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Epidemiological Linkages of Diarrheagenic Vibrio Species from Seawater, Seafood and Patient's Stool and their Antimicrobial Susceptibility Patterns in Zanzibar, Tanzania

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Abstract

Background

Vibrio species are reported to cause diarrhea in developing countries, particularly in Africa. The epidemiological association of Vibrio from seawater, seafood and patients' stool and their antimicrobial susceptibility patterns has yet to be studied in Zanzibar. Therefore, this study aimed to assess epidemiological linkages of diarrheagenic Vibrio species from seawater, seafood and patient's stools and their antimicrobial susceptibility patterns in Zanzibar, Tanzania.

Methods

A cross-sectional study conducted from October, 2019 to February, 2020. Twenty-seven health facilities were selected from west urban region of Zanzibar in Tanzania. Three hundred and three (303) samples of Vibrio species were randomly collected from seawater, seafood and humans for investigation. The samples were cultured using Thiosulphate - citrate - bile salts-sucrose agar and antimicrobial susceptibility **(**AMS) was done by Kirby–Bauer disc diffusion method. Data were analysed using Statistical Package for Social Sciences (SPSS) software (16.0 version). Descriptive statistics in frequencies and proportions were used to summarize the information collected. The multivariable analysis was used to determine the linkages of diarrheagenic Vibrio species from seawater, seafood and stool from patient with diarrhoea whereby $p < 0.05$ was considered statistically significant.

Results

Following the investigation, it was found that seawater had 60 (60.0%) of *Vibrio cholerae*, 57 (57.0%) were from seafood and 20(19.4%) from the patients' stool. Similarly, 22.0% of *V. parahaemolyticus* were identified from seawater, 21.0% from seafood and 14.5% from patient's stool. About 12.0% of *V. vulnificus* were identified from seawater,10.0% from seafood and 4.8% from patient's stool while 6.0% of similar *V. alginolyticus* from seawater, 5.0% from seafood and 2.9% from patient's stool. All Vibrio species presented sufficient susceptibility to chloramphenicol, ciprofloxacin and doxycycline with a varying pattern to trimethoprim-sulfamethoxazole.

Conclusion

A high proportion of diarrheagenic Vibrio species were isolated from seawater and low from patients with diarrhea. Among the tested antibiotics, three (chloramphenicol, ciprofloxacin and doxycycline) were found to be most effective for Vibrio species however. Vibrios were found to be developing resistance to four antibiotics of the commonly used antibiotics (ampicillin, erythromycin, nalidixic acid and trimethoprim-sulfamethoxazole).
Interventions against diarrhoea, that address the role of Vibrio species and their corresponding antimicrobial susceptibility patterns are needed.

Keywords*: Epidemiological linkage, Diarrhoea, Vibrio species, Seafood, Seawater, Patient's stool, Antimicrobial susceptibility.*

Introduction

Diarrhoea 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 among the Vibrio species that cause diarrhoea in Africa resulting from consumption of raw and or insufficiently cooked seafoods (6–14). Seawater temperature plays a key role in determining the types of Vibrio species at the time of harvesting oyster or shellfish in coastal habitats (15,16).

The one health approach is the most recommended method to tackle antimicrobial resistance. The common antibiotics for treatment of diarrhea caused by Vibrio species 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 (13,17). At present, azithromycin, ciprofloxacin and doxycycline or quinolone are recommended by WHO for treatment Vibrio infections (13, 15). 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 options due to culture and natural habitat 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. other 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 seawater, seafood and patient's stool and their antimicrobial susceptibility patterns in Zanzibar, Tanzania.

Methods

Study design, Site and Population

A cross-sectional study design was used covering public health facilities in the three districts of the west urban region, namely urban district, west 'A' district and west 'B' district, from October, 2019 to February, 2020 mainly focused on adults aged above 18 years with diarrhoea attending health facilities. Random sampling was used to select nine public health facilities in each district. The twenty-seven selected health facilities were Mnazi mmoja, Chumbuni, Sebleni, Rahaleo, Kwamtipura, Kidongo chekundu, Kidutani, Shaurimoyo, Mpendae, Fuoni, Kombeni, Magogoni, Kiembe Samaki, Fuoni kibondeni, Shakani, Bwefum, Chukwani, Kisauni, Mbweni matrekta, Mtofaani, Selem, Bubwisudi, Chuini, Kizimbani, Kianga, Beit-el-Ras and Kibweni. All of them are public health facilities treating patients free of charge and hence provide health care to most people in the area. On other hand, out of the six handling fish sites, three main sites, that is Buyu, Kizingo and Ngalawa were selected based in West Urban region.

Sample collection storage and transportation

Three types of samples comprising of seawater, seafood and stool from patients were collected during the study. Hundred samples of seawater (250ml) from three sites Kizingo, Buyu and Ngalawa along the seashore of west urban region were collected, later filtered using membrane filters with pore sizes of 0.5µm. One hundred samples of seafoods included raw clams were collected at Kizingo, Buyu and Ngalawa handling sites of fishermen in Zanzibar. One hundred three of patients stool samples were collected from 27 health facilities using sterile plastic containers. The samples were packed in a cool box with icepacks at 4^{o}C, potential temperature for Vibrio species such as *V. cholerae, V. parahaemolyticus, V. vulnificu* and *V. alginolyticus* and sent to the microbiology department of the pathology laboratory at Mnazi Mmoja hospital in Zanzibar for further analysis.

Sample Size Determination

Population proportion formula was employed using desired characteristics of 27% (9)from diarrhea cases as calculated below.

Fishers formula: $n=Z^2pq/r^2$ (25)

Where: n = Desired sample size; p = Proportion of the population with a desired characteristic which will be 27% (9), ; q= 1-p z= standard deviation desired degree of accuracy. Where z is 1.96 if the degree of confidence is 95%, r= Degree of error is 5%. Therefore, n was found to be 303.

The characteristics of patients whom stool samples were collected from adult patients with diarrhoea aged above 18 years of age presenting with diarrhoea frequency > three times per 24hours, either bloody or watery diarrhea included suspected cases of cholera. The age and work environment of patients have been shown in relation to what has been studied. The samples were collected during rainy and dry season with unsuspected and suspected cholera epidemic.

Bacterial culture, isolation, and identification

In the laboratory, stool samples were inoculated in alkaline peptone water (APW), followed by culture on a selective media Thiosulfate citrate bile salts sucrose (TCBS) medium (Oxoid Humpshine UK) then were incubated at 25^{o}C for 24 hours. Vibrio species that are able to metabolize sucrose, such as both *V. cholerae* and *V. alginolyticus* form yellow colonies on TBCS agar, especially *V. cholerae* has the ability to form large yellow mucoidal colonies on the same medium, whereas other pathogenic species like *V. parahaemolyticus* and *V. vulnificus* produces green colonies. Similar above procedure was used for seawater laboratory analysis. In addition, seafoods samples were cut in 5g small pieces and APW was added to enrich *Vibrio cholerae.* Its alkalinity nature suppresses the growth of intestinal commensals, and *V. cholerae* grows rapidly. Then later 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. The bacterial isolates were identified using oxidase test, Voges Proskauer test, and NaCl at 0% and 6%.

Agglutination Test

The agglutination test was used to confirm isolates, and they were tested with antisera - polyvalent, Inaba, Ogawa and 0139 (Mast Diagnostics, 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. This is one of the easiest and quickest methods that can be used to test the antibiotic sensitivity of a bacterial

isolate. Antimicrobial susceptibility testing with discs is a simple, rapid method and provides a reproducible means of testing bacterial sensitivity to various antibiotics and chemotherapeutic agents. Pure overnight cultures of *Vibrio species* isolates were mixed with sterile saline and after matching with 0.5 McFarland standards were inoculated in Mueller-Hinton Agar. Antibiotic discs were placed on to the Agar. *Vibrio cholerae* isolates were tested against ceftriaxone (CTX, 30µg), ciprofloxacin (CIP. 5µg), doxycycline (DOX, 30µg), chloramphenicol (C, 30µg), erythromycin (ERY, 20µg), ampicillin (AMP, 10µg), trimethoprim-sulfamethoxazole (SXT, 25µg)/co-trimoxazole (CO, 25µg), tetracycline (TET, 20µg), nalidixic acid (NA, 30µg) and azithromycin (AZM, 15µg). Inhibition diameter zone readings was recorded according to Clinical and Laboratory Standards Institute (CLSI) (24) and results were reported as sensitive (S), intermediate (I) and resistance (R).

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. Written informed consent was obtained from adult patients with diarrhoea aged above 18 years of age before collection of information. The patients' result of any investigation remained confidential while results of identified organisms and their antimicrobial susceptibility profile were referred to attending physicians for treatment. The samples were safely discarded and isolates stored in deep freezer for further investigation.

Data handling and statistical analysis

Data were initially compiled in an MS Excel spreadsheet and statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (16.0 version). Descriptive statistics were calculated and summarized in frequency and proportions. Epidemiological Linkages of Diarrheagenic Vibrio Species from Sea water, Seafood and Patient's Stools were determined using multivariable logistic regression. Statistical analyses focused on variables potentially associated with typical species of Vibrio isolates (*V. cholerae, V. parahaemolyticus, V. vulnificus* and *V. alginolyticus*) from seawater, seafood and stool from patients with diarrhea, p values < 0.05 were considered statistically significant.

Results

A total of 303 samples from seawater (100), seafood (100) and stool samples (103) from diarrheal patients were included in the study. The culture results revealed *V. cholerae* isolates of 60 (60.0%) from seawater, 57(57.0%) from seafood, and 20 (19.4%) from

patients' stool samples. 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 patients stool samples. *V. alginolyticus* 6(6.0%) from seawater, 5(5.0%) from seafood samples and 3(2.9%) from patients stool samples. Other enteric bacterial species: *Salmonella* were 4(3.8%) and *Escherichia coli* were 2(1.9%). Unknown species were 11 (11.0%) from seawater, 10(10.0%) from seafood and 6(5.8%) from patient's stool sample as shown in Table 1 and Figure 1.

Table 1: Biochemical Results from Seawater, Seafood and Stool sample

Vibrio species	Oxidase test	Voges Proskauer	NaCl 0%	NaCl 6%	No. of Isolates	Proportions
V. cholerae from seawater	+	+	+	+	60	60.0
V. cholerae from seafood	+	+	+	+	57	57.0
V. cholerae from stools	+	+	+	+	20	19.4
V. parahaemolyticus from seawater	+	+	-	+	22	22.0
V. parahaemolyticus from seafood	+	+	-	+	21	21.0
V. parahaemolyticus from stool	+	+	+	+	15	14.5
V. vulnificus from seawater	+	+	-	+	12	12.0
V. vulnificus from seafood	+	+	-	+	10	10.0
V. vulnificus from stool	+	+	-	+	5	4.8
V. alginolyticus from seawater	+	+	-	+	6	6.0
V. alginolyticus from seafood	+	+	-	+	5	5.0
V. alginolyticus from stool	+	+	-	+	3	2.9
Other enteric bacterial species from stool						
Salmonella	-	+	-	+	4	3.8
E. coli	-	-	-	+	2	1.9
Unknown from seawater	+	-	+	+	11	11.0
Unknown from seafood	+	-	-	+	10	10.0
Unknown from stool	+	-	-	+	6	5.8

The plus sign (+) represents ≥ 90% detected positive; and minus sign (-) represents < 10% detected negative.

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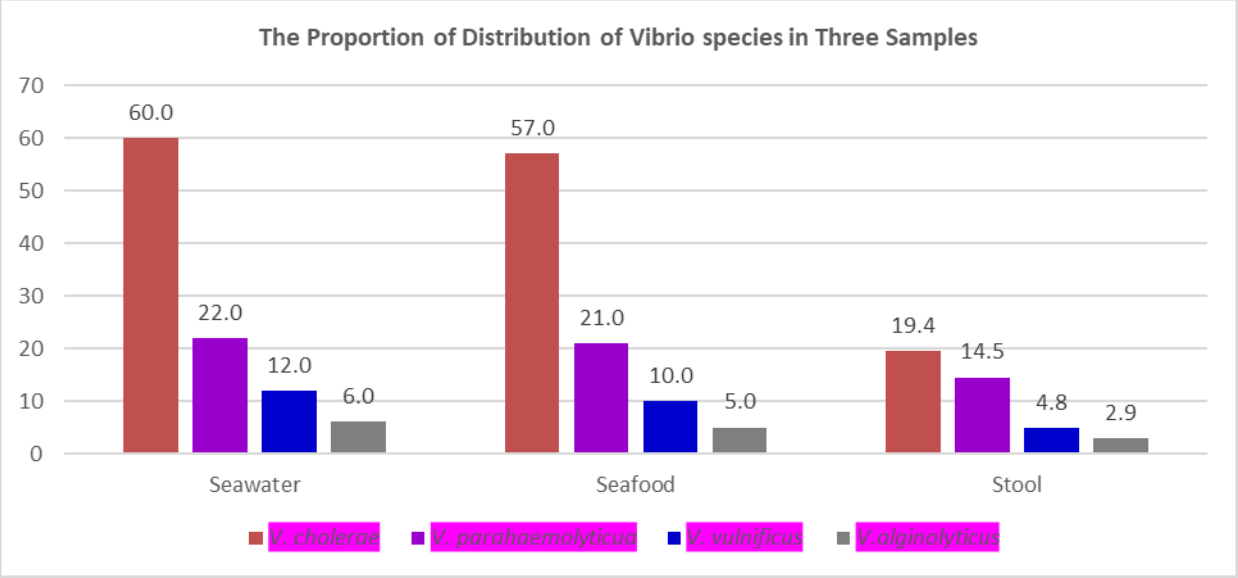

Figure 1. The Proportional Distribution of Vibrio species isolated from three different samples

Antibiotic Susceptibility Test from Patient's Stool Samples

The Vibrio specie isolates displayed different rates of susceptibility to the evaluated antibiotics (Table 2). *Vibrio cholerae* isolates were highly susceptible to chloramphenicol 20(100%), ciprofloxacin 20(100%), doxycycline 20(100%) and trimethoprim-sulfamethoxazole 15(75.0%), but they were less susceptible to ampicillin 5(25.0%), erythromycin 5(25.0%) and nalidixic acid 5(25.0%). The isolates exhibited high resistance to nalidixic acid 15(75.0%) while low resistance to ampicillin 10(50.0%) and erythromycin 5(25.0%). The results indicate no resistance to chloramphenicol, ciprofloxacin, doxycycline and trimethoprim-sulfamethoxazole.

Table 2: Antibiotic susceptible pattern of Vibrio cholerae from patients

Antimicrobial	S	R	I
Ampicillin	5(25.0)	10(50.0)	5(25.0)
Erythromycin	5(25.0)	5(25.0)	10(50.0)
Chloramphenicol	20(100)	0(0.0)	0(0.0)
Nalidixic acid	5(25.0)	15(75.0)	0(0.0)
Ciprofloxacin	20(100)	0(0.0)	0(0.0)
Doxycycline	20(100)	0(0.0)	0(0.0)
Trimethoprim-sulfamethoxazole	15(75.0)	0(0.0)	5(25.0)

Abbreviations: S Sensitive, R Resistant and I Intermediate

The *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 pattern against nalidixic acid 11(73.3%), trimethoprim-sulfamethoxazole 5 (33.3%) and ampicillin 4 (26.6%). The results indicated no resistance and intermediate to chloramphenicol, ciprofloxacin and doxycycline as shown in Table 3.

Table 3: Antibiotic susceptible pattern of V. parahaemolyticus from Patient

Antimicrobial	S	R	I
Ampicillin	8 (53.3)	4 (26.6)	3 (20.0)
Erythromycin	10 (66.6)	2 (13.3)	3 (20.0)
Chloramphenicol	15 (100)	0 (0.0)	0 (0.0)
Nalidixic acid	3 (20.0)	11 (73.3)	1(6.6)
Ciprofloxacin	15 (100)	0 (0.0)	0 (0.0)
Doxycycline	15 (100)	0 (0.0)	0 (0.0)
Trimethoprim-sulfamethoxazole	7 (46.6)	5 (33.3)	3 (20.0)

Abbreviations: S Sensitive, R Resistant and I Intermediate

The *Vibrio vulnificus* isolates were susceptible to doxycycline 5(100%), ciprofloxacin 5(100%), chloramphenicol 4(80%) and erythromycin 3(60.0%). Nevertheless, *V. vulnificus* isolates were reported with different resistances against nalidixic acid 2(40.0%), trimethoprim-sulfamethoxazole 2 (40.0%) and ampicillin 2(40.0%). The report indicates no resistance to chloramphenicol, ciprofloxacin and doxycycline as shown in Table 4.

Table 4: Antibiotic susceptible pattern of V. vulnificus from patient

Antimicrobial	S	R	I
Ampicillin	2 (40.0)	2 (40.0)	1 (20.0)
Erythromycin	3 (60.0)	1 (20.0)	1 (20.0)
Chloramphenicol	4 (80.0)	0 (0.0)	1 (20.0)
Nalidixic acid	1 (20.0)	2 (40.0)	2(40.0)
Ciprofloxacin	5 (100)	0 (0.0)	0 (0.0)
Doxycycline	5 (100)	0 (0.0)	0 (0.0)
Trimethoprim-sulfamethoxazole	2 (40.0)	2 (40.0)	1 (20.0)

Abbreviations: S for Sensitive, R for Resistant and I for Intermediate

The *Vibrio alginolyticus* isolates 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.

Table 5: Antibiotic susceptible pattern of V. alginolyticus from Patient (N / %)

Antimicrobial	S	R	I
Ampicillin	1(33.3)	1(33.3)	1(33.3)
Erythromycin	1(33.3)	1(33.3)	1(33.3)
Chloramphenicol	3(100)	0(0.0)	0(0.0)
Nalidixic acid	1(33.3)	1(33.3)	1(33.3)
Ciprofloxacin	3(100)	0(0.0)	0(0.0)
Doxycycline	3(100)	0(0.0)	0(0.0)
Trimethoprim-sulfamethoxazole	1(33.3)	1(33.3)	1(33.3)

Abbreviations: S for Sensitive, R for Resistant and I for Intermediate

Multidrug resistant (MDR) patterns of *Vibrio cholerae* isolates were demonstrated to three antibiotics: nalidixic acid, ampicillin and erythromycin. The *Vibrio parahaemolyticus, Vibrio vulnificus* and *Vibrio alginolyticus* isolates exhibited multidrug resistance to four antibiotics: nalidixic acid, ampicillin, erythromycin and trimethoprim-sulfamethoxazole. This shows that *Vibrio cholerae* was less resistant to drugs than *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 seawater, seafood and patient's stool. Previous reports from WHO indicated that seawater isolates had more *V. cholerae, V. parahaemolyticus, V. vulnificus* and *V. alginolyticus* isolates followed by seafood and low isolates were in patients' stools. The higher amount of Vibrio spp. in the coastal human settlements is due to seawater temperature (1). The evidence of seawater 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.

The epidemiological linkage between Vibrio species isolates from seawater, seafood and stool from patients with diarrhea was significantly correlated, p = 0.01. However, our study has found a decrease of proportions of *Vibrio cholerae* in seawater 60(60.0%), seafoods 57(57.0%) and patient's stool 20(19.4%). Although we could not find a direct explanation of the causes of a slight decline of proportions in such epidemiological linkages, we are confident that *Vibrio cholerae* is the main causes of diarrhoea outbreak in Zanzibar similar to the study conducted in Uganda, which reported proportions of these species in stool samples were 33.7% (12) and 33% (11). While our study reported a proportion of 19.4% the difference is mainly due to sample size. Likewise, the study on causes of diarrhoea outbreaks in India reported a proportion of 3.93%, which is lower than what we found in this study (20) which might be due to the prior usage of antibiotics by the patients.

The interest of *V. parahaemolyticus* is 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 seawater as a reservoir, contaminated seafood and to human via cold seafoods. 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. The data for this study were collected from patients who were adults above 18 years and live around the same study areas where seawater and seafood were collected in the west urban region. This study also coincides with a previous study which indicated that the consumption of raw and/or undercooked seafood is the main reason behind cholera outbreaks (7).

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.% to the same antibiotic. Similar studies in Zanzibar reported *Vibrio cholerae* were susceptible to ciprofloxacin 100% while they had high resistance of 93% to nalidixic acid (7). In addition

several studies reported *Vibrio cholerae* to be highly resistant to nalidixic acid 83.2% in Kenya (21), 98% in Zambia (10), 100% in Nepal (22) and 100% in Iran (23).

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 (21), 60% in Zambia (10), 100% in Nepal (22) and 100% in (12). The resistance to erythromycin were observed higher in other studies conducted in Iran100%, (23), Nepal 90.9% (22) and Kenya 53.47% (21) 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 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

A high proportion of diarrheagenic Vibrio species were isolated from seawater and low from patients with diarrhea. Among the tested three antibiotics: chloramphenicol, ciprofloxacin and doxycycline were found to be most effective for Vibrio species however, resistance is developing to four commonly used antibiotics: ampicillin, erythromycin, nalidixic acid and trimethoprim-sulfamethoxazole. Interventions against diarrhoea, that address the role of Vibrio species and the corresponding antimicrobial susceptibility patterns are needed.

Competing interests

The authors declare that they have no competing interests.

Author contribution

KMK developed the concept note, study design, data collection, laboratory work, data analysis, and interpretation of data as well as initial development of the manuscript. BM made substantial contributions to design, acquisition of data, analysis, and interpretation of data. KO has made substantial contributions to the interpretation of data and initial draft the manuscript and led the final write up of the manuscript. MD, LN supervisors who worked tirelessly with great contribution on proposal development and manuscript formation. All the authors have been involved in drafting the manuscript and revising it critically for important intellectual content, and; have given final approval of the version to be published.

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Availability of data and materials

The datasets were used and analyzed during the current study is available from the corresponding author on a reasonable request.

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Abbreviations

MDR	Multidrug Resistant
TCBS	Thiosulfate - Citrate - Bile – Salt
SOP	Standard Operating Procedure

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