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Occurrence of *Vibrio* Species in Marine Sources Surroundings Bonaire, Dutch Caribbean

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ABSTRACT

There is a lack of information on presence of vibrios in the marine environment in the Caribbean. The aim of this study was to determine the occurrence of *Vibrios* in the coastal waters of Bonaire. Fifty samples of marine water collected at different depths from various sources around Bonaire were examined for the presence of vibrios. Species identification was confirmed by KB007 HiVibrio™, Identification Kit and TOFEL-MALDI. Forty of the samples contained *Vibrio alginolyticus*, 33 yielded *V. parahaemolyticus* and 29 showed presence of *V. vulnificus* / *V. cholerae*. Regarding total colony counts in the sample, 47.4% of the colonies were *V. alginolyticus*, 35.2% were *V. parahaemolyticus*, and 17.4% represented *V. vulnificus* /*V. cholerae*. Further, of the 25 surface samples from various sites, 14 had a colony count percentage of 50% or greater number of *V. alginolyticus*. Another 10 sites had a colony count percentage of 50% or greater for *V. parahaemolyticus*; three of them had a colony count percentage of 50% or greater for *V. vulnificus* / *V. cholerae*. The present study constitutes the first study of its kind providing evidence of the prevalence of pathogenic *Vibrio* species, viz. *V. alginolyticus, V. parahaemolyticus,* and *V. vulnificus* / *V. cholerae* in marine water from the Dutch Caribbean.

Keywords: Marine waters, Bonaire, *Vibrio* species, and Dutch Caribbean.

INTRODUCTION:

Vibrios are Gram negative, curved rods that are oxidase positive and facultative anaerobes. They are abundant in aquatic environment (Thompson *et al.,* 2004). Among the more than seventy different Vibrio species inhabiting marine, estuarine, and freshwater ecosystems, eight are recognized as clinically important, namely, *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus, V. mimicus*, *V. alginolyticus*, *V. harveyi*, *V. metschnikovii*, and *V. cincinnatiensis* (Farmer, 2006). These organisms are halophilic (salt-loving) and thus most species reat species require salt in the growth media to indicate their presence (Murray *et al.,* 2013). Toxigenic strains of *V. cholerae* 01 have been detected by PCR from the Latin American cholera epidemic (Fields *et al.,* 1992). According to the CDC estimates, vibriosis causes 80,000 illnesses each year in the United States, about 52,000 of these illnesses resulting from eating contaminated food in the United States annually (CDC 24/7). Toxigenic *Vibrio cholerae* are found after O1 in water and seafood, Haiti (Hill *et al.,* 2011). The species clinically most significant for humans are *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus*, and within these three species, there are different serogroups (Thompson *et al.,* 2004). There are currently 200 serogroups of *V. cholerae* identified and it is important to note that not all sero groups have been

found to be pathogenic (Daniel and Shefaei, 2004). Of great importance, however, are *V. cholerae* O1 and O139 since these serogroups produce the cholera toxin that are responsible for epidemic Cholera (Dutta *et al.,* 2013; Murray *et al.,* 2013) require salt in the growth media to indicate their presence (Murray *et al.,* 2013). According to the CDC estimates, vibriosis causes 80,000 illnesses each year in the United States, about 52,000 of these illnesses resulting from eating contaminated food in the United States annually (CDC 24/7). Toxigenic *Vibrio cholerae* O1 in water and seafood, Haiti (Hill *et al.,* 2011). The species clinically most significant for humans are *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus*, and within these three species, there are different serogroups (Thompson *et al.,* 2004).

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stomach acid secretion (higher pH), there is risk of acquiring a *Vibrio* infection with symptoms of severe gastroenteritis (Murray *et al.,* 2013). *Vibrio* infections contracted through an open wound initially result in severe pain at the injury site leading to numbness and if medical attention is delayed, septicaemia may develop (Hoffman *et al.,* 2012). It is in this context that investigation on occurrence of pathogenic *Vibrios* in marine sources is of medical and public health importance. Oliver *et al.* (1982) in a study of sampling water from North Carolina to Georgia found *V. vulnificus*, *V. cholerae*, *V. mimicus*, and *V. fluvial* in the water, on the sediment and on the marine life. A study from Saudi Arabia (Elhadi 2013) found *V. alginolyticus, V. parahaemolyticus, V. vulnificus, V. mimicus* and *V. cholerae* non-O1/ non-O139 in the coastal waters. Isolation of *Vibrio parahemolyticus*, *V. vulnificus*. and *V. fluvialis* from frozen imported raw fish been reported from Egypt (Khalil *et al.,* 2014). Another study performed in Chesapeake Bay found *V. vulnificus* in water samples obtained during the summer months but not in the winter months (Wright *et al.,* 1996). These studies indicate the *Vibrio* bacteria are autochthonous in various locations specifically in the coastal waters of the US, Gulf of Mexico, Americas, and the Caribbean. So far there are no reported studies documenting the presence of *Vibrio* species in coastal waters of the Dutch Caribbean.

Therefore, this study aims to provide data on the presence of *Vibrio* species in the marine environment surrounding Bonaire in the southern region of the Caribbean. This study is particularly important because the local economy of Bonaire relies heavily on sightseeing activities that include diving and snorkelling as well as on the local fishing industry. It is imperative to be aware of the presence of *Vibrio* bacteria and their potential effects on the marine ecology, Finally, the study is significant for the health of tourists and locals, who may acquire *Vibrio* wound infection after sustaining injures while contacting coral and submerged rock formations (Poutier *et al.,* 2012).

METHODOLOGY:

Sampling

A total of 50 samples of marine water were collected in 15 mL sterile culture tubes from various sources around Bonaire at different depths, with water temperature and pH values recorded for each sample. Twenty-five of these samples originated from surface waters (referred as surface samples), around Bonaire. Scuba diving was performed to obtain 13 samples collected from 20 feet to 50 feet deep (referred to as shallow samples) and 12 samples from a depth of 60 to 130 feet (referred to as deep samples) from many of the sites from where surface sample originated.

Measurement of pH, temperature, and depth of samples

Temperature and depth measurements were accurately measured for each water sample using a dive computer. The pH of the sample was measured utilizing a standard pH paper.

Preparation of medium for isolation of Vibrios

A selective and differential medium called CHRO Magar™ Vibrio purchased from Dalynn Biologicals, Calgary, Alberta was used. The four common species of *Vibrio, V. alginolyticus*, *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* are differentiated on the CHROMagar plate by colony colour; *V. alginolyticus* appears colour less or opaque white, *V. parahaemolyticus* appears mauve, and *V. cholerae/ V. vulnificus* both appear blue on this medium. The medium was prepared as per CHROMagar™ *Vibrio* protocol. A quantity of 37.3 Grams of the medium was mixed into 0.5L of sterile boiling water in a 500 mL Erlenmeyer flask. This mixture was allowed to cool before it was poured into sterile polystyrene petri dishes (100 mm X 10 mm). After the media had solidified, the dishes were stored inverted at 4^{O}C.

Incubation temperature

At the commencement of this project, it was necessary to determine the desired incubation temperature. One ml quantities of three water samples were inoculated on plates of CHROMagar™ with the help of a sterile glass spreader and plates incubated at 37^{0}C in the Laboratory of Department of Health, Bonaire (Bonlab) and observed for growth of vibrions after 24-30 hours of incubation. These were compared to samples from the same sites that were similarly inoculated and incubated at room temperature (25-30^{0}C) in the laboratory of SJSM. Growth was comparable on the plates; therefore, incubation at room temperature in SJSM Lab was used for all other samples.

Processing of samples

The petri dishes were allowed to come to room temperature and 1.0 mL of each sample was placed on the plate using sterile plastic 3 mL pipettes. A glass

spreader, sterilized using isopropyl alcohol and flame, was used to evenly spread the samples over the plates. The plates were then incubated inverted at 30^{O}C for 24 hours. To determine if incubating at 30^{O}C was comparable to the standard 37^{O}C four identical water samples were incubated at BonLab at 37^{0}C for 24 hours and compared. Post incubation the number of colony growths on the plates were recorded. One additional plate with no sample applied was used as a control for this study and incubated at BonLab at 37^{0}C to assess possible contamination of the plates during preparation. Finally, two water samples were tested: one from tap water supplying each household and another sample of inland water consisting of overflow effluent from the island's salt drying fields. To determine the total bacterial count, one ml quantities of each of four representative samples including one each collected from the north (Oil Slick), south (Chez Hines), east (Sorobon) and west (Yellow submarine) areas of the island were plated onto a standard nutrient agar (non-specific media) plate and incubated at 30^{O}C for 24 hrs. Each individual growing colony was considered to have originated from one bacterium and the bacterial count calculated. Gram staining was performed for three samples. One was of blue colony of the sample from Karel's site on CHROMagar^{TM} (*Vibrio cholerae*/ *Vibrio vulnificus*). Three were of the isolated colonies on Nutrient agar of the samples from Oil slick, Sorobon and Yellow submarine sites. Data analysis included constructing graphs and pie charts to summarize the raw colony counts from the various collection sites. First, a graph depicting the number of sampling sites that containing relative amounts of each *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus / V. cholerae* was constructed Nutrient agar. Lastly, a graph depicting numbers of colonies of the various *Vibrio* bacteria that divides the sites based on direction (north, south, east, and west) was constructed.

Oxidase testing of Vibrio isolates

The CHROMagar^{TM} *Vibrio* protocol suggested using oxidase testing to provide further proof of *Vibrio* identification. Oxidase testing was performed on one white colony, one pink colony and one blue colony. These were inoculated onto oxidase discs and a blue colour change indicated a positive oxidase reaction. The fourth oxidase test was conducted as a control to determine that the oxidase test materials themselves were accurate and was performed on a sample from the nutrient agar. This sample showed Gram positive, catalase positive cocci and coagulase negative. It was presumed to be either *S. epidermidis* or *S. saprophyticus*, both being oxidase negative.

KB007 HiVibrio^{TM} Identification Kit testing and confirmation by TOFEL-MALDI

In a final attempt to further determine greater specificity of the various *Vibrio* bacteria isolated from the CHROM ^{TM} *Vibrio*, five fresh samples were utilized in KB007 HiVibrio^{TM} Identification Kit. Four of the samples were completed together, the samples were first plated on CHROMagar^{TM} *Vibrio* to isolate *Vibrio* bacteria, and these were then inoculated on to nutriaent agar and allowed to grow. After incubation, the samples were then inoculated into the wells of the testing kit following KB007 HiVibrio^{TM} Identification Kit protocols. The fifth sample was plated onto CHROMagar^{TM} *Vibrio* and then directly inoculated into the wells of the testing kit. Further Confirmation by TOFEL-MALDI was accomplished in Division of Microbiology, Department of Microbiology, PGI, Chandigarh, India. The number of colonies of pathogenic *Vibrio* species in samples from different locations in Bonaire (with their pH, depth, and temperature of the samples) is provided in **Table 1**. Number of colonies of pathogenic Vibrio species in samples from different locations in Bonaire (with their pH, depth, and temperature of the samples) are reported in **Table 2**. The average temperature for the surface, shallow and deep samples was 82.2^{0}F±0.9, 79.8^{0}F±0.6 and 80.3^{0}F±0.8 respectively. The average pH for surface and shallow samples was 7.1±0.3 whereas that for all deep samples was 7 (**Table 1**) *Vibrio alginolyticus*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*/*Vibrio cholerae* were recovered on CHROMagar^{TM} plates inoculated with marine water samples from almost all the sites tested. There were only two samples that did not yield any vibrio growth. The control tested for plate contamination during their production was negative thus confirming that the colonies that grew were in fact bacteria from the test samples. To confirm that the island's drinking water supply was safe from *Vibrios*, a plate was inoculated with a simple tap water sample; it did not show growth of any *Vibrio* species. The number of samples positive for pathogenic vibrio species in depicted in **Fig. 1**. It was found that 40 of the samples contained *V. alginolyticus*, 33 contained *V. parahaemolyticus* and 29 yielded blue colonies representing either *V. vulnificus*

or *V. cholerae*. It should be noted here that differentiation between *V. vulnificus* and *V. cholerae* could not be performed with the *Vibrio* identification kit. The plates were further examined for the number of various colonies present in the water samples according to sites of collection of the samples. The sample obtained from oil slick (west) had approximately 80 distinct colonies present (http://www.cdc.gov/features/ds.foodnet%202012). (http:www.food safetynews.com/2014/). The sample obtained from Sorobon (east) had approximately 120 distinct colonies. Samples from Chez Hines (south) and Yellow Submarine (west) yielded too numerous colonies to count. **Fig. 2** shows the percentages of the *Vibrio* species present at the surface of the water at different sites. It was determined that 47.4% of the colonies were *V. alginolyticus*, 35.2% were *V. parahaemo-lyticus* and 17.4% represented *V. vulnificus/ V. cho-lerae*. Next, to assess a breakdown of the number of various colonies per sample plate, the percentages for each sample were examined in consideration to the depth of the sample collection. 25 surface samples from various sites and among these 14 of them had a colony count percentage of 50% or greater number of *V. alginolyticus*. Further, there were 10 sites that had a colony count percentage of 50% or greater for *V. parahaemolyticus*, 6 sites that had a colony count percentage of 50% or more for *V. alginolyticus*, 4 sites had a colony count percentage of 50% or more for *V. parahaemolyticus* and 2 sites (Alice in Wonderland and Margate Bay) had a col-ony count of 50% or more for *V. vulnificus/ V. cholerae*, and finally there were 3 sites, viz, Chez Hines, Karels, and Sweet Dreams that had a colony count percentage of 50% or greater for *V. vulnificus/ V. cholerae*. There was one site (The Lake) that did not grow any *Vibrio* species. Lastly regarding the samples from deep sites, it was observed that 5 sites contained a colony count percentage of 50% or more for *V. alginolyticus*, 4 sites had a colony count percentage of 50% or more for *V. parahaemolyticus.*

There were 2 sites, the lake and Berri reef that had a colony count percentage of 50% or more for *V. cholerae*. Once again there was one site (Acquarius) that did not grow any *Vibrio*. Finally, regarding the total colony counts from each site delineating the prevalence of the various *Vibrio* bacteria according to geographic direction, the East and North, the grossly demonstrated *V. alginolyticus* and *V. parahaemolyticus* as the predominant species. Although differentiation between *V. vulnificus* and *V. cholerae* was not possible due to limited testing facility, we confirmed that these colonies as well as colonies of other two species, viz. *V. alginolyticus* and *V. parahaemolyticus* were also Gram negative and oxidase positive, the characteristics of *Vibrio* species (Murray *et al.,* 2003). The colony counts in the West were too small to determine any predominant species. The present investigation constitutes the first study of its kind from the Dutch Caribbean, evidencing the prevalence of *Vibrio* species, viz. *V. alginolyticus, V. parahaemolyticus,* and *V. vulnificus /V. cholerae* in marine water in and around Bonaire. Coexistence of these species in water samples from some of the sites is a noteworthy observation. The presence of these organisms in the marine water was not unexpected due to their ubiquitous nature (Depaula *et al.,* 2010; Elhad, 2013; Oliver *et al.,* 1982). *Vibrio fulnicus*, an opportunistic human pathogen is commons in estuarine environment existing as symbiont of oysters (Wright *et al.,* 1996). Ingestion of oysters can result in septecaemia in 95% of persons with underlying chronic diseases, resulting in more than 59% mortality (Wright *et al.,* 1996). Several *Vibrio* species are of great interest to the health departments of most countries since the presence of a few pathogenic strains can cause significant morbidity and mortality to those who are exposed and susceptible (Ho Ho 2021; http://www.cdc.gov/features/dsfoodnet 2012/; http:/www.foodsafetynews.com/2014/08/; Oliver, 2005). Monitoring of *Vibrio* bacterial infection among sea foods is particularly important to reduce this risk (De Paula *et al.,* 2010; Cheshire, 2014; Romesh *et al.,* 2014). Thus, due to the ubiquitous nature of *Vibrio* species, proper food safety techniques should be used when consuming seafood because *Vibrios* can be detected even in frozen seafood (Khalil *et al.,* 2014). Two studies from the Caribbean also found *Vibrio alginolyticus* and *Vibrio vulnificus* present on corals (Cervino *et al.,* 2008; Morrow *et al.,* 2012).

An increase in yellow blotch/band disease in corals caused by the rise in water temperature was also observed in some parts of the Caribbean including Bonaire (Cervino *et al.,* 2008). It is note-worthy that the sample from Sorobon where the water temperature is 4^{O}C higher than that in other sites showed significantly elevated bacterial counts for *V. parahaemolyticus* and *V. alginolyticus*. Interestingly, the shallow waters (depth of 1 to 2 feet) of the effluent

overflow from the salt evaporation fields yielded higher counts of *V. alginolyticus* than that of *V. vulnificus* was also observed in North Carolina during drought periods when salinity values increase (Drake, 2008). *Vibrio vulnificus* and *V. parahaemolyticus* are known to be very pathogenic for humans (Poirier *et al.,* 2012). It is interesting to note that pandemic *Vibrio parahemolyticus* from UK water and shellfish produce (Powel *et al.,* 2013). Bonaire possesses water-processing facilities that desalinate seawater to provide drinking water since there are no natural water reservoirs on the island. We performed a test on this water to assess for *Vibrio* content and but did not detect any, possibly because this water would not have encountered wastewaters. When the inhabitants of the island use inland waters as a water source, as in other islands, weather disturbances can cause contaminations and cholera outbreaks as seen in Haiti's past (CDC 24/7. Saving lives and protecting people). There is also a greater risk of infections when lacerations and open wounds become exposed to *Vibrio* bacteria. The increased number of scuba diving and snorkeling by tourists as well as local fishermen being exposed to the coastal waters with open skin injuries pose a significant health risk for infection and septicaemia (http://www.cdc.gov/features/dsfoodnet2012/). It is important for the local healthcare authorities to be aware of the presence of potentially pathogenic *Vibrio* species present in the local waters.

CONCLUSION:

This study supports that *Vibrios* exist as autochthonous species throughout the coastal waters ranging from the colder water of the north-eastern US to the Gulf. Of the species under investigation, the most prevalent was *Vibrio alginolyticus* in both number of sites and number of colonies; *Vibrio alginolyticus* was also the most prevalent organism (38%) in a study performed in Saudi Arabia (Elhadi, 2013). This is significant observation because *alginolyticus* causes Yellow Blotch disease among coral species (Elhadi, 2013). Bonaire corals may already be at risk for developing this disease based on the amount of *Vibrio alginolyticus* present. The spread of *Vibrio* infections in response to climate change and extreme heat waves has been observed in several countries in the North Atlantic and North Sea regions, including the Caribbean countries. Increased water temperatures caused by atmospheric warming and increased salinity gradients caused by sea-level rise raise concerns about the effect of climate change on the geographic range of *Vibrio vulnificus*, a species associated with oyster and shellfish and occurring in the US southeast, and the potential for increased exposure risk of human infections and (Froelich and Oliver, 2013; Deeb *et al.,* 2018). A noteworthy publication is the report of the 0ccurrence and virulence properties of *Vibrio* and Salinivibrio isolates from tropical lagoons of the southern Caribbean Sea (Fernández-Delgado *et al.,* 2017). Of particular concern to human health are *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*, though most environmental strains are not pathogenic. Our study has established the presence of *Vibrio* species in the coastal waters of Bonaire. The rising prevalence of pathogenic Vibrio species in marine waters has led to a surge of Vibrio infections in humans. Another particularly noteworthy observation revealed in the literature search is the first-time reporting of *Vibrio jasicida* and *V. rotiferianus* at UK shellfish sites (Harrison *et al.,* 2022).

Table 1: Number of colonies of pathogenic *Vibrio*s species in marine water samples (with data on the pH, depth, and temperature of the samples) in different locations in Bonaire.

Date of Collection	Location	pH	Depth (ft)	Tem. F	*Vibrio alginolyticus*	*Vibrio Parahemolyticus*	*Vibrio fulvinicus / V. cholerae*
28.04.2014	Alice in Wonderland	7	0	82	5	0	0
28.04.2014	Alice in Wonderland	7	40	79	1	1	5
28.04.2014	Alice in Wonderland	7	88	79	4	14	6
10.05.2014	Angel City	7	0	83	15	1	13
10.05.2014	Angel City	7	30	80	0	2	1
10.05.2014	Angel City	7	60	80	1	1	0
21.09.2014	Aquarius	7	0	82	1	22	1
21.09.2014	Aquarius	7	45	80	1	2	0
21.09.2014	Aquarius	7	88	80	0	0	0
17.10.2014	Atlantis	7	0	80	25	5	5
17.10.2014	Atlantis	7	35	80	1	1	0

20.09.2014	Beef Reef	7	0	80	3	15	4
20.09.2014	Beef Reef	7	95	83	2	0	3
21.10.2014	Chez Hines	7	0	80	2	2	11
21.10.2014	Chez Hines	8	45	80	19	16	19
21.10.2014	Chez Hines	7	95	80	6	1	2
11.08.2014	Divi	7	0	82	0	1	0
11.08.2014	Eden	7	0	82	5	0	0
11.08.2014	Haito	7	0	82	2	1	0
11.08.2014	Haitp-37	7	0	82	6	0	0
11.08.2014	Karel, s	7	0	82	1	0	0
11.08.2014	Karel, s- 37	7	0	82	5	5	0
11.08.2014	Lac Bay	7	0	80	14	1	2
19.09.2014	Margete Bay	7	50	80	0	0	3
19.09.2014	Margete Bay	7	100	80	0	1	0
10.11.2014	Moat	8	0	83	11	1	1
10.02.2914	Oil Slick	7	0	83	1	1	0
10.02.2914	Oil Slick	7	50	83	36	7	2
10.02.2914	Oil Slick	7	105	80	10	44	4
29.09.2014	Red Beryl	7	0	83	6	2	0
29.09.2014	Red Beryl	7	45	80	6	1	0
29.09.2014	Red Beryl	7	88	80	6	4	0
10.11.2014	Sorobon	7	0	83	19	31	1
10.11.2014	Sorobon Weeds	8	0	83	26	44	1
30.08.2014	Sweat Dreams	7	0	83	8	0	20
30.08.2014	Sweat Dreams	7	50	79	2	0	0
30.08.2014	Sweat Dreams	7	100	82	1	0	0
15.10.2014	The Lake	7	0	82	8	13	4
15.10.2014	The Lake	7	50	80	0	0	0
15.10.2014	The Lake	7	100	81	0	0	2
05.09.2014	Tolo	7	0	83	4	10	4
05.09.2014	Tolo	7	20	80	1	3	0
05.09.2014	Tolo	7	100	80	6	0	0
29.08.2014	Webers Joy	7	0	82	14	7	2
29.08.2014	Webers Joy	7	0	79	38	0	5
29.08.2014	Webers Joy	7	0	80	1	0	0
01.08.2014	Yellow Sub Marine	7	0	82	1	0	0
11.08.2014	Zazu-37	7	0	82	3	3	0
11.08.2014	Control-37	0	0	?	0	0	0
17.10.2014	Tap Water	8		0	0	0	0

The mean and standard deviation (SD for depth and temperature of different types of water were as follows: Surface water - pH: mean = 7.1, SD + 0.3 Depth: mean = 0, SD = 0. Temperature: mean = 82.2, SD = 0.9, Shallow water - Depth: Mean =40.4, SD =9.7, Temperature: Mean =79.8. SD = 0.6. (There was no variation in pH), Deep water – Depth: Mean = 95 there -8, SD = 15.9, Temperature: Mean = 80.3, SD = 0-8 (There in the parenthesis is no variation in pH).

Table 2: Number of colonies of pathogenic Vibrio species in samples from different locations in Bonaire (with their pH, depth, and temperature of the samples.

Date of Collection	Location	pH	Depth (ft)	Temp. F	*Vibrio alginolyticus*	*Vibrio parahemolyticus*	*Vibrio fulvinicus cholerae*
28.04.2014	Alice in Wonderland	7	0	82	5	0	0
28.04.2014	Alice in Wonderland	7	40	79	1	1	5
28.04.2014	Alice in Wonderland	7	88	79	4	14	6
10.05.2014	Angel City	7	0	83	15	1	13
10.05.2014	Angel City	7	30	80	0	2	1
10.05.2014	Angel City	7	60	80	1	1	0
21.09.2014	Aquarius	7	0	82	1	22	1
21.09.2014	Aquarius	7	45	80	1	2	0

21.09.2014	Aquarius	7	88	80	0	0	0
17.10.2014	Atlantis	7	0	80	25	5	5
17.10.2014	Atlantis	7	35	80	1	1	0
20.09.2014	Beef Reef	7	0	80	3	15	4
20.09.2014	Beef Reef	7	95	83	2	0	3
21.10.2014	Chez Hines	7	0	80	2	2	11
21.10.2014	Chez Hines	8	45	80	19	16	19
21.10.2014	Chez Hines	7	95	80	6	1	2
11.08.2014	Divi	7	0	82	0	1	0
11.08.2014	Eden	7	0	82	5	0	0
11.08.2014	Haito	7	0	82	2	1	0
11.08.2014	Haitp-37	7	0	82	6	0	0
11.08.2014	Karel,s	7	0	82	1	0	0
11.08.2014	Karel,s-37	7	0	82	5	5	0
11.08.2014	Lac Bay	7	0	80	14	1	2
19.09.2014	Margete Bay	7	50	80	0	0	3
19.09.2014	Margete Bay	7	100	80	0	1	0
10.11.2014	Moat	8	0	83	11	1	1
10.02.2914	Oil Slick	7	0	83	1	1	0
10.02.2914	Oil Slick	7	50	83	36	7	2
10.02.2914	Oil Slick	7	105	80	10	44	4
29.09.2014	Red Beryl	7	0	83	6	2	0
29.09.2014	Red Beryl	7	45	80	6	1	0
29.09.2014	Red Beryl	7	88	80	6	4	0
10.11.2014	Sorobon	7	0	83	19	31	1
10.11.2014	Sorobon Weeds	8	0	83	26	44	1
30.08.2014	Sweat Dreams	7	0	83	8	0	20
30.08.2014	Sweat Dreams	7	50	79	2	0	0
30.08.2014	Sweat Dreams	7	100	82	1	0	0
15.10.2014	The Lake	7	0	82	8	13	4
15.10.2014	The Lake	7	50	80	0	0	0
15.10.2014	The Lake	7	100	81	0	0	2
05.09.2014	Tolo	7	0	83	4	10	4
05.09.2014	Tolo	7	20	80	1	3	0
05.09.2014	Tolo	7	100	80	6	0	0
29.08.2014	Webers Joy	7	0	82	14	7	2
29.08.2014	Webers Joy	7	0	79	38	0	5
29.08.2014	Webers Joy	7	0	80	1	0	0
01.08.2014	Yellow Sub Marine	7	0	82	1	0	0
11.08.2014	Zazu-37	7	0	82	3	3	0
11.08.2014	Control-37	0	0	?	0	0	0
17.10.2014	Tap Water	8		0	0	0	0

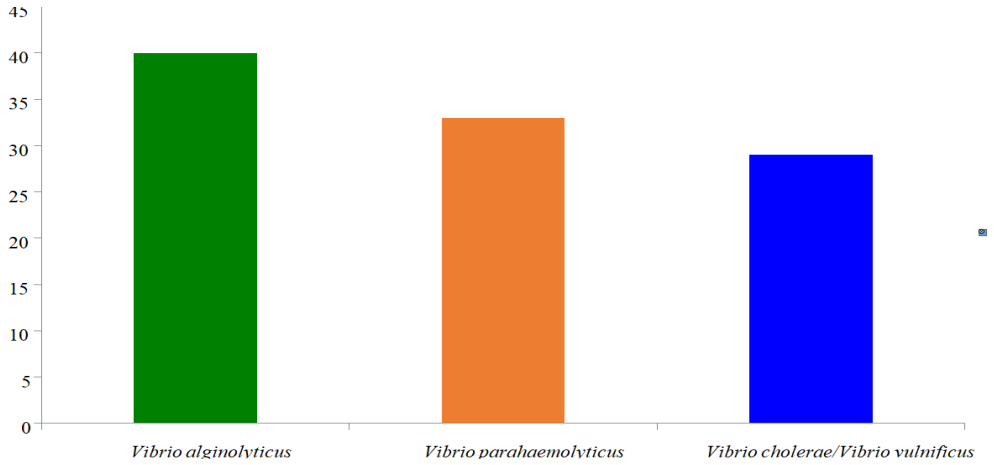

Fig. 1: This graph depicts the number of samples positive for pathogenic Vibrio species.

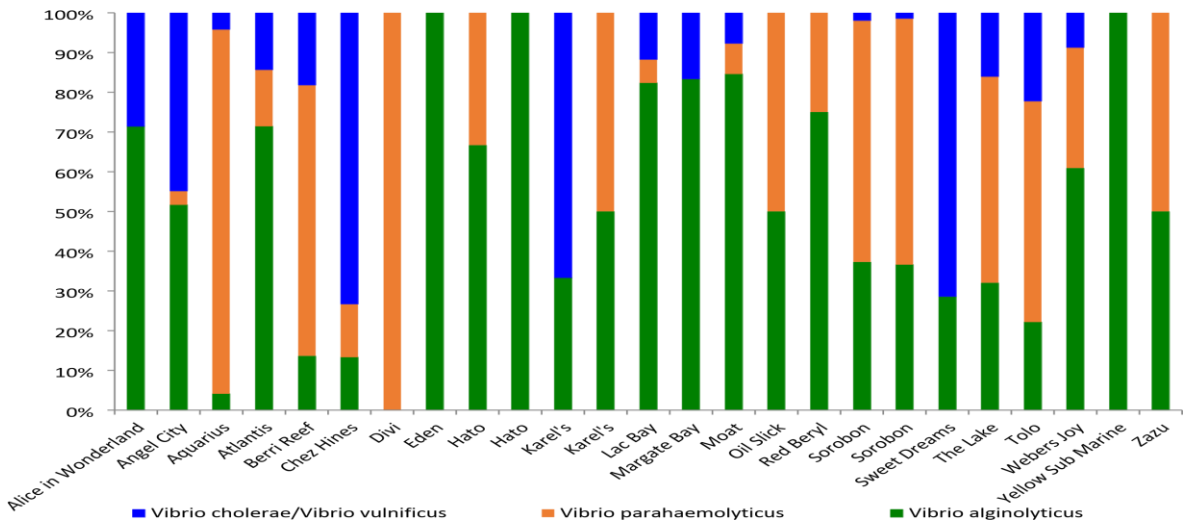

Fig. 2: Bar graph showing the percentages of the *Vibrio* species present at the surface of the water at different sites.

In this figure, the X-axis contains the name of the site where the sample was collected and the Y-axis is the percentage of each species based on the number of colonies counted in that sample. *V. alginolyticus* is represented by green, *V. parahaemolyticus* is represented by pink, and *V. vulnificus/ V. cholera* is represented by blue

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CONFLICTS OF INTEREST:

The authors have no conflict of interest with any individual or organization.

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