Epidemiological Linkages of Diarrheagenic Vibrio Species from Sea water, Seafood and Patients and their Antimicrobial Susceptibility Patterns in Zanzibar, Tanzania

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Research Article

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Abstract

Background

Vibrio species are reported to cause diarrhea in developing countries, particularly in Africa. The typical species of Vibrio have an association with seawater, seafood and patient stool which have been rarely studied in Zanzibar.

Methods

A cross-sectional study was conducted from October, 2019 to February, 2020. Randomly samples were collected to investigate Vibrio species from seawater, seafood to human and vice versa. The samples were cultured using Thiosulphate - citrate - bile salts-sucrose agar and AMS was done by Kirby – Bauer disc diffusion method.

Results

A total of 303 sample participated in the study (60) 60.0% *V. cholerae were* identified from seawater, (57) 57.0% from seafood and (20)19.4% from stool. Similarly, 22.0% of *V. parahaemolyticus* were identified from seawater, 21.0% from seafood and 14.5% from stool. About 12.0% of *V. vulnificus* were identified from seawater,10.0% from seafood and 4.8% from stool while 6.0% of similar *V. alginolyticus* from seawater, 5.0% from seafood and 2.9% from stool. All *Vibrio spp* presented sufficient susceptibility to Chloramphenicol, Ciprofloxacin and Doxycycline with a varying pattern to trimethoprim-sulfamethoxazole.

Conclusion

Vibrio cholerae and *V. parahaemolyticus* isolates are common causative agents of diarrhea in Zanzibar with reservoir in seawater and seafood. Both species presents sufficient susceptibility response to chloramphenicol, ciprofloxacin and doxycycline.

Background

Diarrhea is a frequent discharge of a watery stool accompanied by abdominal cramps, nausea and vomiting, sometimes with fever and chills. The World Health Organization (WHO) estimates about 1.7 billion cases of diarrheal disease worldwide every year (1, 2).There are several pathogenic microorganisms responsible for diarrhoea (3). In 2015, several countries suffered diarrhoea outbreaks that were epidemiologically linked to the consumption of raw oysters due to Vibrio *parahaemolyticus* (4).

It has been reported that most deaths and hospitalization due to diarrhoea occur in developing countries, particularly in Africa (5, 6). The *Vibrio cholera*, *Vibrio parahaemolyticus*, *Vibrio fluvial* and *Vibrio vulnificus* are the among of Vibrio species that cause diarrhoea in Africa (6–12).

Most Vibrio species are pathogens to humans and are found in marine water and are associated with cases of food poisoning, resulted from consumption of raw and or insufficiently cooked sea foods (13). Seawater temperature plays a key role in determining the types of *Vibrio species* at the time of harvest at oyster or shellfish in coastal habitats like *Vibrio parahaemolyticus, Vibrio vulnificus*, and *Vibrio cholerae* (1, 5). They are more prevalent in estuarine and coastal areas, where they live freely in water, sediments plankton and nearly all flora and fauna in coastal environment (5). These are major human health microbial hazards risks causing seafood diseases in the people consume raw or undercooked contaminated seafood's (14). The common vibrio species causing diarrhea are *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio alginolyticus;* Other diarrhoeagenic bacteria species includes *Salmonella, Shigella*, *Klebsiella, Campylobacter, Escherichia, Yersinia* and non-enteric bacteria associated with food poisoning (15, 16).

The one health approach is the most recommended method to tackle antimicrobial resistance. The common antibiotic for treatment of diarrhoea caused by Vibrio spp. These antibiotics are ampicillin, chloramphenicol, tetracycline trimethoprime-sulphamethoxazole. There is an evidence of resistance to ampicillin, chloramphenicol, tetracycline trimethoprime-sulphamethoxazole and other antimicrobials that are commonly used to treat diarrhea in developing countries (17). Unfortunately, limited use of laboratory techniques to test antimicrobial susceptibility has resulted to minimum understanding of the resistance burden and reduced therapeutic efficacy (18).There is a need to understand types of Vibrio spp and efficacy of the available antibiotics to treat diarrhea.

In Zanzibar, seafoods are the most popular and consumed food option due to culture and natural habitant of the islands. For more than 40 years diarrhoeal diseases have been the common public health problems presenting with outbreaks in Zanzibar (19). It is hypothesized that Vibrio species are responsible for most the outbreaks because the outbreak of 2015 presented evidence of Vibrio cholerae isolation (15). However, there are more Vibrio spp than Vibrio cholerae and there is no clear linkage between seafood and diarrhoea in Zanzibar. The aim of this study was to investigate the epidemiological linkages of diarrheagenic vibrio species from marine water, seafood and patients and their antimicrobial susceptibility patterns in Zanzibar, Tanzania.

Materials And Methods

Study design and location

A rapid cross-sectional design was used to collect random biological samples of seawater, seafoods and stool from patient. The patient stool samples were collected 23 health facilities located at in the West Urban Region of Zanzibar and likewise seawater and seafood were collected in West Urban Region of Zanzibar at Kizingo handling site of fishermen.

Sample Collection Storage and Transportation

Hundred samples of marine water 250ml from sea show and from five kilometers from the seashorewas filtered using membrane filters with pore sizes of 0.5µm. One hundred samples of seafoods were collected at Kizingo handling site of fishermen in Zanzibar. Stool samples for this study were collected from 23 health facilities of West Urban region site in Zanzibar. The sample collection was performed from for a period of five months (October, 2019 – February, 2020). A total of 103 samples were randomly collected from diarrhea patients who volunteered to give 25g stool specimen, sterile plastic containers were used for collection of the samples, immediately after sample collection, each sample container was tightly closed, labelled and packed in a cool box at 4^{O}C for transportation. All samples were transported to the to the microbiology department of the Pathology Laboratory at Mnazi Mmoja Hospital Zanzibar for further analysis using conventional microbiological and serology methods where they were stored in a refrigerator at 8^{o} C prior to laboratory analysis.

Bacterial Culture, Isolation, and Identification

In the laboratory, the filters containing the seawater sample were then transferred on thiosulfate – citrate – bile – salt (TCBS) medium (Oxoid Humpshine UK) then were inoculated at 25^{O}C for 24 hours. Presumptive Vibrio species triplicate count was expressed in colony forming units per milliliters (CFU/mL) of water yellow for *V. cholerae* colonies and green for V. *parahaemolyticus* colonies. *V. vulnificus* colonies appeared green on TCBS. The difference between *V. vulnificus* andV. *parahaemolyticus* was the formation of green colonies with a blue centre on TCBS by the later. The*V. alginolyticus* that forms the yellow colonies was differentiated from *V. cholerae* by its ability to form large yellow mucoidal colonies on TCBS.

In addition, seafoods were cut in 5g small pieces aseptically and were shaken into Alkaline Peptone Water (APW) enrichment broth vigorously. The pieces are then removed aseptically with forceps then cultured onto TCBS medium and incubated under 37^{O}C for 48 hours. Vibrio cultures were read after 24 and 48 hours then subjected to identification by colonial appearance. Gram staining, serology and biochemical tests were used to identify the bacteria causing diarrhoea. Ultimately, stool samples were cultured on TCBS agar inoculated in Alkaline Peptone Water at 37^{O}C for 24 to 48 hours. The bacterial isolates were identified using oxidase test, Voges Proskauer test, and NaCl at 0% and 6%. For the oxidase test bacterial colonies were transferred with sterile glass rod to filter paper moistened with oxidase reagent. Rapid appearance of a dark purple color within few seconds was considered a positive reaction. The identified isolates were confirmed as described in the Centers for Infectious Disease Control Manual for cholera diagnosis (20).

Again, bacterial colonies were inoculated in 1% tryptone broth (Oxoid. Humpshine. UK) in the presence of 0 and 6% (wt/vol.) Sodium Chloride (NaCl) and incubated at 37^{O}C for 18 – 24hours in a water bath shaker. This was the test to determine the requirement of Vibrios for Na+. Positive results were determined by examining turbidity. Voges Proskauer Testwas performed by inoculating vibrios in methyl-red and Voges Proskauer medium and incubated at 37^{O}C for 48 hours.

Agglutination Test

The agglutination test was used for confirmation of isolates were tested with antisera - polyvalent, Inaba, Ogawa and 0139 (Mast Diagnosics, Merseyside, United Kingdom) according to manufacturer's instructions. A loop - full colonies of 24 hours growth of *Vibrio spp* from heart infusion agar (HIA) was emulsified in small drop of normal saline on a clean glass slide and was mixed thoroughly. A

drop of antiserum was then added and was mixed well to get a smooth homogenous mixture. The glass slide was tilted back and forth for observation of agglutination. For a positive reaction a strong clumping appeared within 1 minute.

Antimicrobial Susceptibility Testing

Kirby-Bauer disc diffusion method was used for antibiotic sensitivity tests. The 24hour growth culture, standardized to match the 0.5 McFarland standards were inoculated onto Mueller-Hinton agar plates using sterile cotton swabs. Using the same swab, the plates were rotated at 60 degrees and swabbing the surface of the plate. This procedure was repeated one or more times while avoiding hitting the sides of the petri-plate and creating aerosols. The inoculated plate was allowed to stand for 3 to 15 min before applying disks. The antimicrobial disks used were Ceftriaxone (CTX, 30 µg), Ciprofloxacin (CIP. 5 µg), Doxycycline (DOX, 30 µg), Chloramphenicol (C, 30 µg), Erythromycin (ERY, 20 µg), Ampicillin (AMP, 10 µg), Trimethoprim-sulfamethoxazole (SXT, 25 µg)/Co-trimoxazole (CO, 25 µg)), Tetracycline (TET, 20 µg), Nalidixic acid (NA, 30 µg) and Azithromycin (AZM, 15 µg) which were placed on the agar plates. The plates were then incubated overnight at 35˚C for 18 hours. The drug with susceptible zone of inhibition appeared around the antibiotic disc. The size of the zone of inhibition is correlated to the level of antimicrobial activity. Zones of inhibitions of bacterial growth around each antibiotic disc were measured using a sharp-edged ruler based on the Standard Operating Procedure (SOP) adapted from Clinical and Laboratory Standards Institute (21) and results were reported as sensitive (S), intermediate (I) and resistance (R).

Data Analysis

Data were analyzed using STATA version 14 (22). Descriptive statistics in frequencies and proportions were used to describe the magnitude of *Vibrio cholerae,* and other *Vibrio* species(spp) in seawater, seafood and stool from patient with diarrhoea. Correlations were used to determine the relationship between *Vibrio* species from different biological specimen. Patterns of proportions with similar frequencies from different specimen were used to determine the epidemiological linkages between Vibrio species from marine water, seafood and stool from patients with diarrhea.

Ethical Consideration

Ethical approval was granted from the Zanzibar Medical Research Ethics Committee (Ref. No. ZAHREC/02/DEC/2018/6). Permission to conduct the study was sought from the respective health centre authorities. The information about the study was given in writings, and study representative explained the benefits, participation rights and freedom to withdraw from the study at any time. The consent was obtained from adult patients with diarrhoea aged above 18 years of age before collection of information. All patients involved in the study provided signed consents. The participants were assured of the confidentiality of the information and records of their samples. All participants were informed that information collected was not intended to be used for any other purpose except for research study.

Results

A total of 303 samples from seawater (100), seafood (100) and stool samples (103) from diarrheal patients were included in the study. From seawater, culture results revealed *V. cholerae* isolates of 60 (60.0%), from seafood, culture results revealed *V. cholerae* isolates of 57 (57.0%) and from stool sample, culture results revealed *V. cholerae* isolates 20 (19.4%). The culture results for *V. parahaemolyticus* isolates were 22 (22.0%) from seawater, 21 (21.0%) seafood and 15 (14.5%) from stool sample. Similarly, culture results for *V. vulnificus* isolates were 12 (12.0%) from seawater,10 (10.0%) from seafood and from 5 (4.8%) from stool samples. *V. alginolyticus* 6 (6.0%) from seawater, 5 (5.0%) from seafood samples and 3(2.9%) from stool samples. Other enteric bacterial species Salmonella were 4 (3.8%) and *E. coli* were 2 (1.9%). Unknown species were 11 (11.0%) from seawater, 10(10.0%) from seafood and 6 (5.8%) from stool sample as shown in Table 1 and Fig. 1.

Antibiotic Resistance Test From Patient Samples

The *Vibrio specie* isolates displayed different rate of susceptibility to the evaluated antibiotics (Table 2). *Vibrio cholerae* isolates were high susceptible to Chloramphenicol 20 (100%), Ciprofloxacin 20 (100%), doxycycline 20 (100%) and trimethoprim-sulfamethoxazole 15 (75.0%) while low susceptible was to ampicillin 5 (25.0%), erythromycin 5 (25.0%) and nalidixic acid 5 (25.0%). They exhibited high resistance to nalidixic acid 15 (75.0%) while low resistance to ampicillin 10 (50.0%) and erythromycin 5 (25.0%). The result indicates no resistance to Chloramphenicol, Ciprofloxacin, Doxycycline and Trimethoprim-sulfamethoxazole.

The specie of Vibrio parahaemolyticus isolates were susceptible to Doxycycline 15 (100%), Chloramphenicol 15 (100), Ciprofloxacin 15 (100%), erythromycin 10 (66.6%) followed Ampicillin 8 (53.3%) and Trimethoprim-sulfamethoxazole 7(46.6%). However, these isolates showed different resistance against nalidixic acid 11 (73.3%), trimethoprim-sulfamethoxazole 5 (33.3%) and Ampicillin 4 (26.6%). The result indicates no resistance and intermediate to Chloramphenicol, Ciprofloxacin and Doxycycline as shown Table 3.

The specie of Vibrio vulnificus isolate susceptible to Doxycycline 5 (100%), Ciprofloxacin 5 (100%), Chloramphenicol 4 (80%) and erythromycin 3 (60.0%) and Nevertheless, *V. vulnificus* isolates were reported with different resistance against nalidixic acid 2 (40.0%), Trimethoprim-sulfamethoxazole 2 (40.0%), Ampicillin 2 (40.0%) The report indicates no resistance to Chloramphenicol, Ciprofloxacin and Doxycycline as shown in Table 4.

The specie of Vibrio alginolyticus isolate were susceptible to Doxycycline 3 (100%), Ciprofloxacin 3 (100%), and Chloramphenicol 3 (100%). However, *V. alginolyticus* isolates reported resistance against Ampicillin 1 (33.3.%), Erythromycin 1 (33.3%), nalidixic acid 1 (33.3%) and Trimethoprim-sulfamethoxazole 1 (33.3%). The report indicates no resistance Chloramphenicol, Ciprofloxacin and Doxycycline as shown in Table 5.

The results of multiple drug resistant (MDR) patterns of *Vibrio cholerae* isolates exhibited multidrug resistant to three antibiotics nalidixic acid, ampicillin and erythromycin. The *Vibrio parahaemolyticus, Vibrio vulnificus* and *Vibrio alginolyticus* isolates exhibited multidrug resistant to four antibiotics nalidixic acid, ampicillin, erythromycin and trimethoprim-sulfamethoxazole. This show that *Vibrio cholera* were fewer resistant drugs compared to *V. parahaemolyticus, Vibrio vulnificus* and *Vibrio alginolyticus* as shown in Table 2, Table 3, Table 4 and Table 5.

Discussion

This study has provided evidence of epidemiological linkages of diarrheagenic Vibrio Species from seawater, seafood and stool from patients with diarrhea and their Antimicrobial Susceptibility Patterns in West-Urban Region – Zanzibar, Tanzania.

Our results have shown an epidemiological route of vibrio species from sea water, sea food and human stool. Previous reports from WHO presented seawater isolates had more *V. cholera, V. parahaemolyticus, V. vulnificus* and *V. alginolyticus* isolates followed by seafood and low isolates were in patients' stools. There was a theory that seawater temperature is the principal environmental factor increasing the abundance of *Vibrio spp* in the coastal human settlements (1). The evidence of sea water to human stool is consistent with the findings from Rabia and colleagues who reported that major source of diarrhea in Zanzibar 2015/2016 being due to cholera from marine foods.

Despite the epidemiological linkage, our study has found a decrease of proportions of *Vibrio cholera* in seawater 60 (60.0%), seafoods 57 (57.0%) and stool 20 (19.4%). Although we could not find the direct explanation of the causes of a slight decline of proportions in such epidemiological linkages, we are confident to mention that *Vibrio cholerae* are the main causes of diarrhoea outbreak in Zanzibar as a similar study in Uganda reported from these species in stool samples 33.7% (12) and 33% (11) while our study reported 19.4% mainly due to sample size barrier. The study in India on causes of diarrhoea outbreak reported 3.93% which is lower than what we found (23).

The interest of *V. parahaemolyticus being the second cause of diarrhoea* in Zanzibar is obvious despite the higher proportion of 14.5% stool samples than expected rate of 31.0% and 21.0% in sea water and sea food respectively. Previous study in Zanzibar reported 16% of *V. parahaemolyticus* in stool samples (9). This may imply that *V. parahaemolyticus* can be found in other community setting of Zanzibar as a new habitat. On the other hand our study has presented a consistent pattern of *Vibrio vulnificus* 5% and *Vibrio alginolyticus* 3% that proved such an interesting epidemiological linkage in Zanzibar (9).

We are hereby presenting scientific evidence that Vibrio cholerae and other Vibrio spp are transmitted through sea water as a reservoir, contaminated seafood and to human via cold sea foods. This finding is in-line with other studies conducted in Zanzibar (7, 9). Another study (11) describes that people of all ages might contract the disease but more mobile members of the community usually adults are at higher risk and are more frequently affected because of their greater exposure to sea water, seafood and other possible sources of contamination, such as foods, drinks taken outside the home. As evidenced in previous study sea foods has been implicated with cholera outbreaks (7) as a result of consumption of raw seafood and/or undercooked seafood.

Looking into treatment options, we found all *Vibrio spp* isolates *being* highly susceptible to Chloramphenicol 100%, Ciprofloxacin 100% and Doxycycline 100%. However, our study, reports high resistance of Vibrio cholerae (75.0%) and *Vibrio parahaemolyticus* (73.3%) to

nalidixic acid followed by *Vibrio vulnificus* 40.0% and *Vibrio alginolyticus* 33.3.% on the same antibiotic. Similar studies in Zanzibar reported *Vibrio cholerae* were susceptible to Ciprofloxacin 100% while nalidixic acid had high resistance of 93% (7). In addition several studies reported Vibrio cholerae to be highly resistance to nalidixic acid 83.2% in Kenya (24), 98% in Zambia (10), 100% in Nepal (25) and 100% in Iran (26).

The pattern of lower resistance to nalidixic acid 48% was reported in Uganda (12). Other patterns of resistance in our study were observed in *Vibrio cholerae* to ampicillin 50.0%, *Vibrio parahaemolyticus* 26.6%, *Vibrio vulnificus* 40.0%) and *Vibrio alginolyticus* 33.3%. The Vibrio cholerae isolates in previous studies reported high resistance patterns to ampicillin 60% in Kenya (24), 60% in Zambia (10), 100% in Nepal (25) and 100% in (12). The resistance to Erythromycin were observed higher in other studies conducted in Iran100%, (26), Nepal 90.9% (25) and Kenya 53.47% (24) which were inconsistency with our study. This indicates a reduced efficacy in the treatment of diarrheagenic Vibrio species in patients. *Vibrio species* isolates in our study exhibited multidrug resistant patterns to at least three antibiotics namely nalidixic acid, ampicillin and erythromycin.

We report the epidemiological linkage and the resistance patterns of vibrio species in Zanzibar to guide the first-line and second line drugs of choice for treatment of cholera in the subsequent reviews of the Zanzibar Standard Treatment Guideline (ZSTG) for *Vibrio species* as needed by Ministry of Health, 2016. Moreover, those medicines are commonly available in the pharmacies and accessible to anyone who needs them. This is a wakeup call for the Ministry of Health of Zanzibar to initiate a long-term surveillance program to monitor and identify the changes in the rate of antimicrobial pattern of these bacteria of public health concern. Antibiotics remain the most important therapy for successful management of vibrio infections; however, these inexpensive and widely available antimicrobials can no longer be used empirically. This will provide appropriate control measures for antimicrobial resistance pathogens in Zanzibar.

Conclusion

There is an epidemiological linkage of Seawater, Seafood and human as evidences in the stool samples presenting with similar Vibrio species (*Vibrio cholera, Vibrio parahaemolyticus, Vibrio vulnificus* and *Vibrio alginolyticus*) among patients with diarrhoeal disease in Zanzibar, Tanzania. The species of *V. cholera, V. parahaemolyticus, V. vulnificus* and *V. alginolyticus* are susceptible to chloramphenicol, ciprofloxacin and doxycycline while resistance to nalidixic acid, ampicillin, erythromycin and trimethoprim-sulfamethoxazole.

Recommendations

There is need to tackle the epidemiological linkage of Vibrio species from seawater, seafood and human while adhering to the susceptibility patterns to guide the rational use of antibiotics to health care providers and community as a key prerequisite of case management of diarrhoeal diseases in Zanzibar, Tanzania.

Abbreviations

MDR Multiple Drug Resistant; TCBS Thiosulfate - Citrate - Bile – Salt; SOP Standard Operating Procedure

Declarations

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Availability of data and materials

Other additional materials are available upon request from the corresponding author.

Authors' contributions

KMK: Principal investigator of the study, concept development, study design, data collection, laboratory work, data analysis, critically reviewed the data and drafted the manuscript; BM: data analysis, critically reviewed the data and drafted the manuscript; KO: supervision of data collection, critically reviewed the data and drafted the manuscript; MD: supervision of data collection, data analysis, critically reviewed the data. LN: Concept development, supervision of data collection, data analysis, critically reviewed the data. All authors gave final approval of the version to be published and agree to be accountable for all aspects of the work. All authors read and approved the final manuscript.

Competing interests: Authors declare no competing interests

Ethics approval and consent to participate

Ethical clearance was obtained from Ministry of Health Zanzibar Medical Research Ethical Committee with IRB approval number of ZAHREC/02/DEC/2018/6 while the official permission was also obtained from department of preventive health services at the Ministry of Health Zanzibar. In addition, written informed consent was obtained from the adult patients ages 18 years to above who were eligible to be recruited for those who had the capacity to understand the study information. All participants were informed about the purpose of the study, and all the methods were performed in accordance with the relevant guidelines and regulations. The individual results of any investigation remained confidential. All identified cases of patients with diarrhea were referred to attending physicians for treatment.

Consent for publication

This study does not contain any individual or personal data.

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Tables

Tables 1 to 5 are available in the Supplementary Files section.

Figures

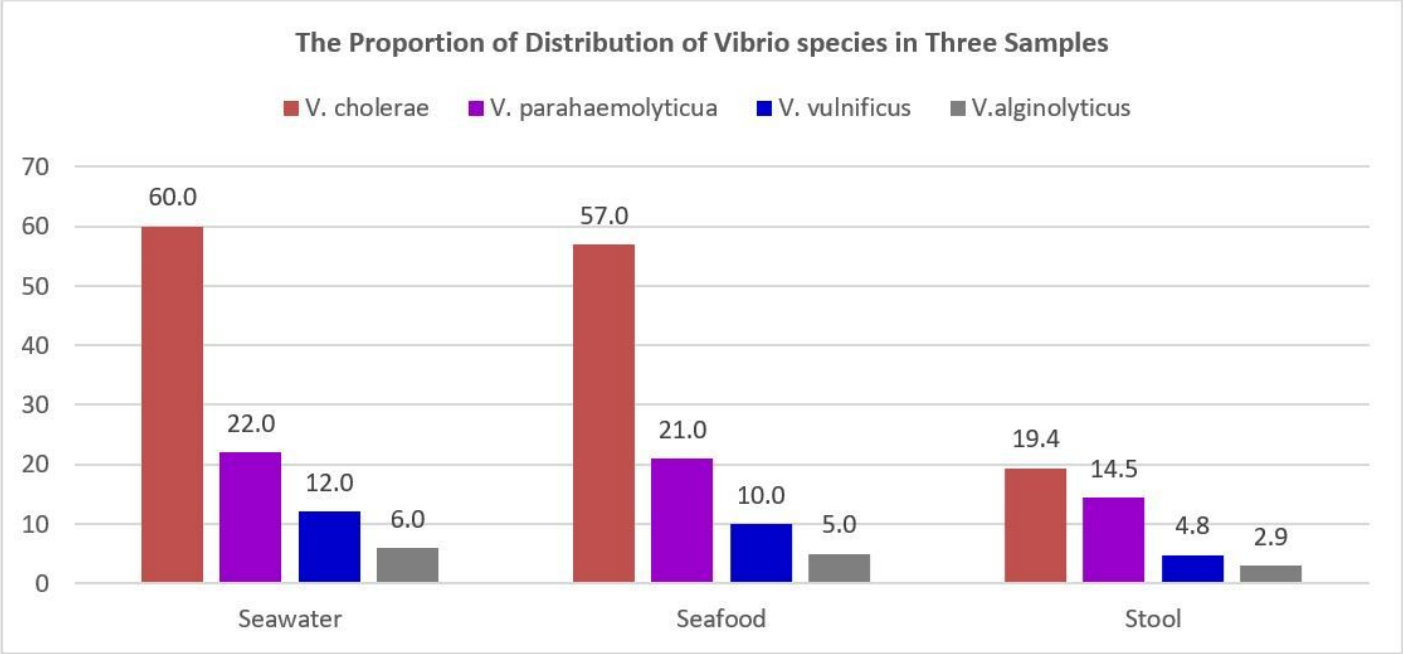

Figure 1

The Proportional Distribution of *Vibrio spp* isolated from different Three specimens

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables.pdf