cholera* and gastroenteritis. Contamination of seafood with *Vibrio* may lead to illnesses like *cholera* and gastroenteritis [1]. *Cholera*, a highly deadly bacterial disease that causes diarrhoea is a common phenomenon observed in various parts of the globe, especially India. The two major outbreaks were observed in 1997-2000 (68 outbreaks) and in 2009-2017 (559 outbreaks) in India [2].

In this study, fish like Mackerels *(Rastrelliger kanagurta)*, White mullets *(Mugil curema)*, Lady fishes *(Elops saurus)* and Sawfish *(Pristis pectinata)* were selected, which are commonly consumed by the local people of Mumbai as it is cheap and easily available. These fishes were collected and examined for the ~~existence~~ presence of bacteria., ~~the~~ Ggram-negative bacteria ~~were~~ isolated from them were~~and~~ characterised; and using biochemical assay, the genus of *Vibrio* was identified. *Vibrio* are found in seawater, brackish water and freshwater; they are gram-negative bacteria that are small, comma-shaped microorganisms that are facultative anaerobes (without oxygen requirements), do not produce spores, and can survive in a salty environment. *Vibrio* species that are majorly pathogenic to humans are *V. cholerae*, *V. parahemolyticus*, and *V. vulnificans*. It was found responsible for gastroenteritis, wound infections, and septicaemia [3]. Other species like *Vibrio alginolyticus*, *Vibrio fluvialis*, *Vibrio mimicus*, *Vibrio furnissii*, etc. are less severe but have pathogenic potential for humans [4]. People consume food that is contaminated with such bacteria, and the bacteria cross the gastric barrier and infect the intestine. They form colonies and multiply in the intestine, releasing toxins. This bacterium spreads fast to those who are immunocompromised, and also to those individuals who use antacids and have GI tract-related disorders.

The purpose of this study is to isolate and identify *Vibrio* species and to explore the occurrence of *Vibrio cholera*, *Vibrio parahaemlyticus* and *Vibrio vulnificans* in fish and the potential risks they pose to human health. In addition, measures such as monitoring

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fish and seafood products for these pathogens, implementing appropriate sanitation measures in seafood processing plants, and educating people about the risks of consuming raw or undercooked seafood can help to reduce the incidence of *Vibrio*-associated foodborne diseases.

2. MATERIALS AND METHODS

For the present study, the investigation was divided into two parts

2.1 Isolation and identification of *Vibrio* species through biochemical test.

2.2 To understand the awareness among people about the bacterial diseases caused by fish consumption using Google Survey Form.

2.1 Isolation and Identification of *Vibrio* through biochemical test

The isolation and identification of *Vibrio* was done as described in Bacteriological Analytical Manual by FDA 2004 and Bergey's Manual [5][6].

Sampling Method: A total of 120 fish were collected from three different fish markets in Mumbai. Mackerel (N = 30), white mullet (N = 30), ladyfish (N = 30), and sawfish (N = 30) samples were collected from local markets in the study area. From one location, the collection of samples was done twice; five fish were collected each time. The samples were collected using polythene bags, labelled, and transported to the laboratory for analysis.

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Table: 01 Fish sample collection from three location in batches.

Name of Fish	Name of Location	Collection	No. of Fishes	Total Fishes Collected
Mackerel	Location 01: Malad	Collection 1	5	30 Mackerels were collected
		Collection 2	5	
	Location 02: Andheri	Collection 1	5	
		Collection 2	5	
	Location 03: Versova	Collection 1	5	
		Collection 2	5	
White Mullet	Location 01: Malad	Collection 1	5	30 White Mullets were collected
		Collection 2	5	
	Location 02: Andheri	Collection 1	5	
		Collection 2	5	
	Location 03: Versova	Collection 1	5	
		Collection 2	5	
Lady Fish	Location 01: Malad	Collection 1	5	30 Lady Fish were collected
		Collection 2	5	
	Location 02: Andheri	Collection 1	5	
		Collection 2	5	
	Location 03: Versova	Collection 1	5	
		Collection 2	5	
Saw Fish	Location 01: Malad	Collection 1	5	30 Saw Fish were collected
		Collection 2	5	
	Location 02: Andheri	Collection 1	5	
		Collection 2	5	
	Location 03: Versova	Collection 1	5	
		Collection 2	5	
				total = 120

The methodology for the isolation of *Vibrio species* from fish samples can be summarized as follows:

Sample preparation: Samples of fish muscles were obtained and homogenized ~~it~~ with distilled water.

Enrichment: 1 ml of the sample was suspended in 9 ml of Alkaline Peptone Water (APW) with pH-8.2 and incubate it at 37°C for exactly 24 hours.

Isolation: Loop full of the surface aliquot from the APW culture and streak it onto the surface of Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar. Incubate the plate at 37°C for overnight.

Examination: TCBS plates were examined for yellow and green colonies that resemble *Vibrio* species. Single colony of each colour was picked and inoculate it onto Nutrient Agar (NA). Incubate the NA at 37°C for overnight.

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BIOCHEMICAL TESTING: Biochemical Testing of *Vibrio* was done as described in the Bacteriological analytical manual by FDA 2004 and Bergey's Manual. Following biochemical~~,~~ tests were performed on the isolated *Vibrio* culture: gram staining [7], growth in different salt concentrations (0%, 3%, 6%, 8%, and 10% NaCl), V~~v~~oge's P~~p~~roskauer test, lysine decarboxylase, sucrose test, indole test, nitrate reduction test, triple sugar iron (TSI) test, oxidase test, string test [5][6].

3. RESULTS AND DISCUSSION

In the present study, 120 samples of fish were collected and grouped according to the location. After enrichment and isolation on selective media TCBS agar, biochemical tests were performed. As a result, *Vibrio cholera* was found in 60 samples; *Vibrio vulnificans* was found in 10 samples; and *Vibrio parahaemolyticus* was found in 10 samples. As a result, three distinct *Vibrio* species were discovered. In 10 samples, *Vibrio* was absent.

Gram staining was performed on all the samples; all samples were gram-negative.

Mackerel fishes from Market 1 (Malad) were grouped as sample S1, and small yellow colonies were observed; the oxidase test was positive, as were the lysine, sucrose, and NO3 tests. Hence, the sample was interpreted as *Vibrio cholera*.

Mackerel fishes from Market 2 (Andheri) grouped as sample S2, small green colonies were observed, growing in 0%, 3%, and 6% concentrations of salt. Positive results were obtained from lysine and NO3, but VP, sucrose, and indole tests were negative. Both the oxidase test and the string test were positive. As in the TSI test, the butt was H2S positive. Hence, the sample was interpreted as the absence of *Vibrio*.

Mackerel fishes from Market 3 (Versova) were grouped as sample S3, small yellow colonies were observed, oxidase test and string test were positive, and Lysine, sucrose and NO3 test were positive. Bacterial growth was observed in 0%, 3%, 6%, and 8% salt concentrations. Hence, the sample was interpreted as *Vibrio cholera*.

White mullet fishes from Market 1 (Malad) were grouped as sample S4, small green colonies were observed, growth in 0% and 3% concentrations of salt. Positive results were obtained from lysine and NO3, but VP, sucrose, and indole tests were negative. Both the oxidase test and the string test were positive. Hence, the sample was interpreted as *Vibrio vulnificans*.

White mullet fishes from Market 2 (Andheri) grouped as sample S5, small green colonies were observed, growing in 0%, 3%, and 6% concentrations of salt. Positive results were obtained from lysine and NO3, but VP, sucrose, and indole tests were

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negative. Both the oxidase test and the string test were positive. As in the TSI test, the butt was H2S positive. Hence, the sample was interpreted as the absence of *Vibrio*.

White mullet fishes from Market 3 (Versova) grouped as sample S6 small green colonies were observed with growth at 0% and 3% concentration of salt. Positive results were obtained from lysine and NO3, but VP, sucrose, and Indole tests were negative. Both the oxidase test and the string test were positive. As in the TSI test, the slant was alkaline. Hence, the sample was interpreted as *Vibrio parahaemolyticus*.

Lady fishes from Market 1 (Malad) were grouped as sample S7, small green colonies were observed, growth in 0% and 3% concentrations of salt. Positive results were obtained from lysine and NO3, but VP, sucrose, and indole tests were negative. Both the oxidase test and the string test were positive. Hence, the sample was interpreted as *Vibrio vulnificans*.

Lady fishes from Market 2 (Andheri) were grouped as sample S8, small yellow colonies were observed, the oxidase test and string test were positive, and Lysine, sucrose and NO3 tests were positive. Bacterial growth was observed at 0% and 3% salt concentrations. Hence, the sample was interpreted as *Vibrio cholera*.

Lady fishes from Market 3 (Versova) were grouped as sample S9, small yellow colonies were observed, the oxidase test and string test were positive, and Lysine, sucrose and NO3 tests were positive. Bacterial growth was observed in 0%, 3%, 6%, and 8% salt concentrations. Hence, the sample was interpreted as *Vibrio cholera*.

Sawfishes from Market 1 (Malad) were grouped as sample S10, and small yellow colonies were observed; the oxidase test was positive, as were the lysine, sucrose, and NO3 tests. Hence, the sample was interpreted as *Vibrio cholera*.

Sawfishes from Market 2 (Andheri) were grouped as sample S11, small yellow colonies were observed, the oxidase test and string test were positive, Lysine, sucrose and NO3 test were positive. Bacterial growth was observed at 0% and 3% salt concentrations. Hence, the sample was interpreted as *Vibrio cholera.*

Sawfishes from Market 3 (Versova) grouped as sample S12, small green colonies were observed with growth at 0% and 3% concentration of salt. Positive results were obtained

from lysine and NO3, but VP, sucrose, and Indole tests were negative. Both the oxidase test and the string test were positive. As in the TSI test, the slant was alkaline. Hence, the sample was interpreted as *Vibrio parahaemolyticus*.

It can be concluded that *Vibrio cholera* species were found in samples of Mackerel and Sawfish collected from Malad (Market 1) and Versova (Market 3). Lady Fish collected from Andheri and Versova also shows the presence of *Vibrio cholera*. *Vibrio vulnificans* was found in white mullets and ladyfish collected from Malad. *Vibrio parahaemolyticus* was found in white mullets and sawfish collected from Versova. Mackerel and white mullets collected from Andheri Market show the absence of *Vibrio* species.

Table 2. Observation and Interpretation table for biochemical test for Vibrio

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Also it would be better if all the data are summarised into a table, than listing it in separate paragraphs like this. Since table is given below, a sentence like - The results of biochemical test are summarised in the table below - would be sufficient

Table 02: Observations and Interpretations Table for Biochemical Test for *Vibrio*

Name of Fish	Name of Location	Colonies on TCBS plate	Gram staining	Growth in					VP	Lysine	Sucrose	Indole	NO_3 reduction	TSI				String Test	Oxidase test	Interpretation
				0% salt	3% salt	6% salt	8% salt	10% salt						butt	Slant	H_2S	Gas			
Mackerel	Location 01: Malad	Small Yellow	Negative	+	-	-	-	-	-	+	+	-	+					Positive	Positive	*V. cholera*
	Location 02: Andheri	Small Green	Negative	+	+	+	-	-	-	+	-	-	+			Butt Positive		Positive	Positive	*NA*
	Location 03: Versova	Small Yellow	Negative	+	+	+	+	-	-	+	+	-	+					Positive	Positive	*V. cholera*
White Mullet	Location 01: Malad	Small Green	Negative	+	+	-	-	-	-	+	-	-	+					Positive	Positive	*V. vulnificus*
	Location 02: Andheri	Small Green	Negative	+	+	+	-	-	-	+	-	-	+			Butt Positive		Positive	Positive	*NA*
	Location 03: Versova	Small Green	Negative	+	+	-	-	-	-	+	-	-	+		Alkaline			Negative	Positive	*V. parahaemolyticus*
Lady Fish	Location 01: Malad	Small Green	Negative	+	+	-	-	-	-	+	-	-	+					Positive	Positive	*V. vulnificus*
	Location 02: Andheri	Small Yellow	Negative	+	+		-	-	-	+	+	-	+					Positive	Positive	*V. cholera*
	Location 03: Versova	Small Yellow	Negative	+	+	+	+	-	-	+	+	-	+					Positive	Positive	*V. cholera*
Saw Fish	Location 01: Malad	Small Yellow	Negative	+	-	-	-	-	-	+	+	-	+					Positive	Positive	*V. cholera*
	Location 02: Andheri	Small Yellow	Negative	+	+		-	-	-	+	+	-	+					Positive	Positive	*V. cholera*
	Location 03: Versova	Small Green	Negative	+	+		-	-	-	+	-	-	+		Alkaline			Negative	Positive	*V. parahaemolyticus*

SURVEY ON CONSUMPTION OF FISH AND ITS IMPACT ON HEALTH: Survey was conduction to understand the consumption of fish food by the people and weather they are aware about the bacterial disease caused by consumption of fishes.

In a Google survey form, various questions were asked, such as which fish are commonly consumed by people, how often they consume them, how much they consume, the method of cooking fish, whether they experience any medical symptoms or illness after consuming fish, and whether they are aware of the bacteria and contamination. 140 responses were received in the survey.

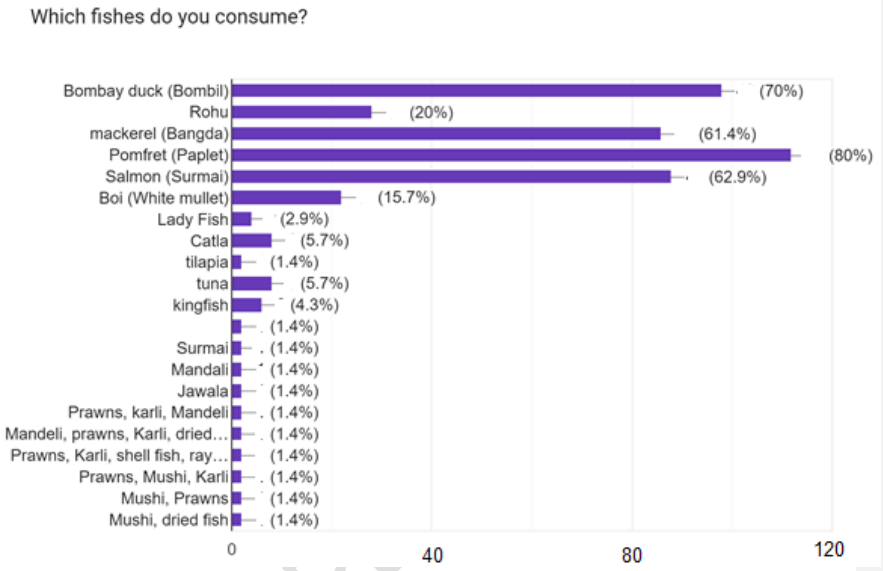

Fig. 1. Responses of Survey Question 1

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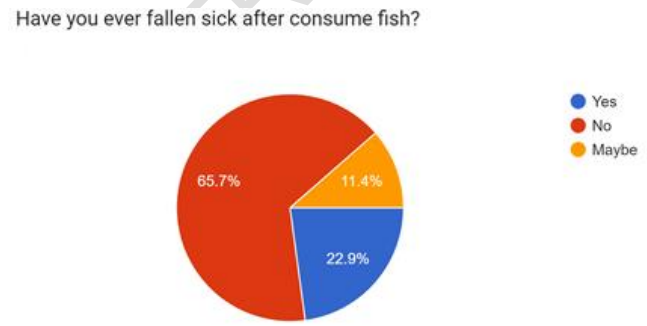

Fig. 2. Responses of Survey Question 2

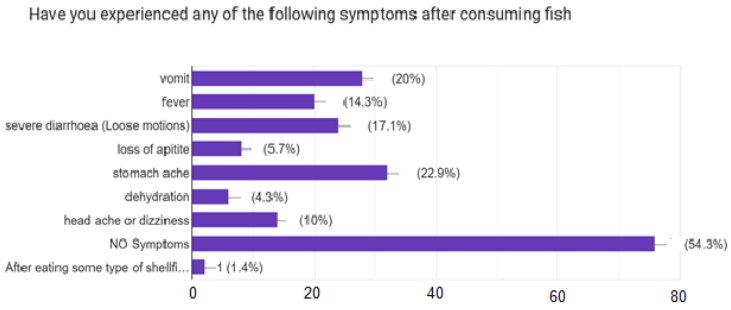

Fig. 3. Responses of Survey Question 3

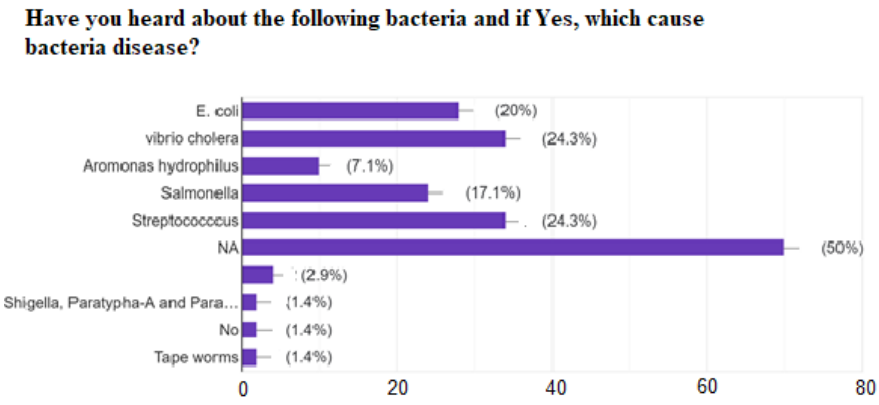

Fig. 4. Responses of Survey Question 4

In the present study, *Vibrio cholera*, *Vibrio parahaemolyticus*, *Vibrio vulnificans* are the three different species identified from the fish samples, which were collected from Mumbai fish market. From which *Vibrio cholera* was identified from Mackerels and Saw fish, which were collected from Malad and Versova. Lady Fish collected from Andheri and Versova, also shows presence of *Vibrio cholera*. *Vibrio parahaemolyticus* was identified from the white mullets and saw fish collected from Versova. *Vibrio vulnificans* was identified from the white mullets and Lady Fish which were collected from Malad. No *Vibrio* species were identified from the Mackerels and White Mullets, which were collected from Andheri. Similarly, another study from north-western Mumbai where 140 samples of various fishes and shell fishes were examined for pathogenic strain of *V. parahaemolyticus* [8], as *Vibrio*

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parahaemolyticus was identified from Versova, in this study it signifies that these species of *Vibrio* are frequently identified in this region. Studies from south of India is comparable as they have identified the similar species of *V. parahaemolyticus* using Biochemical test and PCR [9] [10] [11]. Along the coast of South west India, according to the study 93.87% of samples were identified as *Vibrio parahaemolyticus* [12]. In kolkata, 90 samples were collected and 60 samples were positive for *V. parahaemolyticus* [13]. These studies from over all India gives us a brief idea about the spread of *Vibrio parahaemolyticus* in various parts of India.

Similar study was carried out by K. Arunagiri and associates, who collected twenty fish and crab samples from fish stores in Kanchipuram and Walajapet. It was observed that 21 species of *Vibrio* were identified from fin fish and other aquatic materials [14], and the current study indicated that three of the 21 species were common.

In a similar investigation by Mookerjee and co-workers, 120 surface water samples were collected for testing from three separate locations along the Hooghly River in order to test the water for *Vibrio*. In the water samples five enteropathogenic *Vibrio* species, including *Vibrio cholera*e (35%), *Vibrio parahaemolyticus* (22.5%), *Vibrio* mimicus (19.1%), *Vibrio* alginolyticus (15.8%), and *Vibrio* vulnificus (11.6%), were present [15]. Among these five species of *Vibrio*, the three species identified in present study were common, however their sample is river water whereas we isolated *Vibrio* from marine fishes.

Other than India different Asian countries have also identified *Vibrio* in the fishes. In Sri lanka, seven biochemical tests were performed on 159 samples collected from Mutwal and Chilwa and twelve species were thus isolated and identified within the family *Vibrionaceae*, including *A. hydrophila*, *V. cholerae*, *V. metschnikovii*, *V. parahaemolyticus*, *V. carchariae*, *P. shigelloides*, *V. vulnificus*, *V. damsela*, *V. mimicus*, *V. furnissii*, and *V. fluvialis* [16]. Among these species the three speices identified in this study are same. TCBS is the selective media used for the culture of *Vibrio*. Chythanya and co-workers in their study of clinical samples, most nontarget microbes were eliminated using a selective medium like TCBS agar. In the research, bacteria like *V. vulnificus*, *V. parahaemolyticus*, *V. damsela*, and *V. fluvialis* were recovered from moribund shrimps on TCBS agar [17] while, two of the species *V.*

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parahaemoyticus and *V. vulnificans* are common to present study. Nambabi and associates have isolated and identified various *vibrio* species and they concluded that various intrinsic and extrinsic factors are responsible for the deterioration of the fishes [18].

Survey was conducted and it was found that people consume fish food, some of them observe various symptoms and fell sick, contamination of fish can be a major reason. Most of the people are unaware about the various bacteria that are human pathogens and may cause diseases. It is necessary to educate people about sanitization and how to preventabout contamination of such food. As people are moderately aware about the ocean pollution and contamination, it is necessary to have holistic approach for making new policies [19]. Without public awareness and support, it is difficult to protect the marine protection acts [20].

4. CONCLUSION

In the fish markets of Mumbai, the fishes were biochemically tested using various parameters (Gram staining, growth in different salt concentrations, VP test, Lysine, sucrose, indole, TSI, string test, oxidase test) for the presence of gram-negative bacteria *Vibrio* and 10 out of 12 groups were identified with *Vibrio cholera*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*. The presence of these species indicates that there is contamination of the seafood with bacteria which may cause diseases like *Cholera* and gastroenteritis. These species' existence suggests that the fishes have been contaminated with bacteria, which could result in illnesses like *cholera* and gastroenteritis. Novel methods like PCR or 16S-23S sequencing can be used to enhance the identification protocols. To determine the extent of the contamination and the risk of illness, more samples, including fish and other crustaceans, need to be examined. The research might assist the officials in adoptingtaking the appropriate measures to prevent the spread of illness. Additionally, a survey was conducted through Google google fForms to understand the consumption of fish food by the people and whether they are aware of the bacterial disease caused by the consumption of fish. It was found that people consumed various types of fish including marine and freshwater fishes. People also consume raw fish delicacies and use various cooking techniques for fish. Very few people are aware of the bacteria and its related diseases.

REFERENCE

[1] Carboni G. P. (2021). The enigma of Pacini's *Vibrio cholerae* discovery. *Journal of medical microbiology*, *70*(11), 10.1099/jmm.0.001450. https://doi.org/10.1099/jmm.0.001450

[2] Chatterjee, P., Kanungo, S., Bhattacharya, S. K., & Dutta, S. (2020). Mapping *cholera* outbreaks and antibiotic resistant *Vibrio cholera*e in India: An assessment of existing data and a scoping review of the literature. *Vaccine*, *38 Suppl 1*, A93–A104. https://doi.org/10.1016/j.vaccine.2019.12.003

[3] Johnson, C. N., Bowers, J. E., Griffitt, K. J., Molina, V., Clostio, R. W., Pei, S., Laws, E. R., Paranjpye, R. N., Strom, M. S., Chen, A., Hasan, N. A., Huq, A., Noriea, N. F., Grimes, D. J., & Colwell, R. R. (2012b). Ecology of *Vibrio parahaemolyticus* and *Vibrio* vulnificus in the Coastal and Estuarine Waters of Louisiana, Maryland, Mississippi, and Washington (United States). *Applied and Environmental Microbiology*, *78*(20), 7249–7257. https://doi.org/10.1128/aem.01296-12

[4] Baker-Austin, C., Oliver, J. D., Alam, M., Ali, A., Waldor, M. K., Qadri, F., & Martinez-Urtaza, J. (2018). *Vibrio* spp. infections. Nature Reviews Disease Primers, 4(1), 1–19. https://doi.org/10.1038/s41572-018-0005-8

[5] FDA (2004) Bacteriological analytical manual, chapter 9. US Food and Drug Administration, Center for Food Safety

[6] Brenner, D. J., Krieg, N. R., & Staley, J. R. (2007). *Bergey's Manual® of Systematic Bacteriology: Volume 2: The Proteobacteria, Part B: The Gammaproteobacteria*. Springer Science & Business Media.

[7] BARTHOLOMEW, J. W., & MITTWER, T. (1952). The Gram stain. *Bacteriological reviews*, *16*(1), 1–29. https://doi.org/10.1128/br.16.1.1-29.1952

[8] Ayyappan, M. V. (2018, July 26). *Distribution of Potentially Pathogenic Vibrio parahaemolyticus in Seafood and the Aquatic Environment of Mumbai, India*. https://epubs.icar.org.in/index.php/FT/article/view/81772

[9] Raghunath, P., Acharya, S., Bhanumathi, A., & Karunasagar, I. (2008). Detection and molecular characterization of *Vibrio parahaemolyticus* isolated from seafood

harvested along the southwest coast of India. *Food Microbiology*, *25*(6), 824–830. https://doi.org/10.1016/j.fm.2008.04.002

[10] Chakraborty, R. D., Surendran, P., & Joseph, T. C. (2008). Isolation and characterization of *Vibrio parahaemolyticus* from seafoods along the southwest coast of India. *World Journal of Microbiology & Biotechnology*, *24*(10), 2045–2054. https://doi.org/10.1007/s11274-008-9708-4

[11] Sudha, S., Divya, P. S., Francis, B., & Hatha, A. a. M. (2012b). Prevalence and distribution of *Vibrio parahaemolyticus* in finfish from Cochin (south India). *Veterinaria Italiana*, *48*(3), 269–281.

[12] Deepanjali, A., Kumar, H., & Karunasagar, I. (2005). Seasonal Variation in Abundance of Total and Pathogenic *Vibrio parahaemolyticus* Bacteria in Oysters along the Southwest Coast of India. *Applied and Environmental Microbiology*, *71*(7), 3575–3580. https://doi.org/10.1128/aem.71.7.3575-3580.2005

[13] Pal, D., & Das, N. (2010). Isolation, identification and molecular characterization of *Vibrio parahaemolyticus* from fish samples in Kolkata. *European Review for Medical and Pharmacological Sciences*, *14*(6), 545–549.

[14] Arunagiri, K., Jayashree, K., & Sivakumar, T. (2013). Isolation and identification of *Vibrio*s from marine food resources. *International Journalof Current Microbiology and Applied Science*.

[15] Mookerjee, S., Batabyal, P., Sarkar, M. H., & Palit, A. (2015). Seasonal Prevalence of Enteropathogenic *Vibrio* and Their Phages in the Riverine Estuarine Ecosystem of South Bengal. *PLOS ONE*, *10*(9), e0137338. https://doi.org/10.1371/journal.pone.0137338

[16] Jayasinghe, C., Ahmed, S., & Kariyawasam, M. (2010). The Isolation and Identification of *Vibrio* Species in Marine Shrimps of Sri Lanka. *Journal of Food and Agriculture*, *1*(1). https://doi.org/10.4038/jfa.v1i1.1838

[17] Chythanya, R., & Karunasagar, I. (2002). Inhibition of shrimp pathogenic *Vibrio*s by a marine Pseudomonas I-2 strain. *Aquaculture*, *208*(1–2), 1–10. https://doi.org/10.1016/s0044-8486(01)00714-1

[18] N, S. M. N. N. (2012). The role, isolation and identification of *Vibrio* species on the quality and safety of seafood. *Biotechnology and Molecular Biology Reviews*, *7*(2). https://doi.org/10.5897/bmbr11.024

[19] Gelcich, S., Buckley, P., Pinnegar, J.K., Chilvers, J., Lorenzoni, I., Terry, G., Guerrero, M.,
Castilla, J.C., Valdebenito, A., Duarte, C.M. 2014. Public awareness, concerns, and
priorities about anthropogenic impacts on marine environments. Proceedings of the
National Academy of Sciences 111(42): 15042-15047.
[20] Fletcher, S., Potts, J. S., Heeps, C., Pike, K. 2009. Public awareness of marine environ-
mental issues in the UK. Marine Policy 33(2): 370-375

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