



Molecular Detection and Distribution of Six Medically Important *Vibrio* spp. in Selected Freshwater and Brackish Water Resources in Eastern Cape Province, South Africa

Oluwatayo E. Abioye^{1,2,3*}, Ayodeji Charles Osunla^{1,2,4} and Anthony I. Okoh^{1,2,5}

¹SAMRC Microbial Water Quality Monitoring Centre, University of Fort Hare, Alice, South Africa, ²Applied and Environmental Microbiology Research Group, Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa, ³Department of Microbiology, Obafemi Awolowo University, Ife, Nigeria, ⁴Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Nigeria, ⁵Department of Environmental Health Sciences, University of Sharjah, Sharjah, United Arab Emirates

OPEN ACCESS

Edited by:

Rodrigo Gouvea Taketani,
Rothamsted Research, United Kingdom

Reviewed by:

Eiji Arakawa,
National Institute of Infectious
Diseases (NIID), Japan
Helena D. M. Villela,
Federal University of Rio de Janeiro,
Brazil

*Correspondence:

Oluwatayo E. Abioye
abioyethayor@gmail.com

Specialty section:

This article was submitted to
Aquatic Microbiology,
a section of the journal
Frontiers in Microbiology

Received: 15 October 2020

Accepted: 29 March 2021

Published: 02 June 2021

Citation:

Abioye OE, Osunla AC and
Okoh AI (2021) Molecular Detection
and Distribution of Six Medically
Important *Vibrio* spp. in Selected
Freshwater and Brackish Water
Resources in Eastern Cape Province,
South Africa.
Front. Microbiol. 12:617703.
doi: 10.3389/fmicb.2021.617703

Water resources contaminated with pathogenic *Vibrio* species are usually a source of devastating infection outbreaks that have been a public health concern in both developed and developing countries over the decades. The present study assessed the prevalence of six medically significant *Vibrio* species in some water resources in Eastern Cape Province, South Africa for 12 months. We detected vibrios in all the 194 water samples analyzed using polymerase chain reaction (PCR). The prevalence of *Vibrio cholerae*, *Vibrio mimicus*, *Vibrio fluvialis*, *Vibrio vulnificus*, *Vibrio alginolyticus*, and *Vibrio parahaemolyticus* in freshwater samples was 34, 19, 9, 2, 3, and 2%, and that in brackish water samples was 44, 28, 10, 7, 46, and 51%, respectively. The population of the presumptive *Vibrio* spp. isolated from freshwater (628) and brackish water (342) samples that were confirmed by PCR was 79% (497/628) and 85% (291/342), respectively. Twenty-two percent of the PCR-confirmed *Vibrio* isolates from freshwater ($n = 497$) samples and 41% of the PCR-confirmed *Vibrio* isolates from brackish water samples ($n = 291$) fall among the *Vibrio* species of interest. The incidences of *V. cholerae*, *V. mimicus*, *V. fluvialis*, *V. vulnificus*, *V. alginolyticus*, and *V. parahaemolyticus* amidst these *Vibrio* spp. of interest that were recovered from freshwater samples were 75, 14, 4, 6, 1, and 1%, whereas those from brackish water samples were 24, 7, 3, 3, 47, and 18%, respectively. Our observation during the study suggests pollution as the reason for the unusual isolation of medically important vibrios in winter. Correlation analysis revealed that temperature drives the frequency of isolation, whereas salinity drives the composition of the targeted *Vibrio* species at our sampling sites. The finding of the study is of public health importance going by the usefulness of the water resources investigated. Although controlling and preventing most of the factors that contribute to the prevalence of medically important bacteria, such as *Vibrio* species, at the sampling points might be difficult, regular monitoring for creating health risk awareness will go a long way to prevent possible *Vibrio*-related infection outbreaks at the sampling sites and their immediate environment.

Keywords: pathogenic *Vibrio* spp., temperature, salinity, public-health, brackish-water, freshwater

INTRODUCTION

The *Vibrio* genus is made up of over one hundred species (Romalde et al., 2014; Fernández-delgado et al., 2015) of which about 12 have been associated with human infections. The three major human pathogens in the *Vibrio* genus are *Vibrio cholerae*, *Vibrio vulnificus*, and *Vibrio parahaemolyticus* (Guardiola-avila et al., 2015; Kokashvili et al., 2015; Jones, 2017). Pathogenic members of the *Vibrio* genus are very common in the aquatic environment and can cause water- and food-related infections (Ajin et al., 2016; Kirchberger et al., 2016; Machado and Bordalo, 2016; Shaheen et al., 2016). Infections caused by these human pathogenic *Vibrio* species include cholera and vibriosis, e.g., wound infections, septicemia, and gastroenteritis, which is often self-limiting (Baker-Austin et al., 2018). Human pathogenic *Vibrio* species include *V. carchariae*, *V. mimicus*, *V. cincinnatiensis*, *V. fluvialis*, *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus* (now *Grimontia hollisae*), *V. furnissii*, *V. metschnikovii*, *V. hollisae*, and *V. damsela* (*Photobacterium hollisae*; Finkelstein, 1996; Levinson and Jawetz, 1996; Turner, 1997; Morris, 2019). The most notable human *Vibrio* pathogens are *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and *V. fluvialis* (CDC, 1999; Finkelstein et al., 2002; Kothary et al., 2003; Chakraborty et al., 2006). On the other hand, *V. mimicus*, which diverged from a common ancestor as *V. cholerae*, and *V. alginolyticus*, which was formerly classified as *V. parahaemolyticus*, are emerging human pathogens (Mustapha et al., 2013; Guardiola-avila et al., 2015). *V. cholerae* causes cholera, whereas other human pathogenic members of the *Vibrio* genus cause infections generally refer to as vibriosis (Baker-Austin et al., 2018). The vehicles of transmission of the etiological agents of cholera and vibriosis to humans are water and food most especially seafood (Di et al., 2017). Water- and food-related diseases continue to be a huge problem for humanity (Tantillo et al., 2004). To circumvent the scourge of cholera and vibriosis, localized monitoring of the environment for etiological agents of the infections was recommended, and this should be done without prejudice to either *Vibrio*-related outbreak is ongoing or not (CDC, 2015). To better protect human health, monitoring the environment for agents of waterborne and foodborne infections, such as human pathogenic *Vibrio* spp., has been emphasized in the literature (European Commission, 2001; Baffone et al., 2006; Jones and Oliver, 2009). Members of the *Vibrio* genus are halophiles; thus, they are not expected to be found in freshwater resources, and this may explain why studies on the prevalence of human pathogenic *Vibrio* spp. in freshwater are relatively small. However, the ability of *Vibrio* spp. to adapt to varying ecological niches (Ceccarelli and Colwell, 2014; Payne et al., 2016; Osunla and Okoh, 2017) supported the need to investigate the role of freshwater resources in the spread of etiological agents of cholera and vibriosis.

Of the approximately 139 serogroups of *V. cholerae*, only O1 and O139 cause pandemic and epidemic cholera, but the non-O1/non-O139 serogroups also cause sporadic cholera-like infections (Glenn Morris, 1994; Rippey, 1994; Sharma et al., 1998; Deen et al., 2019). On the other hand, *V. parahaemolyticus*

causes most of the seafood-related diarrhea infections in Florida (Klontz et al., 1993; Letchumanan et al., 2015), and it causes septicemia occasionally (Rippey, 1994; Letchumanan et al., 2015). *V. vulnificus* biotype 1 is the most deadly of all *Vibrio* species because of its high invasiveness and its fatality rate that is higher than that of any other bacteria (Oliver et al., 1991; Oliver and Bockian, 1995; Bisharat, 2002; Bisharat et al., 2005; Drake et al., 2007). *V. fluvialis* commonly cause food poisoning (Bhattacharjee et al., 2010; Kanungo et al., 2012; Chowdhury et al., 2013), and it is an emerging pathogen that possesses epidemic potentials (Vinothkumar et al., 2013). Cholera and vibriosis outbreaks had been reported from several parts of the globe. The isolation of these pathogens from various aquatic milieu around the globe (Sarkar et al., 1985; Venkateswaran et al., 1989; Barua and Greenough, 1991; Kazi et al., 2005; Binh et al., 2009; Safa et al., 2009; Grim et al., 2010; Reimer et al., 2011; Esteves et al., 2015; Okoh et al., 2015; Di et al., 2017; Abioye and Okoh, 2018) has been reported. The occurrence of cholera in the Eastern Cape Province (ECP) has been well documented over the years (Mudzanani et al., 2003; Mema, 2008). The previous studies carried out in our laboratory (Applied and Environmental Microbiology Research Group, Department of Biochemistry and Microbiology, University of Fort Hare, South Africa) confirmed the occurrence of medically important *Vibrio* species in some wastewater treatment plants (WWTPs) of the ECP and their receiving watershed, especially those that discharge poorly treated final effluent (Igbiosa et al., 2009, 2011a,b; Nongogo and Okoh, 2014; Okoh et al., 2015).

These previous findings support the need for spatial and temporal investigation of the presence and distribution of these bacterial species in freshwater resources in the province since there is interconnectivity between WWTPs receiving watershed and other freshwater resources most especially rivers. Going by the scarcity of freshwater resources in South Africa, this kind of investigation is needful since all available freshwater resources in the country are potential alternatives to regular sources of treated freshwater during freshwater scarcity. Some of the anthropogenic activities that contribute to the occurrence of pathogenic *Vibrio* spp. in the aquatic environment include indiscriminate dumping of refuses into water bodies, discharge of poorly treated wastewater from the treatment plant into receiving watershed, run-off from farms especially farmland treated with manure, and defecating and urinating into water bodies by humans and grazing animals (Nevondo and Cloete, 2001; USEPA, 2001; Rees et al., 2010; Abakpa et al., 2013). Contamination due to the aforementioned factors is evident around some of the freshwater resources in the ECP. This is not a surprise since most of the ECP waterbodies are not well protected (Christine et al., 2013). Diseases outbreak in Eastern Cape as a result of watershed contaminated with poorly treated effluent from WWTPs has occurred in the past (Cottle and Deedat, 2002). Unfortunately, recent studies showed that the problem persists in the ECP (Mema, 2008; Nongogo and Okoh, 2014; Okoh et al., 2015; Edokpayi et al., 2017). Poorly treated WWTP's effluent has been identified as a significant contributor of pathogens to the water milieu of the ECP (Okeyo et al., 2018).

Vibrio spp. of medical importance including four of the six *Vibrio* spp. focused on in this study have been reported from various WWTPs of Eastern Cape and their receiving watershed. A significant amount of *V. cholerae*, *V. parahaemolyticus*, *V. metschnikovii*, *V. fluvialis*, and *V. vulnificus* were reportedly isolated from final effluents in some WWTPs in Nkonkobe rural community, Chris Hani, and Amathole district municipalities (Igbinosa et al., 2009, 2011a,b; Nongogo and Okoh, 2014). A more comprehensive study on final effluents of 14 WWTPs in Amathole and Chris Hani district municipalities reported 66.8% *Vibrio* spp. prevalence of the 1,000 randomly selected isolates recovered from the WWTPs. Of the 300 confirmed *Vibrio* spp., 68.2% belong to one of *V. parahaemolyticus*, *V. fluvialis*, and *V. vulnificus* (Okoh et al., 2015). The aforementioned is of potential health risk to individuals using watershed for recreational, agricultural, and domestic purposes most especially at the downstream of WWTP final effluents discharge points. In some studies from other provinces of South Africa, the isolation of *Vibrio harveyi*, *V. parahaemolyticus*, *V. cholerae*, *V. mimicus*, and *V. vulnificus* from tap, borehole, and dam in North West province (Maje et al., 2020) and *V. cholerae* from four WWTPs located in Gauteng Province (Dungeni et al., 2010) has also been reported. Health risks that an individual using these water resources could be exposed to include gastrointestinal infections caused by *V. parahaemolyticus*, *V. mimicus*, and *V. fluvialis*; wound infections caused by *V. vulnificus* and *V. harveyi*; cholera caused by *V. cholerae*; and cholera-like bloody diarrhea caused by *V. fluvialis* (Ramamurthy et al., 1994; Igbinosa and Okoh, 2010; Jones, 2017; Brehm et al., 2020).

The occurrences of dysfunctional WWTPs that discharge unacceptable final effluent (Herbig and Meissner, 2019) and the lack of adequate protection for surface water in the ECP suggest that pathogenic vibrios could have contaminated some water resources in the province. Although extensive work has been done on the contribution of WWTPs to the abundance of *Vibrio* spp. in the province, however, work on major rivers, their tributaries, and brackish water resources is still limited.

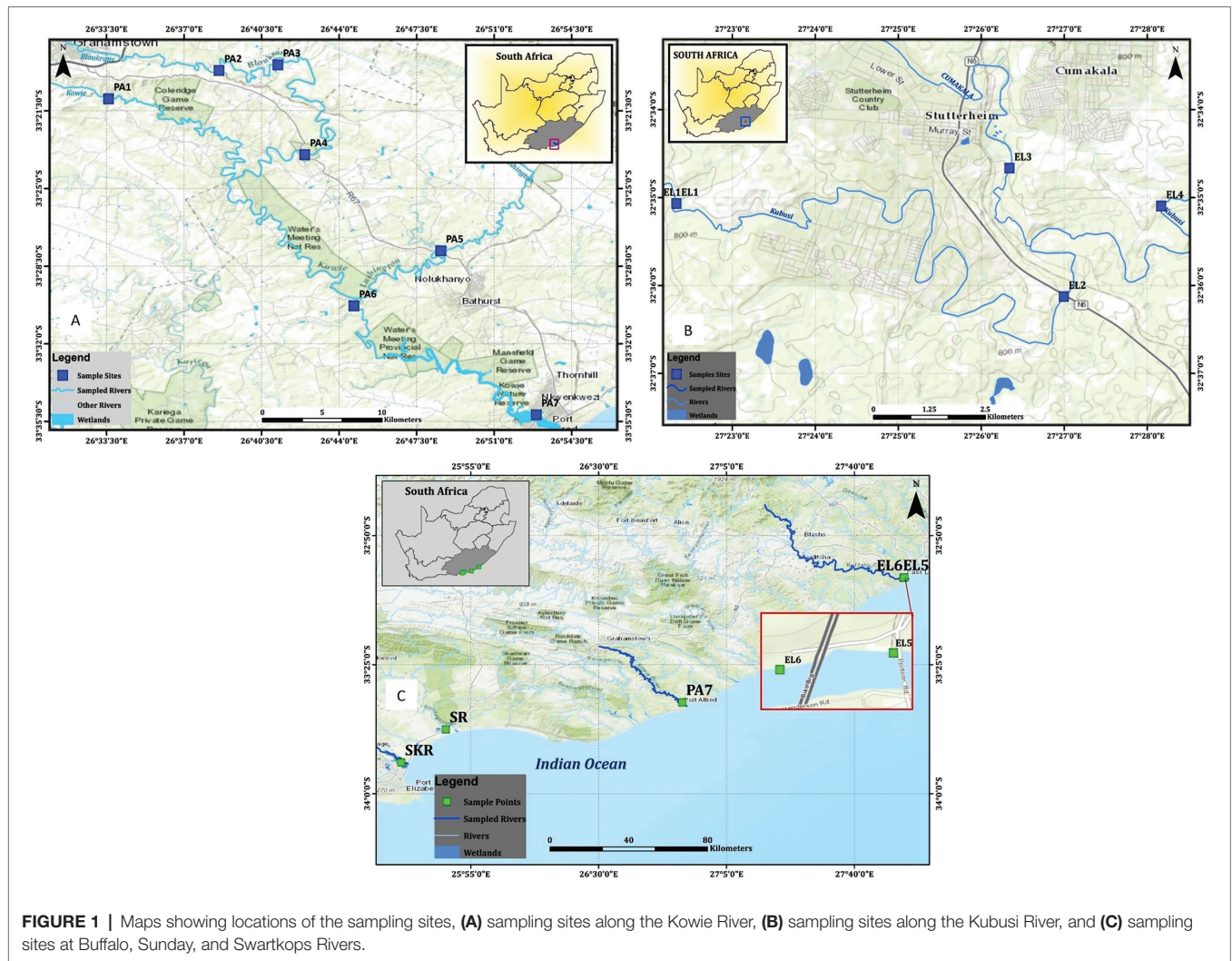
The findings of Momba et al. (2006), which confirmed the presence of toxigenic *V. cholerae* in the surface and groundwater of rural locality of Nkonkobe Local Municipality of Amathole District, Eastern Cape Province, South Africa, showed that surface water is of significant importance to the understanding of the abundance of *Vibrio* spp. in the province water milieu. However, the study by Momba et al. (2006) reported for toxigenic *V. cholerae* alone but not for non-cholera causing *Vibrio* pathogens of public health importance. In addition, the study was carried out more than a decade ago, and it does not cover all the important surface water resources in the province. The only one report found on the occurrence of *V. vulnificus* while developing this manuscript was from the KwaZulu-Natal beach, but the information was found in local news (Comins, 2012) rather than in research articles publishing outlets. The other few reports on occurrences of cholera and non-cholera causing *Vibrio* pathogens in rivers were carried out in KwaZulu-Natal, Mpumalanga, and Limpopo provinces,

South Africa (Bessong et al., 2009; Madoroba and Momba, 2010; Ntema et al., 2014; Marie and Lin, 2018). Although studies on occurrences of cholera and non-cholera causing *Vibrio* pathogens have been carried out in the ECP in the past, they were limited to up, final effluents discharge points, and some meters downstream of the receiving watershed for the WWTPs (Igbinosa et al., 2009, 2011a,b; Nongogo and Okoh, 2014; Okoh et al., 2015). Therefore, our understanding of the abundance and distribution of *Vibrio* spp. beyond the targeted points along the receiving watershed of the WWTPs and rivers that do not serve as receiving watershed for WWTPs in the province is still unclear. Based on the aforementioned, information on the occurrence of the etiological agents of cholera and vibriosis in South Africa freshwater and brackish water resources at the moment is limited most especially in the ECP. Going by the advice of the CDC (2017) to monitor the environment for *Vibrio* pathogens as a means of preventing cholera and vibriosis outbreaks, this study assesses the microbial quality of some important freshwater and brackish water sources in the ECP based on the presence or absence of six medically important *Vibrio* pathogens. The six medically important *Vibrio* spp. targeted were *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. fluvialis*, *V. mimicus*, and *V. alginolyticus*. The last two have not been reported from this region before now. It is intended that the study will contribute to the information needed for the epidemiology of the etiological agents of cholera and vibriosis in the aquatic milieu of the province most especially rivers and brackish water resources. It was also anticipated that this study will encourage *Vibrio* pathogens monitoring programs in the brackish and freshwater milieu of the ECP most especially those that humans access regularly.

MATERIALS AND METHODS

Study Areas and Sample Collection

Water samples were collected from freshwater and brackish water resources located along Kowie and tributaries, Kubusi, Sunday, Swartkops, and Buffalo Rivers as shown in **Figure 1**. Two freshwater dams in Amathole District Municipality were also included in the study, and their coordinates are S32°46.507'E026°51.604' and S32°47.406'E026°50.821'. The dams are used for fishing by the local anglers. The water sampling method employed is as documented in the previous study carried out in our laboratory (Igbinosa et al., 2011b; Okoh et al., 2015). Briefly, water samples were collected at 3–5 different points at each sampling site into 1-L sterile sterilin bottles by midstream dipping of sample bottles at 25–30 cm down the water column. The sample bottle cap was replaced while the sample bottle was still beneath the water column, transferred into cooler boxes containing ice, and afterwards transferred to the Applied and Environmental Microbiology Research Group (AEMREG) laboratory, University of Fort Hare, Alice. All the samples collected were analyzed within 6 h of collection, and samples were collected once a month for 1 year. The characteristics of each of the sampling sites are given in **Table 1**.



Temperature, Salinity, and Presumptive *Vibrio* Density Determination

The salinity and temperature were determined using a multiparameter ion-specific meter (version HI98195; Hanna Instruments) following the manufacturer's guidelines. The presumptive *Vibrio* density (PVD) was determined following the membrane filtration technique described in the literature (Nongogo and Okoh, 2014). A 100 ml of raw or diluted water samples, as the case may be, was filtered with the aid of a vacuum pump using a 0.45 μm membrane filter. Afterwards, the resulting membrane filters were placed on sterile thiosulfate citrate bile salts sucrose (TCBS) plates and incubated for 24–48 h at 37°C in triplicates. The mean of the yellow and green colonies on the TCBS triplicate plates per sample was recorded as PVD.

Total *Vibrio* spp. Density

The most probable number coupled with polymerase chain reaction (MPN-PCR) method detailed in the literature was adapted for determining the total *Vibrio* spp. density (TVD) in water samples (Ahmad et al., 2011; Copin et al., 2012;

Ramos et al., 2014; Abioye and Okoh, 2018). As a statistical-based method, MPN only estimates the viable numbers of bacteria in a sample by the principle of extinction dilution, i.e., inoculating liquid medium in 10-fold dilutions to a dilution factor at which no turbidity is observed after the incubation period. Unfortunately, the turbidity observed could be due to the presence of other bacteria other than the organism of interest. To overcome this shortcoming and make the methods more accurate and precise, MPN turbid tubes were subjected to PCR to ascertain the presence of the organism of interest. In this approach, MPN turbid tubes that tested negative for the organism of interest when subjected to PCR are counted as false positive and are not useful for calculating the density. Thus, this approach corrects for the shortcoming of using only MPN for determining the density of specific bacteria species. This is called the MPN-PCR method. Briefly, a 10-fold serial dilution of water sample was prepared up to the power of five, and afterwards, 1 ml of aliquots from raw and each of the diluted water sample was aseptically introduced into test tubes containing 10 ml of sterile freshly prepared alkaline peptone

TABLE 1 | Sampling site characteristics.

Sample sites/coordinates	Codes	Site peculiarities
Kowie River site 1 (S33°20.953'E026°33.601')	PA1	Very close to the source of Kowie River and devoid of any notable human activities during the sampling period. Wild animals have unrestricted access to the water resource at this point.
Bloukrans River site 2 (S33°19.658'E026°38.561')	PA2	It is one of the sites along the Kowie River tributary. It is used for agricultural purposes and serves as receiving watershed for wastewater treatment plant.
Bloukrans River site 3 (S33°21.218'E026°43.561')	PA3	It is one of the sites along the Kowie River tributary. It is used for agricultural purposes.
Bloukrans River site 4 (S32°23.478'E026°42.456')	PA4	It is one of the sites along the Kowie River tributary. It is used for agricultural and spiritual purposes.
Lashinton River site 5 (S32°27.783'E026°48.584')	PA5	It is one of the sites along the Kowie River tributary. It is used for agricultural purposes. An awful smell at this site suggests discharge of improperly treated effluent at the upstream.
Kowie River site 6 (S33°30.265'E026°44.679')	PA6	Use for fishing activities.
Kowie River Estuary site 7 (S33°35.193'E026°53.009')	PA7	It is located in Port Alfred. It is used for recreational activities, e.g., leisure fishing and swimming.
Kubusi River site 8 (S32°35.026'E027°22.311')	EL1	Not many human activities occur here, but the upstream is located within a game reserve. It is located at the outskirts of Stutterheim.
Kubusi River site 9 (S32°35.686'E027°25.417')	EL2	It is located around one of the local Stutterheim settlements. Free-range cows drink water and defecate at this point. The river is used for agricultural purposes some meters upstream of this site.
Kumukala River 10 (S32°34.675'E027°26.350')	EL3	It is one of the tributaries of Kubusi River. It serves as a final effluent receiving watershed for treatment plants. We observed eutrophication and algal bloom at this site. It is very close to Kumukala-Kubusi confluence. Kubusi River is used for an agricultural purpose just immediately after this site.
Kubusi River site 11 (S32°35.078'E027°28.168')	EL4	Eutrophication and algal bloom occurred at this site. Some agricultural activities were going on around this site.
Buffalo River Estuary site 12 (S33°01.357'E027°53.604')	EL5	An estuarine located along the Buffalo River. It receives industrial effluents, and it is used for fishing activities.
Buffalo River Estuary site 13 (S33°01.385'E027°53.470')	EL6	An estuarine located along the Buffalo River. It receives industrial effluents, and it is used for fishing activities. A fish slaughterhouse/cutting plant discharges its untreated effluents at the sampling site.
Sunday River Estuary site 14 (S33°41.580'E025°49.563')	SR	An estuary that is located close to Port Elizabeth. Serious fishing and recreational activities occur at this site.
Swartkops River Estuary site 15 (S33°51.444'E025°35.940')	SKR	It is an estuary located in Port Elizabeth. Serious fishing and recreational activities occur at this site. Companies and wastewater treatment plants surround the river. The water smells awful, and this suggests that the surrounding companies might be discharging poorly treated effluents into the river.
DAM 1 site 16 (S32°46.507'E026°51.604')	ALD1	The dam is used by the University of Fort Hare for agricultural purposes. The presence of cow droppings at the edge of the dam suggests that free-range cows visit the site to drink water. Local fishermen also fish from the dam, especially during the summer season. It is close to Alice wastewater treatment plant.
DAM 2 site 17 (S32°47.406'E026°50.821')	ALD2	The dam is used by the University of Fort Hare for agricultural purposes. The presence of cow droppings at the edge of the dam suggests that free-range cows visit the site to drink water. Local fishermen also fish from the dam, especially during the summer season. Tyume, which is the sources of the dam that serves as receiving watershed for a couple of wastewater treatment plants.

water (APW) in triplicates and incubated at 37°C for 24 h. Turbid tubes were separated, and total genomic DNA was extracted from each turbid tube by the boiling method (Maugeri et al., 2006). For the total genomic DNA extraction, 1 ml of aliquot from the turbid tubes was aseptically transferred into a sterile microcentrifuge tube and centrifuged for 2 min at a speed of $11,000 \times g$ using a MiniSpin microcentrifuge. After centrifugation, the supernatant was discarded while 200 μ l of sterile distilled water was added to the cell pellet at the bottom of the microcentrifuge tubes and vortexed to form a solution containing evenly distributed cells. The cells in the solution were afterwards lysed with the aid of an AccuBlock (Digital dry bath; Labnet) for 15 min at 100°C, and the solution of the lysed cells was centrifuged at a speed earlier mentioned. The supernatant that was the genomic extraction was subjected to 25 μ l PCR reaction to confirm the presence of *Vibrio* spp. using a primer that targets a variable region of *16S rRNA* gene that

is specific for members of the *Vibrio* genus. Furthermore, to determine the absolute density of each of the targeted *Vibrio* spp., DNA templates from MPN tubes that were positive for *Vibrio* spp. were subjected to another round of PCR that targeted each of the six *Vibrio* spp. using species-specific primers used for delineation (Table 2). The composition of the PCR reaction was 5 μ l of DNA template, 12.5 μ l of one Taq 2X Master Mix Standard Buffer (BioLabs, UK), 1 μ l each of 10 μ M of forward and reverse primers, and 5.5 μ l of nuclease-free water. The concentration of the DNA in templates ranges between 80 and 195 ng/ μ l through the experiment. The positive controls used for the PCR assay were *V. parahaemolyticus* (DSM 10027), *V. vulnificus* (DSM 10143), *V. fluvialis* (DSM 19283), *V. mimicus* (DSM 19130), and *V. alginolyticus* (DSM 2171) and one locally isolated *V. cholera*. The TVD was determined by extrapolating the equivalent MPN values for turbid tubes that were positive for members of the *Vibrio* genus and each of the targeted *Vibrio*

TABLE 2 | List of species-specific primers.

Species	Sequence	Size bp	References
<i>V. cholerae</i>	F: 3'-CAC CAA GAA GGT GAC TTT ATT GTG-5'	304	GOEL et al., 2010; de Menezes et al., 2014
	R: 3'-AGG ATA CGG CAC TTG AGT AAG ACTC-5'		
<i>V. parahaemolyticus</i>	F: 3'-GCA GCT GAT CAA AAC GTT GAG T-5'	897	Tarr et al., 2007; Okoh et al., 2015
	R: 3'-ATT ATC GAT CGT GCC ACT CAC-5'		
<i>V. vulnificus</i>	F: 3'-GTC TTA AAG CGG TTG CTG C-5'	410	Wong and Chow, 2002; Okoh et al., 2015
	R: 3'-CGC TTC AAG TGC TGG TAG AAG-5'		
<i>V. fluvialis</i>	F: 3'-GAC CAG GGC TTT GAG GTG GAC GAC-5'	217	Osorio and Klose, 2000; Chakraborty et al., 2006
	R: 3'GGT TTG TCG AAT TAG CTT CAC C-5'		
<i>V. mimicus</i>	F: 3'-GGTAGCCATCAGTCTTATCACG-5'	390	Lei et al., 2000; Shinoda et al., 2004; Sultan et al., 2007; Guardiola-avila et al., 2015
	R: 3'-ATCGTGTCCCAATACTTCACCG-5'		
<i>V. alginolyticus</i>	F: 3'-GAGAACCCGACAGAAGCGAAG-5'	337	Zhou et al., 2007; Wei et al., 2014
	R: 3'-CCTAGTGCGGTGATCAGTGTG-5'		

spp. using bacteriological analytical manual (BAM) Excel spreadsheet (Blodgett, 2015). The detection limit for the MPN-PCR method is <3 MPN/ml (<0.477 log MPN/ml). The forward sequence for the *Vibrio* genus-specific primer used was 3'CGG TGA AAT GCG TAG AGA T5', whereas that for the reverse sequence was 3'TTA CTA GCG ATT CCG AGT TC5'. The thermal cycler condition for the PCR assay was 15 min at 93°C for initial denaturation, 35 cycles of 92°C for 40 s, 57°C for 1 min, and 72°C for 1.5 min for denaturation, annealing, and elongation, respectively, and 75°C for 7 min for the final extension. The resulting amplicons were electrophoresed on 1.5% agarose, and pictures of amplified DNA bands on agarose gel were viewed and taken using an ultraviolet (UV) transilluminator following the manufacturer's guideline. The expected amplicon size was 663 bp (Kwok et al., 2002; Okoh et al., 2015).

Isolation of Presumptive *Vibrio* spp. From Water Samples

To maximize the isolation of the *Vibrio* species of interest, a water sample (100 ml) was filtered as articulated in the Temperature, Salinity, and Presumptive *Vibrio* Density Determination section, and the resulting membrane filter was introduced into a conical flask containing sterile 100 ml APW. The conical flask with its content was perturbed gently for about 1–2 min before incubating the set-up for 24 h at 37°C (Ntema et al., 2010). The set-up was observed on hourly bases, and as soon as there was the formation of the pellicle at the surface of the incubated APW, a loopful just below the pellicle was carefully streaked on sterile TCBS (Ceccarelli et al., 2015). The newly streaked TCBS plates were incubated for 24 h at 37°C. At the expiration of the incubation period, colonies (5–10 per plate) with typical *Vibrio* yellowish and greenish morphology were carefully picked, re-streaked on fresh sterile TCBS, and incubated for 24 h at 37°C. After the incubation period, a colony from TCBS plates with uniform colonies' morphology were transferred to fresh 1% NaCl nutrient agar plates. Resulting colonies were Gram stained and observed under a microscope to ensure the purity of the isolated presumptive *Vibrio* species to be stocked. Twenty percent glycerol stock of pure

isolates were prepared afterwards and stored at –80°C for PCR analysis.

Molecular Identification of Presumptive *Vibrio* Isolates

A total genomic DNA was extracted from the presumptive *Vibrio* isolates using the boiling method as described earlier in the Total *Vibrio* spp. Density section. The DNA was extracted from a colony of an 18-h-old pure culture of presumptive *Vibrio* spp. isolates. A solution of the colony prepared in a microcentrifuge containing 200 µl of sterile distilled water was boiled, and total genomic DNA was afterwards extracted from the cell solutions as earlier described in the Total *Vibrio* spp. Density section. This was followed by the confirmation of all the presumptive isolates as member of the *Vibrio* genus or otherwise following the PCR protocol detailed in the Total *Vibrio* spp. Density section. All PCR-confirmed *Vibrio* isolates were further delineated into the six *Vibrio* spp. targeted in this study.

Delineation of the PCR-Confirmed *Vibrio* Species

A set of species-specific primers were employed in PCR assay for delineating confirmed *Vibrio* spp. into the *Vibrio* species of interest. The respective primers employed for the identification of *V. cholerae*, *V. mimicus*, *V. fluvialis*, *V. vulnificus*, *V. alginolyticus*, and *V. parahaemolyticus* targeted the conserved region on *OmpW*, *vhm*, *ToxR*, *GroEl*, *GyrB*, and *fla E* genes that are specific for the organisms of our interest. *V. cholera* and *V. mimicus* were simultaneously identified using a duplex PCR protocol, whereas *V. vulnificus*, *V. fluvialis*, and *V. alginolyticus* were also simultaneously identified by employing a triplex PCR protocol. *V. parahaemolyticus* isolates were identified using a simplex PCR protocol. The primer sequences, sources, and expected amplicon sizes are given in **Table 2**, and all the PCR protocols were as earlier articulated in our previous study (Abioye and Okoh, 2018). The resulting amplicons were electrophoresed, bands on gels were viewed, and their pictures were taken using a UV transilluminator following the manufacturer's guideline.

Statistical Analysis

Results for freshwater and brackish water samples were distinctively treated on seasonal and annual bases. Density across sites was statistically compared using the Kruskal-Wallis test and the Games-Howell *post hoc* test at $p \leq 0.05$. Spearman correlation analysis was carried out to understand the relationship between PVD, TVD, temperature, and salinity at our sampling sites since salinity and temperature have been reported to modulate the ecology of *Vibrio* spp. differently at different geographical locations. The frequency of isolation (relative density) and absolute density of each of the six targeted *Vibrio* spp. were also correlated with temperature and salinity to establish species-based relationship. Kruskal-Wallis test and Spearman correlation analysis were used because our variables (temperature, density, salinity, and frequency of isolation) were not normally distributed.

Excel version 2013 and SPSS version 2020 were used to organize and analyze our data.

RESULT AND DISCUSSION

Temperature, Salinity, and *Vibrio* spp. Density

The average annual temperature and salinity of water samples at the sampling points were as given in **Table 3**. Salinity ranges between 0.06 ± 0.04 PSU at site EL1 and 2.03 ± 0.47 PSU at site PA5 for freshwater sampling sites, whereas it ranges between 16.14 ± 3.91 PSU at site SR and 31.65 ± 4.19 PSU at site PA7 for brackish water sampling sites. The temperature range was between $15.31 \pm 4.25^\circ\text{C}$ at site EL1 and $21.50 \pm 3.67^\circ\text{C}$ at site PA6 for freshwater sampling sites, and it was between $18.74 \pm 2.12^\circ\text{C}$ at site EL6 and $22.01 \pm 4.47^\circ\text{C}$ at site SR for brackish water sampling sites. During the study regime, 194 water samples were collected and analyzed for PVD and TVD. The mean annual PVD and TVD per site (**Figure 2**) differ across the sampling sites. The average annual PVD ranges between 1.23 ± 0.83 log CFU/ml at site ALD2 and 2.92 ± 0.93 log CFU/ml at site EL4 for freshwater samples, whereas that for brackish water samples ranges between 2.36 ± 0.72 log CFU/ml at site PA7 and 2.99 ± 1.0 log CFU/ml at site EL6. On the other hand, the range of annual average TVD was 1.35 ± 0.56 log MPN/ml at site PA1 to 2.63 ± 1.28 log MPN/ml at site EL3 for freshwater samples. The range for brackish water samples was 1.23 ± 0.64 log MPN/ml at site PA7 to 1.83 ± 0.71 log MPN/ml at site SR. The PVD at all sampling sites was more than that at site PA1 except for sites ALD1 and ALD2. This observation was significant at $p \leq 0.05$ for all sampling sites except for sites PA7, EL1, EL2, EL4, ALD1, and SR. A similar scenario was observed for TVD except that TVD at site PA1 was greater than TVD at site PA7. The observation for TVD was only significant for $\text{PA2} > \text{PA1}$. The densities of each of the targeted species are as given in **Supplementary Table SS1B**. A density greater than 0.477 log MPN/ml, which was the maximum detection limit of the three tubes by the five dilutions MPN-PCR density determination method employed, was recorded mainly in summer but scarcely recorded in winter months.

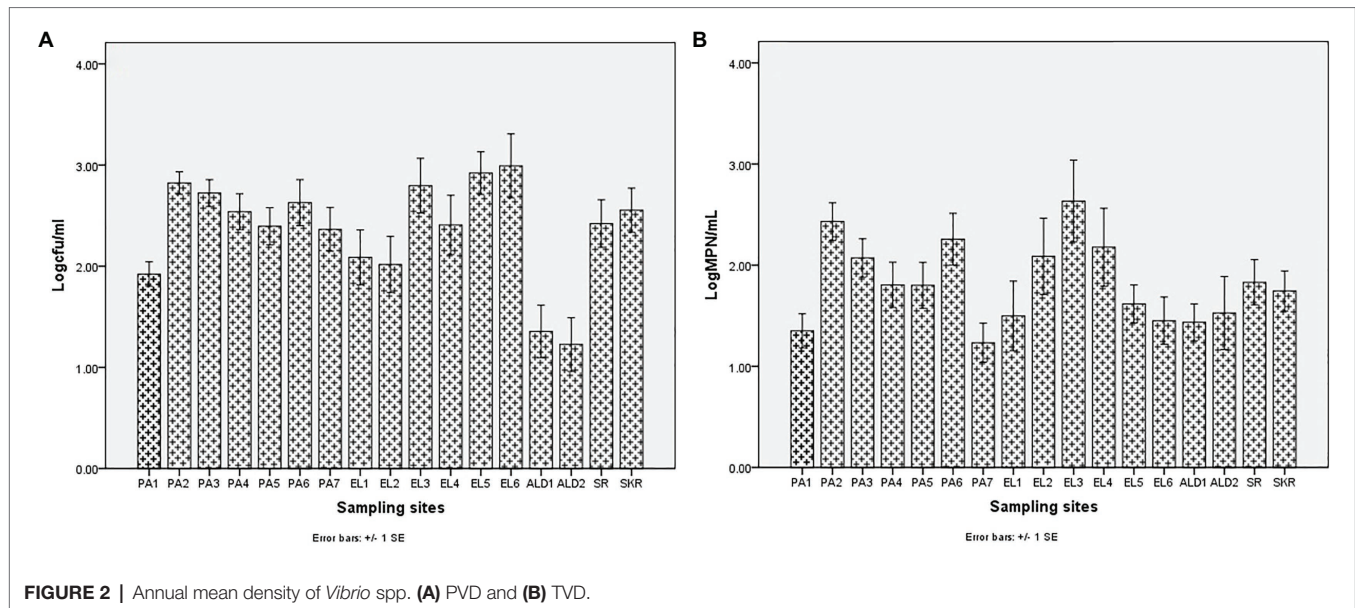
TABLE 3 | Average annual salinity and temperature at the freshwater and brackish water sampling sites.

Sites	Salinity \pm SD (PSU)	Temperature \pm SD ($^\circ\text{C}$)
Freshwater sampling sites		
PA1	0.18 ± 0.04	18.40 ± 3.14
PA2	0.53 ± 0.17	18.02 ± 3.00
PA3	0.69 ± 0.13	18.30 ± 3.11
PA4	0.71 ± 0.11	19.12 ± 3.17
PA5	2.03 ± 0.47	19.67 ± 3.41
PA6	1.70 ± 0.60	21.50 ± 3.67
EL1	0.06 ± 0.04	15.31 ± 4.24
EL2	0.11 ± 0.09	15.61 ± 4.30
EL3	0.13 ± 0.05	16.45 ± 3.80
EL4	0.14 ± 0.19	16.40 ± 4.18
ALD1	0.12 ± 0.07	18.81 ± 5.47
ALD2	0.13 ± 0.12	19.77 ± 4.68
Brackish water sampling sites		
PA7	31.65 ± 4.19	20.10 ± 2.07
EL5	29.14 ± 8.69	19.37 ± 1.46
EL6	27.94 ± 8.84	18.74 ± 2.12
SR	16.14 ± 3.91	22.10 ± 4.47
SKR	28.45 ± 2.29	21.63 ± 3.56

Key: SD = standard deviation

Of the samples with >0.477 log MPN/ml *V. cholerae*, *V. mimicus*, *V. fluvialis*, *V. vulnificus*, *V. alginolyticus*, and *V. parahaemolyticus* densities, 82, 72, 63, 100, 87, and 58%, respectively, were collected in summer months.

Sites PA1, EL1, ALD1, and ALD2 were anticipated to be pristine and have relatively low microbial load because PA1 and EL1 were around the sources of Kowie and Kubusi Rivers, respectively, whereas sites ALD1 and ALD2 were in a controlled environment. However, in some instance, PVD and TVD at these supposedly said pristine sampling sites were greater than PVD and TVD at other sampling sites, e.g., the mean annual *Vibrio* density at PA1 was greater than that at PA7, which are more exposed to anthropogenic activities. The unanticipated observation could be as a result of the easy access and activities of wild and domesticated animals around the water resources at PA1. South Africa water resources have been adjudged unprotected (Christine et al., 2013; Colvin et al., 2016) while domesticated and wild animals activities around surface water generally cause contamination of water resources with pathogens (Cabral, 2010; McAllister and Topp, 2012; Decol et al., 2017; Topalcengiz et al., 2017; Liu et al., 2018). Furthermore, domestic wastes from informal settlements along the riverbanks, effluents from overloaded and poorly maintained treatment plants, polluted runoff, and solid wastes that are potential contributors of pathogens to surface water (Fatoki et al., 2001; Chigor et al., 2012; Edokpayi et al., 2017; Adams et al., 2019) were evident at our sampling sites. In addition, droppings from free-range and grazing cows were observed at most of our freshwater sampling sites. Animals droppings are major contributors of pathogenic *Vibrio* spp., e.g., toxigenic *V. cholerae* into human environments (Dufour et al., 2013; Momba and Azab El-Liethy, 2017; Penakalapati et al., 2017; Delahoy et al., 2018; Munshi et al., 2019). As anticipated, sites SR, SKR, PA7, EL5, and EL6 located at estuaries where *Vibrio* spp. are commonly isolated



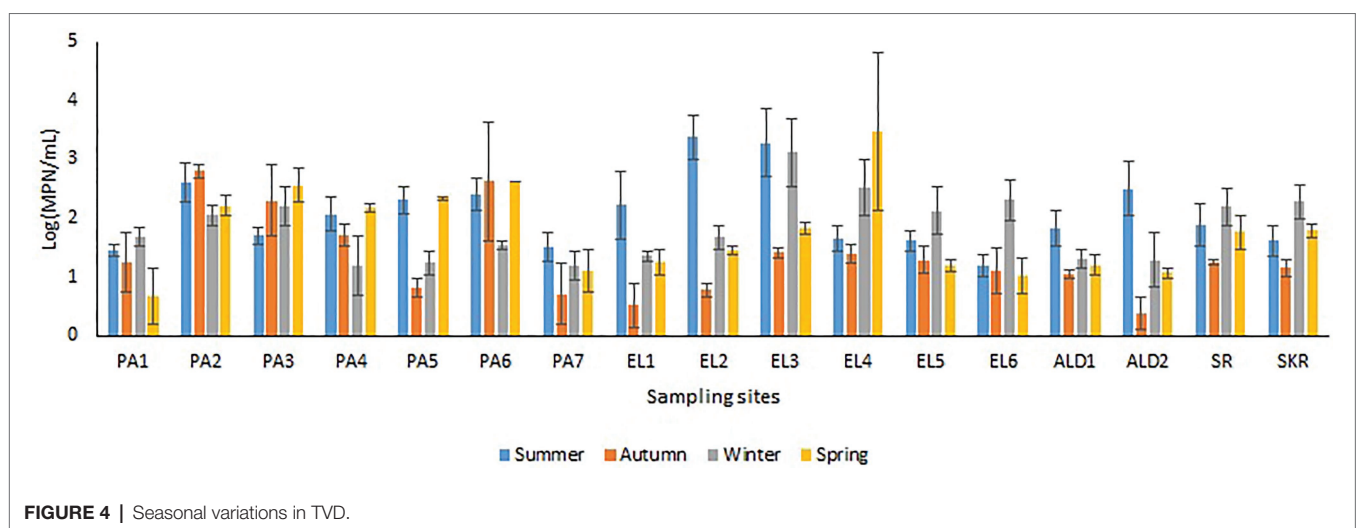
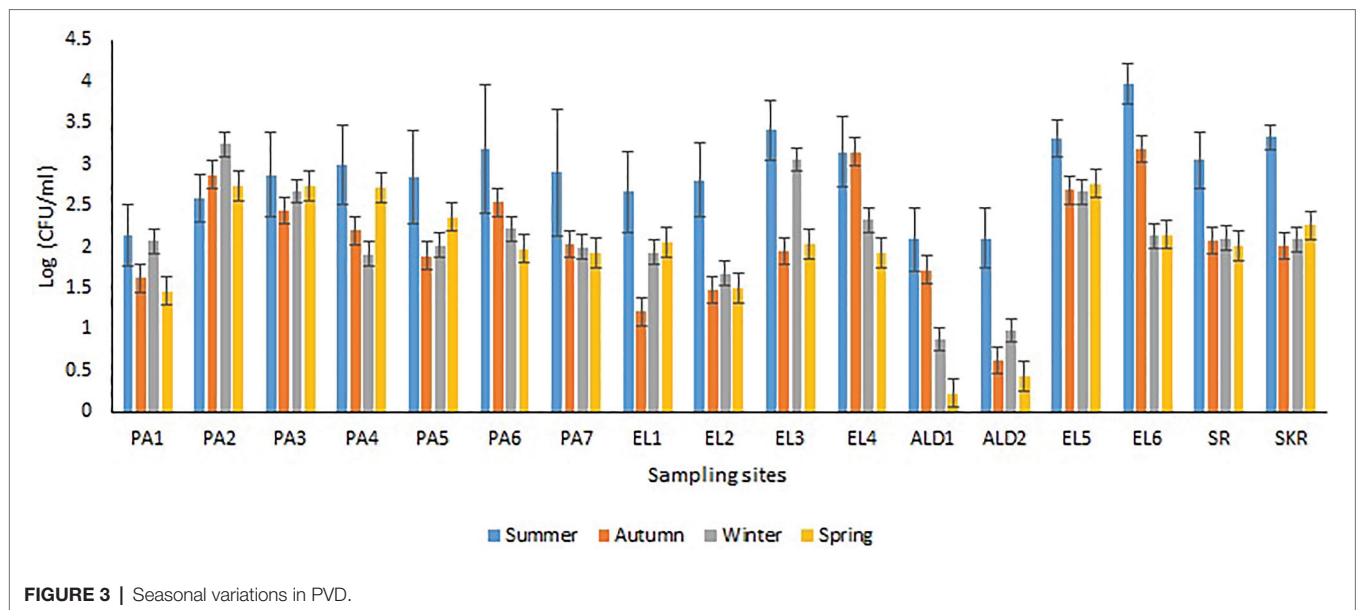
and site EL6 that also serves as receiving watershed for fish slaughterhouse/cutting plant had relatively high *Vibrio* density than some of the freshwater sampling sites. Reports have shown that effluents from fish slaughterhouses contribute significantly to *Vibrio* spp. load in receiving watershed (Novotny et al., 2004; Skall and Olesen, 2011). Surprisingly, the densities of *Vibrio* at some of the freshwater sampling sites were also significantly more than those at some of the brackish water sampling sites (Figures 2A,B). This observation could be as a result of the type of industrial effluent and other pollutants that are usually discharged into the water environments around the brackish water sampling sites (Adeniji et al., 2017a,b). Industrial waste has been reported to modulate the microbial biodiversity of the aquatic ecosystem (Li et al., 2017; Jordaan et al., 2019), and this could be on a downward trend for microbial populations when such waste is toxic rather than nutrient-rich. *Vibrio* spp. density (PVD and TVD) also varies across seasons with relatively high density recorded in summer as earlier reported (Igbinoso et al., 2011a,b; Kokashvili et al., 2015); however, few exceptions were observed (Figures 3, 4). A relatively high PVD and TVD in winter recorded at sites PA2 and EL3 can be traced to pollution. The two sites serve as receiving watershed for dysfunctional WWTPs (MacLennan, 2019), and dysfunctional WWTPs usually contaminate the water system with pathogens (Edokpayi et al., 2017). The highest density for each of the targeted *Vibrio* spp. (Supplementary Table SS1B) was recorded at sites (PA2, PA5, EL3, SR, and SKR) where we observed a high level of pollution.

Statistically speaking, a significantly positive weak correlation ($r = 0.201$, $p = 0.018$) between PVD and salinity was observed for freshwater but a significantly negative weak correlation ($r = -0.327$, $p = 0.014$) for brackish water samples. The correlation between TVD and salinity was not significant for freshwater ($r = 0.058$, $p = 0.503$) and brackish water ($r = -0.163$, $p = 0.203$) samples. The correlation

coefficients between PVD vs. temperature ($r = 0.133$, $p = 0.121$ for freshwater; $r = 0.2$, $p = 0.14$ for brackish water) and TVD vs. temperature ($r = 0.018$, $p = 0.252$ for freshwater; $r = -0.068$, $p = 0.619$ for brackish water) were not significant too. The lack of correlation between salinity, temperature, and *Vibrio* density had been reported in the literature (Randa et al., 2004; De Souza Costa Sobrinho et al., 2010; Paranjpye et al., 2015; Nilsson et al., 2019). The finding suggests that the contribution of salinity and temperature to the *Vibrio* genus density at our sampling sites is small, and that some other physicochemical and biological factors could be key to *Vibrio* density dynamics at our sampling sites. Interestingly, the literature has shown that parameters, such as dissolved oxygen and chemical and biological oxygen demands, have a strong relationship with *Vibrio* spp. density and the aforementioned parameters are good indexes for detecting water resources polluted with organic waste that may contain pathogens (Sharma and Chaturvedi, 2007; Prasanthan et al., 2011; Mezgebe et al., 2015; Di et al., 2017; Rabea et al., 2019). Unfortunately, organic waste pollution can occur at any time and cause unexpected offshoot in microbial density and diversity in an ecological niche. This, thus, explain the observation of the highest *Vibrio* density in seasons other than summer at some of our sampling sites because we observed that sources of organic waste pollutants are close to these sampling sites.

Effects of Salinity and Temperature on the Density, Frequency of Isolation, and Seasonality of the Targeted *Vibrio* Species

The frequency of isolation of each of the *Vibrio* species of interest in freshwater and brackish water samples per sampling month and the impact of temperature and salinity on the



frequency of detection are given **Figure 5**. The seasonal variation in the frequency of isolation of the *Vibrio* spp. of interest is given in **Figure 6**. The results show that the organism of interest was frequently isolated in summer than other seasons. Surprisingly, while the isolation frequency of organisms of interest in freshwater samples followed a similar pattern as salinity and temperature, only temperature did for brackish water samples. It is an established fact that salinity and temperature are important environmental factors that influence *Vibrio* spp. density, but the literature has also shown that the correlation between these factors and *Vibrio* spp. density differs for different geographical locations and sample types (Kelly, 1982; Kaspar and Tamplin, 1993; Fukushima and Seki, 2004; Randa et al., 2004; Igbinsola et al., 2011a; Takemura et al., 2014; Kokashvili et al., 2015; Colwell, 2016; Urquhart et al., 2016). For example, Kokashvili et al. (2015) observed a linear correlation between the two

factors and frequency of isolation of some medically important *Vibrio* species but a negative correlation between temperature and *V. metschnikovii* at the marine and freshwater sampling sites. In addition, Randa et al. (2004) observed a significant moderate negative correlation between salinity and abundance of *V. vulnificus* in estuarine water samples, whereas Colwell (2016) reported a non-significant negative correlation between temperature and the abundances of *V. parahaemolyticus* and *V. vulnificus* in estuary water samples. In this study, the frequency of isolation of at least one of the targeted species from freshwater samples showed a significant positive relationship with salinity and temperature (**Table 4**). However, only temperature had a significant positive relationship with the frequency of isolation of at least one of the targeted *Vibrio* spp. in brackish water samples. Interestingly, correlation coefficients and *p* values for the relationship between frequency of isolation of at least one

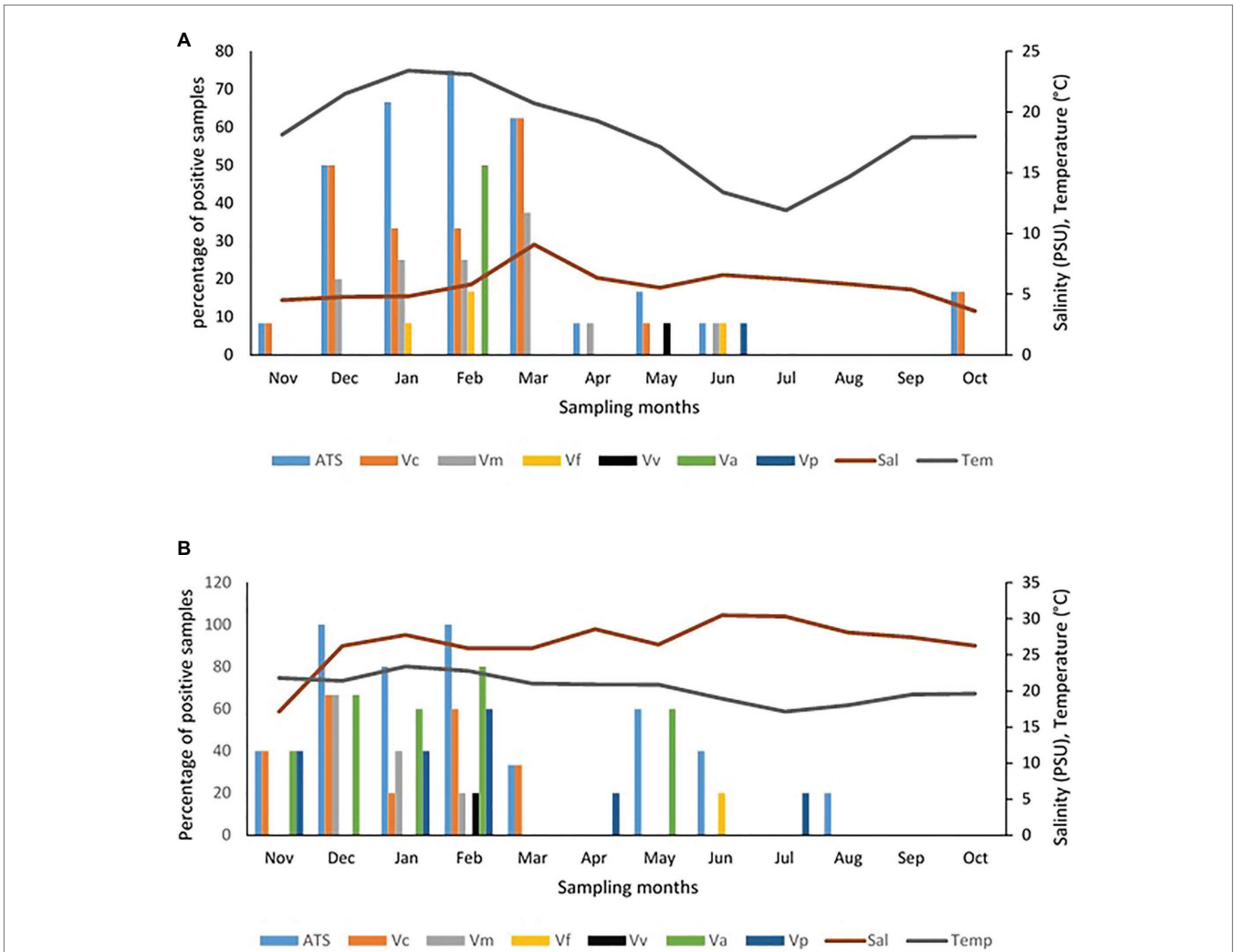


FIGURE 5 | Influence of temperature and salinity on the detection of targeted *Vibrio* spp. in water samples. **(A)** Freshwater samples, **(B)** brackish water samples. Key: Va = *V. alginolyticus*, Vc = *V. cholerae*, Vf = *V. fluvialis*, Vm = *V. mimicus*, Vp = *V. parahaemolyticus*, Vv = *V. vulnificus*, ATS = all targeted *Vibrio* species.

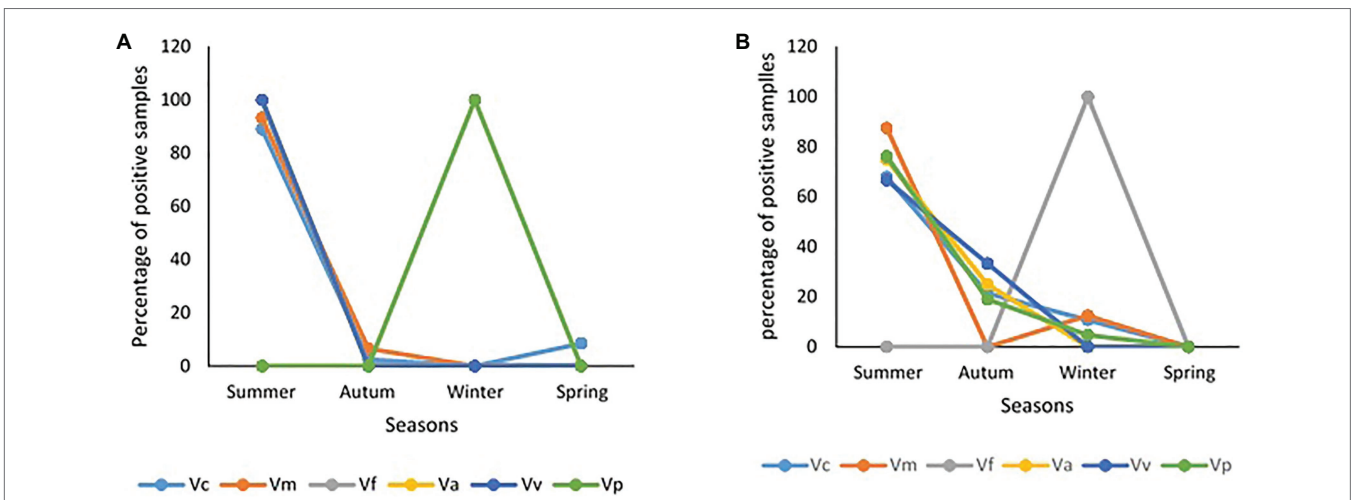


FIGURE 6 | Seasonality of samples positive for targeted *Vibrio* spp. **(A)** freshwater samples, **(B)** brackish water samples.

TABLE 4 | Correlation analysis between the frequency of isolation of targeted *Vibrio* spp. from water samples, temperature, and salinity.

Freshwater				Brackish water			
Organism type	Parameters	R	p	Organism type	Parameters	R	p
FITV	Sal	0.215	0.011	FITV	Sal	0.094	0.497
	Temp	0.462	<0.0001		Temp	0.459	<0.0001
FIVc	Sal	0.33	<0.0001	FIVc	Sal	0.193	0.153
	Temp	0.411	<0.0001		Temp	0.139	0.308
FIVm	Sal	0.028	0.747	FIVm	Sal	-0.010	0.941
	Temp	0.192	0.023		Temp	0.072	0.596
FIVf	Sal	-0.1	0.244	FIVf	Sal	0.121	0.374
	Temp	0.234	0.006		Temp	-0.140	0.305
FIVa	Sal	-0.022	0.801	FIVa	Sal	0.03	0.828
	Temp	0.153	0.073		Temp	0.51	<0.0001
FIVv	Sal	-0.055	0.521	FIVv	Sal	-0.233	0.084
	Temp	-0.064	0.453		Temp	0.351	0.008
FIVp	Sal	-0.061	0.477	FIVp	Sal	-0.057	0.679
	Temp	-0.067	0.435		Temp	0.571	<0.0001

Key: Bolded R and p values show a significant correlation. FIA = frequency of isolation; TV = the targeted *Vibrio* spp.; Va = *V. alginolyticus*; Vc = *V. cholerae*; Vf = *V. fluvialis*; Vm = *V. mimicus*; Vp = *V. parahaemolyticus*; Vv = *V. vulnificus*; %P = percentage prevalence

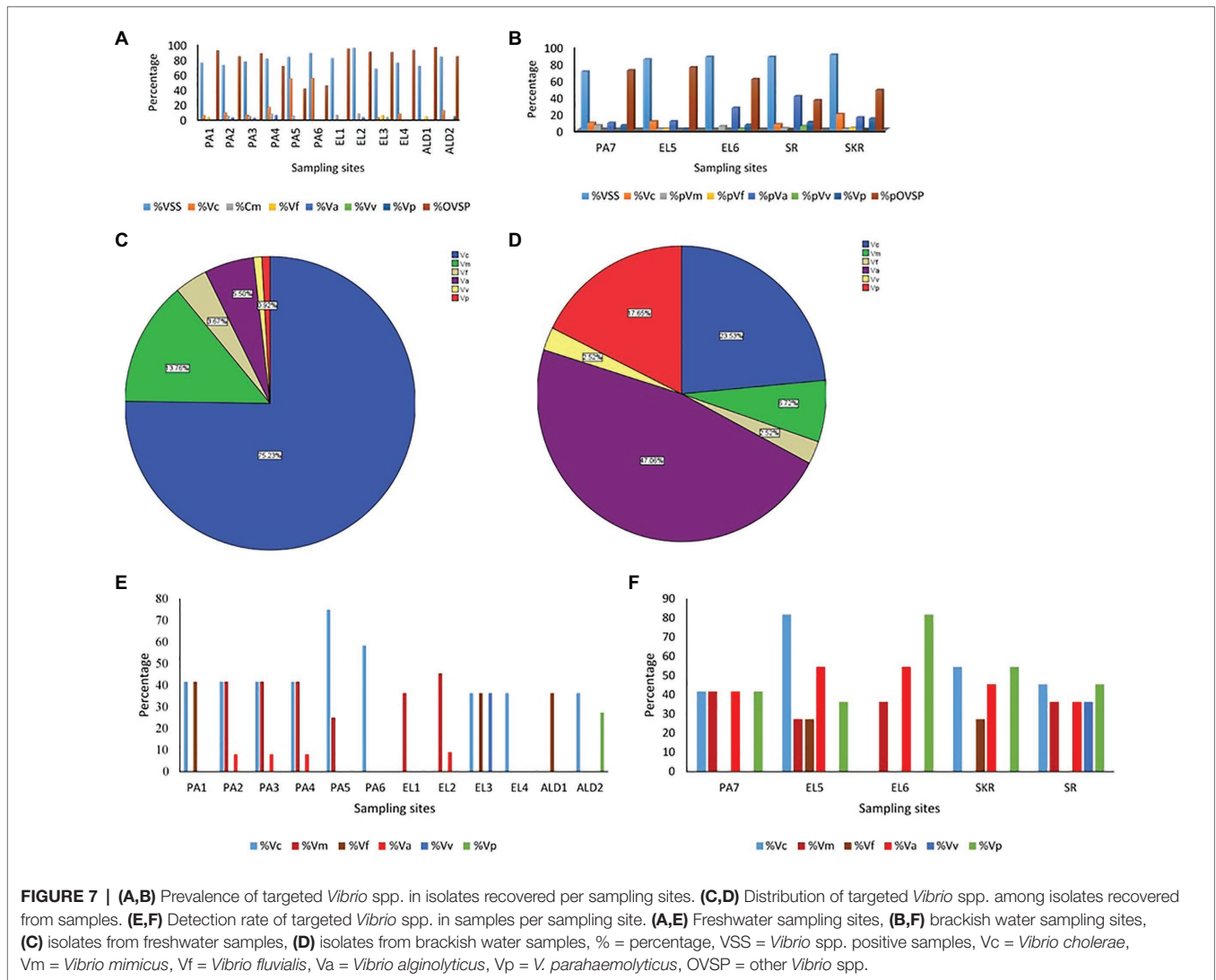
of the *Vibrio* spp. and temperature were very similar for the two water types (Table 4). This shows that temperature modulates the chances of isolating at least one of the *Vibrio* spp. of interest from the two water types in a similar way. However, at species level, the correlation analysis (Table 4) showed that temperature is more effective as guide for the isolation of *V. cholerae*, *V. mimicus*, and *V. fluvialis* (generally called non-halophilic) from freshwater and *V. alginolyticus*, *V. vulnificus*, and *V. parahaemolyticus* (generally called halophilic) from brackish water. In their work, Liu et al. (2016) and Mahoney et al. (2010) reported that temperature is more relevant to the isolation of non-halophilic vibrios from freshwater and halophilic vibrios from brackish water than salinity. Consequently, we adjudged from our data (Figure 5) that a temperature range between 20 and 24°C is the optimum temperature for the isolation of *V. cholerae*, *V. mimicus*, and *V. fluvialis* from freshwater sampling sites and *V. alginolyticus*, *V. vulnificus*, and *V. parahaemolyticus* from brackish water sampling sites. It was further inferred that a salinity range between 5 and 9 PSU should be used in adjunct with a temperature range of 20 and 24°C for the isolation of *V. cholerae* at the freshwater sampling sites. Our submission is in concordance with earlier studies that showed that relatively high frequency of isolation of medically important *Vibrio* spp. is usually achieved in summer when the temperature is above 15°C and salinity is between 5 and 25 PSU (Jones and Summer-Brason, 1998; DePaola et al., 2003; Urquhart et al., 2016).

It is good to point out that targeted *Vibrio* spp. from few samples at sites ALD1, ALD2, EL5, and SKR were isolated in winter in this study. This could be attributed to the level of pollution at these sites as earlier discussed and affirm the possibility of isolating medically important *Vibrio* spp. in usual season because of pollution and contamination that is not season dependent. It has been earlier reported by Kopprio et al. (2020) and Watkins and Cabelli (1985) that warmer temperatures and sewage pollution are reasons for the abundance of pathogenic *Vibrio* spp.

in the aquatic milieu. In addition, the studies carried out by Jaiani et al. (2013) and Janelidze et al. (2011) suggest that temperature affects *Vibrio* abundance, whereas salinity affects *Vibrio* species composition of any ecological niche. It is important to note here that a similar scenario discussed above was observed when absolute densities of targeted *Vibrio* spp. were statistically analyzed (Supplementary Tables SS1B,C; Supplementary Figures SS1A,B).

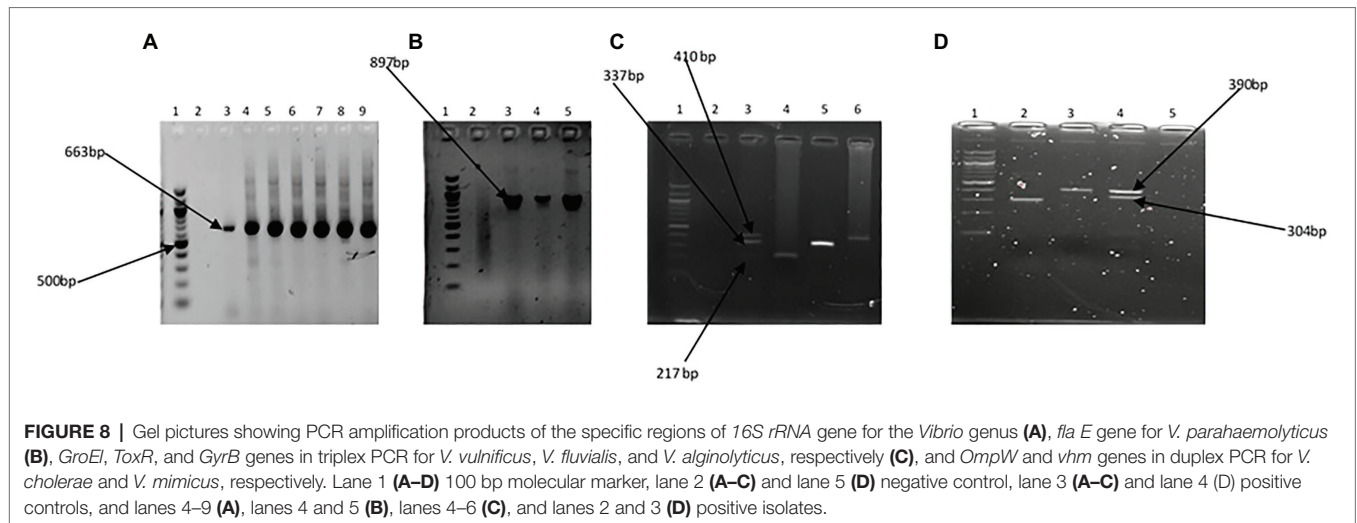
Prevalence of the Six Targeted *Vibrio* Species of Medical Importance

The Kruskal-Wallis test showed that the average annual absolute density of each of the targeted *Vibrio* spp. was significantly different across sites at $p < 0.05$. The Games-Howell *post hoc* test showed the site comparisons with significant differences (Supplementary Table SS1D). The comparisons of absolute density of targeted species between freshwater and brackish water sites that showed significant difference revealed that *V. cholerae* and *V. mimicus* are more abundant in freshwater than in brackish water, whereas *V. vulnificus*, *V. alginolyticus*, and *V. parahaemolyticus* are more abundant in brackish water than in freshwater. Furthermore, seasonal comparison of absolute density (Supplementary Table SS1E) showed that all the targeted species proliferate in summer significantly than in other seasons of the year. The relative abundance of each of the six targeted *Vibrio* spp. in isolates recovered at the sampling sites is shown in Figures 7A,B, whereas the incidence of each of the targeted species among the total *Vibrio* spp. isolates recovered from each of the water sample types is given in Figures 7C,D. The prevalence of each of the targeted *Vibrio* spp. per sampling site is given in Figures 7E,F. At least one of the targeted *Vibrio* species was detected and isolated from each of the sampling sites. The prevalence of *V. cholerae*, *V. mimicus*, *V. fluvialis*, *V. vulnificus*, *V. alginolyticus*, and *V. parahaemolyticus* in freshwater samples was 34, 19, 9, 2, 3, and 2%, respectively. On the other hand, the prevalence



of *V. cholerae*, *V. mimicus*, *V. fluvialis*, *V. vulnificus*, *V. alginolyticus*, and *V. parahaemolyticus* in brackish water samples was 44, 28, 10, 7, 46, and 51%, respectively. A total of 628 and 342 presumptive isolates from freshwater and brackish water samples, respectively, were analyzed in this study. Of the freshwater presumptive isolates, 79% were confirmed as *Vibrio* spp., whereas 85% of presumptive isolates from brackish water samples were confirmed as *Vibrio* spp. Twenty-two and 41% of the PCR-confirmed isolates from freshwater samples and brackish water samples, respectively, fall among the targeted *Vibrio* species. The samples of gel electrophoresis pictures for the confirmation and speciation of *Vibrio* spp. into the six targeted species are given in **Figure 8**. The incidence of *V. cholerae*, *V. mimicus*, *V. fluvialis*, *V. vulnificus*, *V. alginolyticus*, and *V. parahaemolyticus* of all the six targeted *Vibrio* species recovered from freshwater samples was 75, 14, 4, 6, 1, and 1%, whereas that for brackish water was 24, 7, 3, 47, 3, and 18%, respectively. The predominant species of the freshwater samples were *V. cholerae* and *V. mimicus*, whereas those of the brackish water samples

were *V. alginolyticus*, *V. cholerae*, and *V. parahaemolyticus*. The prevalence of the remaining four (*V. fluvialis*, *V. vulnificus*, *V. alginolyticus*, and *V. parahaemolyticus*) of the six targeted *Vibrio* spp. among *Vibrio* isolates from freshwater samples and three (*V. mimicus*, *V. fluvialis*, *V. vulnificus*) of the six targeted *Vibrio* species in brackish water samples were, respectively, low. Our result showed population diversity of targeted *Vibrio* species in freshwater and brackish water samples. Halophilic vibrios were more abundant in brackish water, whereas non-halophilic vibrios were abundant in freshwater samples. The six targeted *Vibrio* spp. were more diverse in brackish water sample than in freshwater sample. These findings corroborate some earlier reports and suggest that salt concentration modulates the diversity of vibrios in water resources (Elhadi, 2013; Mookerjee et al., 2015; de Menezes et al., 2017; Fu et al., 2019). The result (**Figure 7; Supplementary Table SS1A**) of the present study showed that the possibility of contracting infections, such as cholera and cholera-like infections, caused by the non-halophilic vibrios is higher at the freshwater sampling sites most especially



sites PA4, PA5, PA6, and ALD1 where most of the halophilic vibrios were recovered than at the brackish water sampling sites. Likewise, the chances of contracting gastroenteritis, wound infections, and other vibriosis peculiar to halophilic vibrios are higher at the brackish sampling sites most especially sites EL6, SR, and SKR. Furthermore, the coexistence of medically important non-halophilic and halophilic vibrios at the same ecological niche in most of our sampling sites is of public health concern. As the density and diversity of cholera and vibriosis causing *Vibrio* species increase in a single ecological niche, the chances of acquiring the infections they cause also increase. Furthermore, the possibility of exchanging genetic materials, such as virulence and antibiotics resistance determinants, will be high. The coexistence of medically important *Vibrio* species within the same ecological niche has been reported in an earlier study (Kokashvili et al., 2015). Although the present study focused on freshwater and brackish water that have not been investigated for members of the *Vibrio* genus before, *Vibrio* spp. of medical importance including four of the six *Vibrio* spp. focused on in this study have been reported from various WWTPs of Eastern Cape and their receiving watershed. A significant amount of *V. cholerae*, *V. parahaemolyticus*, *V. metschnikovii*, *V. fluvialis*, and *V. vulnificus* were reportedly isolated from final effluents in some WWTPs in Nkonkobe rural community, Chris Hani, and Amathole district municipalities (Igbinsosa et al., 2009, 2011a,b; Nongogo and Okoh, 2014). A more comprehensive study on final effluents of 14 WWTPs in Amathole and Chris Hani district municipalities reported 66.8% *Vibrio* spp. prevalence of the 1,000 randomly selected isolates recovered from the WWTPs. Of the 300 confirmed *Vibrio* spp., 68.2% belong to one of *V. parahaemolyticus*, *V. fluvialis*, and *V. vulnificus* (Okoh et al., 2015). All these studies, including the present one, show that medically important *Vibrio* spp. are present in the aquatic milieu of the ECP. The aforementioned is of potential health risk to individuals using the water resources studied and watershed for recreational, agricultural, and domestic purposes most

especially at the downstream of WWTP final effluents discharge points. In some studies from other provinces of South Africa, the isolation of *V. harveyi*, *V. parahaemolyticus*, *V. cholerae*, *V. mimicus*, and *V. vulnificus* from tap, borehole, and dam in North West province (Maje et al., 2020) and *V. cholerae* from four WWTPs located in Gauteng Province (Dungeni et al., 2010) has also been reported. Health risks that an individual using these water resources could be exposed to include gastrointestinal infections caused by *V. parahaemolyticus*, *V. mimicus*, and *V. fluvialis*; wound infections caused by *V. vulnificus* and *V. harveyi*; cholera caused by *V. cholerae*; and cholera-like bloody diarrhea caused by *V. fluvialis* (Ramamurthy et al., 1994; Igbinsosa and Okoh, 2010; Jones, 2017; Brehm et al., 2020).

SUMMARY AND CONCLUSION

The present study confirmed the presence of *Vibrio* species of medical importance in both freshwaters (Kowie River, Bloukrans River, Lashinton River, Kubusi River, and two dams in Amathole District Municipality) and brackish water (Buffalo, Sunday, Kowie, and Swartkops estuaries) of the Eastern Cape, Province of South Africa. It is worth mentioning that humans have regular contact with more than 88% of our sampling sites and all the sampling sites had not been investigated for the occurrence of *Vibrio* spp. of medical importance before. The predominant species at the sampling sites showed that the chances of contracting cholera and cholera-like infection are high at the freshwater sampling sites than at the brackish water sampling sites. On the other hand, the chances of contracting other vibriosis are high at the brackish water sampling sites than at the freshwater sampling sites. The findings of the study also suggest pollution as the reason for the isolation of medically important vibrios in the unusual season at some of the sampling sites. It was also observed that temperature drives isolation frequency, whereas salinity drives the composition of the targeted *Vibrio* species at both

freshwater and brackish water sampling sites. Although the virulence status of the isolated medically important *Vibrio* species was not elucidated in this study, the confirmation of their presence in the water resources investigated for the first time is a significant finding. This finding is an eye opener to the potential threat that the water resources investigated pose to public health in terms of cholera and vibriosis. Therefore, our findings are of public health importance going by the usefulness of the water resources investigated. Although controlling and preventing most of the identified factors that could be contributing to the prevalence of medically important *Vibrio* species at the sampling points might be difficult, regular monitoring will go a long way to prevent possible *Vibrio*-related infection outbreaks.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

REFERENCES

- Abakpa, G. O., Umoh, V. J., Ameh, J. B., and Al, E. T. (2013). Occurrence of *Vibrio cholerae* in some households engaged in livestock farming in some parts of Zaria, Nigeria. *Adv. Microbiol.* 3, 128–131. doi: 10.4236/aim.2013.31020
- Abioye, O. E., and Okoh, A. I. (2018). Limpet (*Scutellastra cochlear*) recovered from some estuaries in the eastern Cape Province, South Africa act as reservoirs of pathogenic *Vibrio* species. *Front. Public Health* 6:237. doi: 10.3389/fpubh.2018.00237
- Adams, J., Pretorius, L., and Snow, G. (2019). Deterioration in the water quality of an urbanised estuary with recommendations for improvement. *Water SA* 45, 86–96. doi: 10.4314/wsa.v45i1.10
- Adeniji, A., Okoh, O., and Okoh, A. (2017a). Petroleum hydrocarbon fingerprints of water and sediment samples of Buffalo River estuary in the eastern Cape Province, South Africa. *J. Anal. Methods Chem.* 2017:2629365. doi: 10.1155/2017/2629365
- Adeniji, A., Okoh, O., and Okoh, A. (2017b). Petroleum hydrocarbon profiles of water and sediment of alga bay, Eastern Cape, South Africa. *Int. J. Environ. Res. Public Health* 14:1263. doi: 10.3390/ijerph14101263
- Ahmad, N., Ghazali, F. M., Cheah, Y. K., and Chitek, T. Z. T. (2011). Prevalence and quantification of *Vibrio* species and *Vibrio parahaemolyticus* in freshwater fish at hypermarket level. *Int. Food Res. J.* 18, 689–695.
- Ajin, A., Silvester, R., Alexander, D., Nashad, M., and Abdulla, M. H. (2016). Characterization of blooming algae and bloom-associated changes in the water quality parameters of traditional pokkali cum prawn fields along the South West coast of India. *Environ. Monit. Assess.* 188, 1–10. doi: 10.1007/s10661-016-5133-6
- Baffone, W., Tarsi, R., Pane, L., Campana, R., Repetto, B., Mariottini, G. L., et al. (2006). Detection of free-living and plankton-bound vibrios in coastal waters of the Adriatic Sea (Italy) and study of their pathogenicity-associated properties. *Environ. Microbiol.* 8, 1299–1305. doi: 10.1111/j.1462-2920.2006.01011.x
- Baker-Austin, C., Oliver, J. D., Alam, M., Ali, A., Waldor, M. K., Qadri, F., et al. (2018). *Vibrio* spp. infections. *Nat. Rev. Dis. Prim.* 4:8. doi: 10.1038/s41572-018-0005-8
- Barua, D., and Greenough, W. B. (1991). *Cholera*. United States: Springer.
- Bessong, P. O., Odiyo, J. O., Musekene, J. N., and Tessema, A. (2009). Spatial distribution of diarrhoea and microbial quality of domestic water during an outbreak of diarrhoea in the Tshikwi Community in Venda, South Africa. *J. Health Popul. Nutr.* 27, 652–659. doi: 10.3329/jhpn.v27i5.3642

AUTHOR CONTRIBUTIONS

OA and AIO initiated the research topic. AIO provided the materials for the study. OA structured the methods and carried out the statistical analysis and wrote the manuscript. OA and ACO carried out the experiment. ACO and AIO proofread and corrected the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Water Research Commission (grant number: K5 2432), South Africa and Medical Research Council, South Africa.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2021.617703/full#supplementary-material>

- Bhattacharjee, S., Bhattacharjee, S., Bal, B., Pal, R., Niyogi, S. K., and Sarkar, K. (2010). Is *Vibrio fluvialis* emerging as a pathogen with epidemic potential in coastal region of eastern India following cyclone Aila? *J. Health Popul. Nutr.* 28, 311–317. doi: 10.3329/jhpn.v28i4.6036
- Binh, M. N., Je, H. L., Ngo, T. C., Seon, Y. C., Nguyen, T. H., Dang, D. A., et al. (2009). Cholera outbreaks caused by an altered *Vibrio cholerae* O1 El tor biotype strain producing classical cholera toxin B in Vietnam in 2007 to 2008. *J. Clin. Microbiol.* 47, 1568–1571. doi: 10.1128/JCM.02040-08
- Bisharat, N. (2002). *Vibrio vulnificus* infection can be avoided. *Isr. Med. Assoc. J.* 4, 631–633.
- Bisharat, N., Cohen, D. I., Harding, R. M., Falush, D., Crook, D. W., Peto, T., et al. (2005). Hybrid *Vibrio vulnificus*. *Emerg. Infect. Dis.* 11, 30–35. doi: 10.3201/eid1101.040440
- Blodgett, R. (2015). BAM Appendix 2: Most Probable Number from Serial Dilutions | FDA [WWW Document]. Available at: <https://www.fda.gov/food/laboratory-methods-food/bam-appendix-2-most-probable-number-serial-dilutions> (Accessed June 23, 2020).
- Brehm, T. T., Berneking, L., Rohde, H., Chistner, M., Schlickewei, C., Sena Martins, M., et al. (2020). Wound infection with *Vibrio harveyi* following a traumatic leg amputation after a motorboat propeller injury in Mallorca, Spain: a case report and review of literature. *BMC Infect. Dis.* 20:104. doi: 10.1186/s12879-020-4789-2
- Cabral, J. P. S. (2010). Water microbiology. Bacterial pathogens and water. *Int. J. Environ. Res. Public Health* 7, 3657–3703. doi: 10.3390/ijerph7103657
- CDC (1999). Outbreak of *Vibrio parahaemolyticus* infection associated with eating raw oysters and clams harvested from long Island sound--Connecticut, New Jersey, and New York, 1998. *MMWR Morb. Mortal. Wkly Rep.* 48, 48–51.
- CDC (2015). Recommendations for the Use of Antibiotics for the Treatment of Cholera [WWW Document]. Available at: <https://www.cdc.gov/cholera/treatment/antibiotic-treatment.html> (Accessed December 16, 2019).
- CDC (2017). Vibriosis | 2017 Case Definition. Available at: <https://wwwn.cdc.gov/nndss/conditions/vibriosis/case-definition/2017/> (Accessed June 21, 2020).
- Ceccarelli, D., Chen, A., Hasan, N. A., Rashed, S. M., Huq, A., and Colwell, R. R. (2015). Non-O1/non-O139 *Vibrio cholerae* carrying multiple virulence factors and *V. cholerae* O1 in the Chesapeake Bay, Maryland. *Appl. Environ. Microbiol.* 81, 1909–1918. doi: 10.1128/AEM.03540-14
- Ceccarelli, D., and Colwell, R. R. (2014). *Vibrio* ecology, pathogenesis, and evolution. *Front. Microbiol.* 5:256. doi: 10.3389/fmicb.2014.00256
- Chakraborty, R., Sinha, S., Mukhopadhyay, A. K., Asakura, M., Yamasaki, S., Bhattacharya, S. K., et al. (2006). Species-specific identification of *Vibrio*

- fluvialis* by PCR targeted to the conserved transcriptional activation and variable membrane tether regions of the *toxR* gene. *J. Med. Microbiol.* 55, 805–808. doi: 10.1099/jmm.0.46395-0
- Chigor, V. N., Sibanda, T., and Okoh, A. I. (2012). Studies on the bacteriological qualities of the Buffalo River and three source water dams along its course in the Eastern Cape Province of South Africa. *Environ. Sci. Pollut. Res. Int.* 20, 4125–4136. doi: 10.1007/s11356-012-1348-4
- Chowdhury, G., Ghosh, S., Pazhani, G. P., Paul, B. K., Maji, D., Mukhopadhyay, A. K., et al. (2013). Isolation and characterization of pandemic and nonpandemic strains of *Vibrio parahaemolyticus* from an outbreak of diarrhea in north 24 Parganas, West Bengal, India. *Foodborne Pathog. Dis.* 10, 338–342. doi: 10.1089/fpd.2012.1340
- Christine, C., Sindiswa, N., and Imelda, H. (2013). An Introduction to South Africa's water sources areas [WWW Document]. Available at: www.journeyofwater.co.za (Accessed January 14, 2020).
- Colvin, C., Muruvu, D., Lindley, D., and Schachtschneider, K. (2016). Water: Facts and Futures Rethinking South Africa's Water Future [WWW Document]. Available at: http://awsassets.wwf.org.za/downloads/wwf009_waterfactsandfutures_report_web_lowres_.pdf (Accessed January 14, 2020).
- Colwell, J. J. (2016). The Effects of Temperature and Salinity on *Vibrio* Species in Breton Sound Estuary. LSU Master's Theses.
- Comins, L. (2012). Deadly bug hits beaches. Available at: https://www.iol.co.za/news/south-africa/kwazulu-natal/deadly-bug-hits-beaches-1227227 (Accessed June 01, 2020).
- Copin, S., Robert-Pillot, A., Malle, P., Quilici, M. L., and Gay, M. (2012). Evaluation of most-probable-number-PCR method with internal amplification control for the counting of total and pathogenic *Vibrio parahaemolyticus* in frozen shrimps. *J. Food Prot.* 75, 150–153. doi: 10.4315/0362-028X.JFP-11-165
- Cottle, E., and Deedat, H. (2002). The cholera outbreak: a 2000–2002 case study of the source of the outbreak in the Madlebe Tribal Authority areas, uThungulu Region, KwaZulu-Natal. Durban: Health Systems Trust.
- de Menezes, F. G. R., da Neves, S. S., Sousa, O. V., Vila-Nova, C. M. V. M., Maggioni, R., Theophilus, G. N. D., et al. (2014). Detecção de genes de virulência em estirpes de *Vibrio cholerae* isolados de estuários no Nordeste do Brasil. *Rev. Inst. Med. Trop. Sao Paulo* 56, 427–432. doi: 10.1590/S0036-46652014000500010
- de Menezes, F. G. R., Rodriguez, M. T. T., de Carvalho, F. C. T., Rebouças, R. H., Costa, R. A., de Sousa, O. V., et al. (2017). Pathogenic *Vibrio* species isolated from estuarine environments (Ceará, Brazil) - antimicrobial resistance and virulence potential profiles. *An. Acad. Bras. Cienc.* 89, 1175–1188. doi: 10.1590/0001-3765201720160191
- De Souza Costa Sobrinho, P., Destro, M. T., Franco, B. D. G. M., and Landgraf, M. (2010). Correlation between environmental factors and prevalence of *Vibrio parahaemolyticus* in oysters harvested in the southern coastal area of Sao Paulo state, Brazil. *Appl. Environ. Microbiol.* 76, 1290–1293. doi: 10.1128/AEM.00861-09
- Decol, L. T., Casarin, L. S., Hessel, C. T., Batista, A. C. F., Allende, A., and Tondo, E. C. (2017). Microbial quality of irrigation water used in leafy green production in Southern Brazil and its relationship with produce safety. *Food Microbiol.* 65, 105–113. doi: 10.1016/j.fm.2017.02.003
- Deen, J., Mengel, M. A., and Clemens, J. D. (2019). Epidemiology of cholera. *Vaccine* 38, A31–A40. doi: 10.1016/j.vaccine.2019.07.078
- Delahoy, M. J., Wodnik, B., McAilley, L., Penakalapati, G., Swarthout, J., Freeman, M. C., et al. (2018). Pathogens transmitted in animal feces in low- and middle-income countries. *Int. J. Hyg. Environ. Health* 221, 661–676. doi: 10.1016/j.ijheh.2018.03.005
- DePaola, A., Nordstrom, J. L., Bowers, J. C., Wells, J. G., and Cook, D. W. (2003). Seasonal abundance of total and pathogenic *Vibrio parahaemolyticus* in Alabama oysters. *Appl. Environ. Microbiol.* 69, 1521–1526. doi: 10.1128/AEM.69.3.1521-1526.2003
- Di, D. Y. W., Lee, A., Jang, J., Han, D., and Hur, H. G. (2017). Season-specific occurrence of potentially pathogenic *Vibrio* spp. on the southern coast of South Korea. *Appl. Environ. Microbiol.* 83, e02680–e02616. doi: 10.1128/AEM.02680-16
- Drake, S. L., Depaola, A., and Jaykus, L. (2007). An overview of *Vibrio vulnificus* and *Vibrio parahaemolyticus*. *Compr. Rev. Food Sci. Food Saf.* 6, 120–144. doi: 10.1111/j.1541-4337.2007.00022.x
- Dufour, A., Bartram, J., Bos, R., and Gannon, V. (2013). Animal waste, water quality and human health. *Water Intell. Online* 12, 1–10. doi: 10.2166/9781780401249
- Dungeni, M., van Der Merwe, R. R., and Momba, M. N. B. (2010). Abundance of pathogenic bacteria and viral indicators in chlorinated effluents produced by four wastewater treatment plants in the Gauteng Province, South Africa. *Water SA* 36, 607–614. doi: 10.4314/wsa.v36i5.61994
- Edokpayi, J. N., Odiyo, J. O., and Durwoju, O. S. (2017). "Impact of wastewater on surface water quality in developing countries: a case study of South Africa" in *Water quality*. ed. H. Tutu (Rijeka, Croatia: InTech), 401–416.
- Elhadi, N. (2013). Occurrence of potentially human pathogenic *Vibrio* species in the coastal water of the Eastern Province of Saudi Arabia. *Res. J. Microbiol.* 8, 1–12. doi: 10.3923/jm.2013.1.12
- Esteves, K., Hervio-Heath, D., Mosser, T., Rodier, C., Tournoud, M.-G., Jumas-Bilak, E., et al. (2015). Rapid proliferation of *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio cholerae* during freshwater flash floods in French Mediterranean Coastal Lagoons. *Appl. Environ. Microbiol.* 81, 7600–7609. doi: 10.1128/AEM.01848-15
- European Commission (2001). Opinion of the scientific committee on veterinary measures relating to public health on *Vibrio vulnificus* and *Vibrio parahaemolyticus* (in raw and undercooked seafood).
- Fatoki, O. S., Muyima, N. Y. O., and Lujiza, N. (2001). Situation analysis of water quality in the Umtata River catchment. *Water SA* 27, 467–473. doi: 10.4314/wsa.v27i4.4959
- Fernández-delgado, A. M., Sanz, V., Giner, S., Contreras, M., and Michelangeli, F. (2015). Prevalence and distribution of *Vibrio* spp. in wild aquatic birds of the Southern Caribbean Sea, Venezuela, 2011–12. *J. Wildl. Dis.* 52, 621–626. doi: 10.7589/2015-06-154
- Finkelstein, R., Edelstein, S., and Mahamid, G. (2002). Fulminant wound infections due to *Vibrio vulnificus*. *Isr. Med. Assoc. J.* 4, 654–655.
- Finkelstein, R. A. (1996). Cholera, *Vibrio cholerae* O1 and O139, and Other Pathogenic *Vibrios*, Medical Microbiology. University of Texas Medical Branch at Galveston.
- Fu, S., Hao, J., Yang, Q., Lan, R., Wang, Y., Ye, S., et al. (2019). Long-distance transmission of pathogenic *Vibrio* species by migratory waterbirds: a potential threat to the public health. *Sci. Rep.* 9:16303. doi: 10.1038/s41598-019-52791-5
- Fukushima, H., and Seki, R. (2004). Ecology of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in brackish environments of the Sada River in Shimane Prefecture, Japan. *FEMS Microbiol. Ecol.* 48, 221–229. doi: 10.1016/j.femsec.2004.01.009
- Glenn Morris, J. Jr. (1994). "Non-O group 1 *Vibrio cholerae* strains not associated with epidemic disease," in *Vibrio Cholerae and Cholera*. eds. I. K. Wachsmuth, P. A. Blake and O. Olsvik (Wiley: American Society of Microbiology), 103–115.
- Goel, A. K., Jain, M., Kumar, P., Kamboj, D. V., and Singh, L. (2010). Virulence profile and clonal relationship among the *Vibrio cholerae* isolates from ground and surface water in a cholera endemic area during rainy season. *Folia Microbiol.* 55, 69–74. doi: 10.1007/s12223-010-0011-z
- Grim, C. J., Hasan, N. A., Taviani, E., Haley, B., Chun, J., Brettin, T. S., et al. (2010). Genome sequence of hybrid *Vibrio cholerae* O1 MJ-1236, B-33, and CIRS101 and comparative genomics with *V. cholerae*. *J. Bacteriol.* 192, 3524–3533. doi: 10.1128/JB.00040-10
- Guardiola-avila, I., Noriega-orozco, L., and Acedo-félix, E. (2015). Presence of the *Hemolysin* gene of *Vibrio mimicus* in fish and seafood products in Sonora, México. *J. Food Res.* 4, 66–76. doi: 10.5539/jfr.v4n1p66
- Herbig, F. J. W., and Meissner, R. (2019). Talking dirty-effluent and sewage irreverence in South Africa: a conservation crime perspective. *Cogent Soc. Sci.* 5:1701359. doi: 10.1080/23311886.2019.1701359
- Igbinosa, E. O., Obi, L. C., and Okoh, A. I. (2009). Occurrence of potentially pathogenic vibrios in final effluents of a wastewater treatment facility in a rural community of the Eastern Cape Province of South Africa. *Res. Microbiol.* 160, 531–537. doi: 10.1016/j.resmic.2009.08.007
- Igbinosa, E. O., and Okoh, A. I. (2010). *Vibrio fluvialis*: an unusual enteric pathogen of increasing public health concern. *Int. J. Environ. Res. Public Health* 7, 3628–3643. doi: 10.3390/ijerph7103628
- Igbinosa, E. O., Obi, L. C., and Okoh, A. I. (2011a). Seasonal abundance and distribution of *Vibrio* species in the treated effluent of wastewater treatment facilities in suburban and urban communities of Eastern Cape Province, South Africa. *J. Microbiol.* 49, 224–232. doi: 10.1007/s12275-011-0227-x
- Igbinosa, E. O., Obi, L. C., Tom, M., and Okoh, A. I. (2011b). Detection of potential risk of wastewater effluents for transmission of antibiotic

- resistance from *Vibrio* species as a reservoir in a peri-urban community in South Africa. *Int. J. Environ. Health Res.* 21, 402–414. doi: 10.1080/09603123.2011.572278
- Jaiani, E., Kokashvili, T., Mitaishvili, N., Elbakidze, T., Janelidze, N., Lashkhi, N., et al. (2013). Microbial water quality of recreational lakes near Tbilisi, Georgia. *J. Water Health* 11, 333–345. doi: 10.2166/wh.2013.057
- Janelidze, N., Jaiani, E., Lashkhi, N., Tskhedviani, A., Kokashvili, T., Gvarishvili, T., et al. (2011). Microbial water quality of the Georgian coastal zone of the Black Sea. *Mar. Pollut. Bull.* 62, 573–580. doi: 10.1016/j.marpolbul.2010.11.027
- Jones, J. L. (2017). “Chapter 11 - *Vibrio*” in *Foodborne Diseases. 3rd Edn.* eds. C. E. R. Dodd, T. Aldsworth, R. A. Stein, D. O. Cliver and H. P. Riemann (Academic Press), 243–252.
- Jones, M. K., and Oliver, J. D. (2009). *Vibrio vulnificus*: disease and pathogenesis. *Infect. Immun.* 77, 1723–1733. doi: 10.1128/IAI.01046-08
- Jones, S., and Summer-Brason, B. (1998). Incidence and detection of pathogenic “*Vibrio*” sp. in a northern New England estuary, USA. *J. Shellfish Res.* 17, 1665–1669.
- Jordaan, K., Comeau, A. M., Khasa, D. P., and Bezuidenhout, C. C. (2019). An integrated insight into the response of bacterial communities to anthropogenic contaminants in a river: a case study of the Wonderfontein spruit catchment area, South Africa. *PLoS One* 14:e0216758. doi: 10.1371/journal.pone.0216758
- Kanungo, S., Sur, D., Ali, M., You, Y. A., Pal, D., Manna, B., et al. (2012). Clinical, epidemiological, and spatial characteristics of *Vibrio parahaemolyticus* diarrhea and cholera in the urban slums of Kolkata, India. *BMC Public Health* 12:830. doi: 10.1186/1471-2458-12-830
- Kaspar, C. W., and Tamplin, M. L. (1993). Effects of temperature and salinity on the survival of *Vibrio vulnificus* in seawater and shellfish. *Appl. Environ. Microbiol.* 59, 2425–2429. doi: 10.1128/AEM.59.8.2425-2429.1993
- Kazi, A., Mumtaz, A., Nazir, R., Ismail, T., and Akbar, S. (2005). Determination of clobazam by visible spectrophotometry in pure and pharmaceutical preparations. *Proc. Pak. Acad. Sci.* 42:133.
- Kelly, M. T. (1982). Effect of temperature and salinity on *Vibrio* (Beneckea) *vulnificus* occurrence in a Gulf Coast environment. *Appl. Environ. Microbiol.* 44:820. doi: 10.1128/AEM.44.4.820-824.1982
- Kirchberger, P. C., Orata, F. D., Barlow, E. J., Kauffman, K. M., Case, R. J., Polz, M. F., et al. (2016). A small number of phylogenetically distinct clonal complexes dominate a coastal *Vibrio cholerae* population. *Appl. Environ. Microbiol.* 82, 5576–5586. doi: 10.1128/AEM.01177-16
- Klontz, K. C., Williams, L., Baldy, L. M., and Campos, M. (1993). Raw oyster-associated *Vibrio* infections: linking epidemiologic data with laboratory testing of oysters obtained from a retail outlet. *J. Food Prot.* 56, 977–979. doi: 10.4315/0362-028X-56.11.977
- Kokashvili, T., Whitehouse, C. A., Tskhedviani, A., Grim, C. J., Elbakidze, T., Mitaishvili, N., et al. (2015). Occurrence and diversity of clinically important *Vibrio* species in the aquatic environment of Georgia. *Front. Public Health* 3:232. doi: 10.3389/fpubh.2015.00232
- Koppryo, G. A., Neogi, S. B., Rashid, H., Alonso, C., Yamasaki, S., Koch, B. P., et al. (2020). *Vibrio* and bacterial communities across a pollution gradient in the bay of Bengal: unraveling their biogeochemical drivers. *Front. Microbiol.* 11:594. doi: 10.3389/fmicb.2020.00594
- Kothary, M. H., Lowman, H., McCardell, B. A., and Tall, B. D. (2003). Purification and characterization of enterotoxigenic E1 Tor-like hemolysin produced by *Vibrio fluvialis*. *Infect. Immun.* 71, 3213–3220. doi: 10.1128/IAI.71.6.3213-3220.2003
- Kwok, A. Y. C., Wilson, J. T., Coulthart, M., Ng, L. K., Mutharia, L., and Chow, A. W. (2002). Phylogenetic study and identification of human pathogenic *Vibrio* species based on partial hsp60 gene sequences. *Can. J. Microbiol.* 48, 903–910. doi: 10.1139/w02-089
- Lei, S., Miyoshi, S., Kewei, B., Nakamura, M., Hiura, M., Tomochika, K., et al. (2000). Presence of *Hemolysin* genes (vmh, tdh and hlx) in isolates of *Vibrio mimicus* determined by polymerase chain reaction. *J. Health Sci.* 46, 63–65. doi: 10.1248/jhs.46.63
- Letchumanan, V., Yin, W., Lee, L., and Chan, K. (2015). Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from retail shrimps in Malaysia. *Front. Microbiol.* 6:33. doi: 10.3389/fmicb.2015.00033
- Levinson, W., and Jawetz, E. (1996). *Medical Microbiology and Immunology* 4th Edition by Levinson, Warren E., Jawetz, Ernest (1996) Paperback: Amazon.com: Books [WWW Document]. Available at: <https://www.amazon.com/>
- Medical-Microbiology-Immunology-Levinson-Paperback/dp/B011DBKQSO (Accessed June 1, 2020).
- Li, D., Jiang, X., Wang, J., Wang, K., and Zheng, B. (2017). Effect of sewage and industrial effluents on bacterial and archaeal communities of creek sediments in the Taihu Basin. *Water* 9:373. doi: 10.3390/w9060373
- Liu, B., Liu, H., Pan, Y., Xie, J., and Zhao, Y. (2016). Comparison of the effects of environmental parameters on the growth variability of *Vibrio parahaemolyticus* coupled with strain sources and genotypes analyses. *Front. Microbiol.* 7:994. doi: 10.3389/fmicb.2016.00994
- Liu, H., Whitehouse, C. A., and Li, B. (2018). Presence and persistence of *Salmonella* in water: the impact on microbial quality of water and food safety. *Front. Public Health* 6:159. doi: 10.3389/fpubh.2018.00159
- Machado, A., and Bordalo, A. A. (2016). Detection and quantification of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* in coastal waters of Guinea-Bissau (West Africa). *EcoHealth* 13, 339–349. doi: 10.1007/s10393-016-1104-1
- MacLennan, S. (2019). Fish River sewage and Makhanda: DWS answers questions [WWW Document]. Available at: <https://www.grocotts.co.za/2019/06/25/dws-takes-muni-to-court-over-sewage/> (Accessed May 22, 2020).
- Madoroba, E., and Momba, M. N. (2010). Prevalence of *Vibrio cholerae* in rivers of Mpumalanga province, South Africa as revealed by polyphasic characterization. *Afr. J. Biotechnol.* 9, 7295–7301.
- Mahoney, J. C., Gerding, M. J., Jones, S. H., and Whistler, C. A. (2010). Comparison of the pathogenic potentials of environmental and clinical *Vibrio parahaemolyticus* strains indicates a role for temperature regulation in virulence. *Appl. Environ. Microbiol.* 76, 7459–7465. doi: 10.1128/AEM.01450-10
- Maje, M. D., Kaptchouang Tchatchouang, C. D., Manganyi, M. C., Fri, J., and Ateba, C. N. (2020). Characterisation of *Vibrio* species from surface and drinking water sources and assessment of biocontrol potentials of their bacteriophages. *Int. J. Microbiol.* 2020:8863370. doi: 10.1155/2020/8863370
- Marie, V., and Lin, J. (2018). Microbial indicators and environmental relationships in the Umhlangane River, Durban, South Africa. *Open Life Sci.* 13, 385–395. doi: 10.1515/biol-2018-0047
- Maugeri, T. L., Carbone, M., Fera, M. T., and Gugliandolo, C. (2006). Detection and differentiation of *Vibrio vulnificus* in seawater and plankton of a coastal zone of the Mediterranean Sea. *Res. Microbiol.* 157, 194–200. doi: 10.1016/j.resmic.2005.06.007
- McAllister, T. A., and Topp, E. (2012). Role of livestock in microbiological contamination of water: commonly the blame, but not always the source. *Anim. Front.* 2, 17–27. doi: 10.2527/af.2012-0039
- Mema, V. (2008). Impact of Poorly Maintained Wastewater and Sewage Treatment Plants: Lessons From South Africa.
- Mezgebe, K., Gebrekidan, A., Hadera, A., and Weldegebriel, Y. (2015). Assessment of physico-chemical parameters of Tsaeda Agam River in Mekelle City, Tigray, Ethiopia. *Bull. Chem. Soc. Ethiop.* 29, 377–385. doi: 10.4314/bcse.v29i3.5
- Momba, M., and Azab El-Liethy, M. (2017). “*Vibrio Cholerae* And Cholera Biotypes,” in *Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project; Part 3: Specific Excreted Pathogens: Environmental and Epidemiology Aspects - Section 2: Bacteria.* eds. A. Pruden, N. Ashbolt and J. Miller). eds. J. B. Rose and B. Jiménez-Cisneros. MI, UN: Michigan State University E. Lansing. Available at: <http://www.waterpathogens.org/book/Vibrio> (Accessed May 22, 2020).
- Momba, M. N. B., Malakate, V. K., and Theron, J. (2006). Abundance of pathogenic *Escherichia coli*, *Salmonella typhimurium* and *Vibrio cholerae* in Nkonkobe drinking water sources. *J. Water Health* 4, 289–296. doi: 10.2166/wh.2006.011
- Mookerjee, S., Batabyal, P., Sarkar, M. H., and Palit, A. (2015). Seasonal prevalence of Enteropathogenic *Vibrio* and their phages in the riverine estuarine ecosystem of South Bengal. *PLoS One* 10:e0137338. doi: 10.1371/journal.pone.0137338
- Morris, J. (2019). Minor *Vibrio* and *Vibrio*-like species associated with human disease - UpToDate [WWW Document]. Available at: https://www.uptodate.com/contents/minor-vibrio-and-vibrio-like-species-associated-with-human-disease?search=minor-Vibrio-and-Vibrio-like-species-associated-with-human-disease&source=search_result&selectedTitle=1~150&usage_type=default&display_rank=1 (Accessed June 1, 2020).

- Mudzanani, L., Ratsaka-Mathokoa, M., Mahlasela, L., Netshidzivhanii, P., and Mugero, C. (2003). *Cholera*. South African Health Review.
- Munshi, S. K., Roy, J., and Noor, R. (2019). Microbiological investigation and determination of the antimicrobial potential of cow dung samples. *S. J. Microbiol.* 8, 34–37. doi: 10.3329/sjm.v8i1.42437
- Mustapha, S., Mustapha, E. M., and Nozha, C. (2013). *Vibrio alginolyticus*: an emerging pathogen of foodborne diseases. *Maejo Int. J. Sci. Technol.* 2, 302–309.
- Nevondo, T., and Cloete, T. (2001). The global cholera pandemic. Science in Africa [WWW Document]. Available at: <http://www.scienceinfrica.com/global-cholera-pandemic> (Accessed June 1, 2020).
- Nilsson, W. B., Paranjpye, R. N., Hamel, O. S., Hard, C., and Strom, M. S. (2019). *Vibrio parahaemolyticus* risk assessment in the Pacific Northwest: It's not what's in the water. *FEMS Microbiol. Ecol.* 95:fiz027. doi: 10.1093/femsec/fiz027
- Nongogo, V., and Okoh, A. I. (2014). Occurrence of vibrio pathotypes in the final effluents of five wastewater treatment plants in Amathole and Chris Hani District municipalities in South Africa. *Int. J. Environ. Res. Public Health* 11, 7755–7766. doi: 10.3390/ijerph110807755
- Novotny, L., Dvorska, L., Lorencova, A., Beran, V., and Pavlik, V. (2004). Fish: a potential source of bacterial pathogens for human beings. *Vet. Med.* 49, 343–358. doi: 10.17221/5715-VETMED
- Ntema, V. M., Potgieter, N., and Barnard, T. G. (2010). Detection of *Vibrio cholerae* and *Vibrio parahaemolyticus* by molecular and culture based methods from source water to household container-stored water at the point-of-use in South African rural communities. *Water Sci. Technol.* 61, 3091–3102. doi: 10.2166/wst.2010.222
- Ntema, V. M., Potgieter, N., Van Blerk, G. N., and Barnard, T. G. (2014). Investigating the occurrence and survival of *Vibrio cholerae* in selected surface water sources in the KwaZulu-Natal province of South Africa Report to the Water Research Commission.
- Okeyo, A. N., Nontongana, N., Fadare, T. O., and Okoh, A. I. (2018). *Vibrio* species in wastewater final effluents and receiving watershed in South Africa: implications for public health. *Int. J. Environ. Res. Public Health.* 1266:15. doi: 10.3390/ijerph15061266
- Okoh, A. I., Sibanda, T., Nongogo, V., Adefisoye, M., Olayemi, O. O., and Nontongana, N. (2015). Prevalence and characterisation of non-cholerae *Vibrio* spp. in final effluents of wastewater treatment facilities in two districts of the Eastern Cape Province of South Africa: implications for public health. *Environ. Sci. Pollut. Res.* 22, 2008–2017. doi: 10.1007/s11356-014-3461-z
- Oliver, J. D., and Bockian, R. (1995). In vivo resuscitation, and virulence towards mice, of viable but nonculturable cells of *Vibrio vulnificus*. *Appl. Environ. Microbiol.* 61, 2620–2623. doi: 10.1128/AEM.61.7.2620-2623.1995
- Oliver, J. D., Nilsson, L., and Kjelleberg, S. (1991). Formation of nonculturable *Vibrio vulnificus* cells and its relationship to the starvation state. *Appl. Environ. Microbiol.* 57:2640. doi: 10.1128/AEM.57.9.2640-2644.1991
- Osorio, C. R., and Klose, K. E. (2000). A region of the transmembrane regulatory protein ToxR that tethers the transcriptional activation domain to the cytoplasmic membrane displays wide divergence among vibrio species. *J. Bacteriol.* 182, 526–528. doi: 10.1128/JB.182.2.526-528.2000
- Osunla, C. A., and Okoh, A. I. (2017). *Vibrio* pathogens: a public health concern in rural water resources in sub-Saharan Africa. *Int. J. Environ. Res. Public Health* 14, 1–27. doi: 10.3390/ijerph14101188
- Paranjpye, R. N., Nilsson, W. B., Liermann, M., Hilborn, E. D., George, B. J., Li, Q., et al. (2015). Environmental influences on the seasonal distribution of *Vibrio parahaemolyticus* in the Pacific Northwest of the USA. *FEMS Microbiol. Ecol.* 91:fiv121. doi: 10.1093/femsec/fiv121
- Payne, S. M., Mey, A. R., and Wyckoff, E. E. (2016). *Vibrio* iron transport: evolutionary adaptation to life in multiple environments. *Microbiol. Mol. Biol. Rev.* 80, 69–90. doi: 10.1128/MMBR.00046-15
- Penakalapati, G., Swarthout, J., Delahoy, M. J., Mcaliley, L., Wodnik, B., Levy, K., et al. (2017). Exposure to animal feces and human health: a systematic review and proposed research priorities. *Environ. Sci. Technol.* 51, 11537–11552. doi: 10.1021/acs.est.7b02811
- Prasanthan, V., Udayakumar, P., and Ouseph, P. P. (2011). Influence of abiotic environmental factors on the abundance and distribution of *Vibrio* species in coastal waters of Kerala, India. *Indian J. Geo-Mar. Sci.* 40, 587–592.
- Rabea, E., Bahia, B., Youssef, M., Ahlam, F., Ghita El, M., Bouchra, O., et al. (2019). Microbiological and physicochemical characterization of hospital effluents before and after treatment with two types of sawdust. *J. Chem.* 2019, 1–10. doi: 10.1155/2019/3275101
- Ramamurthy, T., Albert, M. J., Huq, A., Colwell, R. R., Takeda, Y., Takeda, T., et al. (1994). *Vibrio mimicus* with multiple toxin types isolated from human and environmental sources. *J. Med. Microbiol.* 40, 194–196. doi: 10.1099/00222615-40-3-194
- Ramos, R. J., Miotto, L. A., Miotto, M., Silveira Junior, N., Cirolini, A., da Silva, H. S., et al. (2014). Occurrence of potentially pathogenic *Vibrio* in oysters (*Crassostrea gigas*) and waters from bivalve mollusk cultivations in the South Bay of Santa Catarina. *Rev. Soc. Bras. Med. Trop.* 47, 327–333. doi: 10.1590/0037-8682-0069-2014
- Randa, M. A., Polz, M. F., and Lim, E. (2004). Effects of temperature and salinity on *Vibrio vulnificus* population dynamics as assessed by quantitative PCR. *Appl. Environ. Microbiol.* 70, 5469–5476. doi: 10.1128/AEM.70.9.5469-5476.2004
- Rees, G., Pond, K., and Kay, D. (2010). Safe management of shellfish and harvest waters. *Water Intell. Online* 12, 1–10. doi: 10.2166/9781780405797
- Reimer, A. R., Domselaar, G. Van Stroika, S., Walker, M., Kent, H., Tarr, C., et al. (2011). Comparative genomics of *Vibrio cholerae* from Haiti, Asia, and Africa. *Emerg. Infect. Dis.* 17, 2113–2121. doi: 10.3201/eid1711.110794
- Rippey, S. R. (1994). Infectious diseases associated with molluscan shellfish consumption. *Clin. Microbiol. Rev.* 7, 419–425. doi: 10.1128/CMR.7.4.419
- Romalde, J. L., Diéguez, A. L., Lasa, A., and Balboa, S. (2014). New *Vibrio* species associated to molluscan microbiota: a review. *Front. Microbiol.* 4:413. doi: 10.3389/fmicb.2013.00413
- Safa, A., Nair, G. B., and Kong, R. Y. C. (2009). Evolution of new variants of *Vibrio cholerae* O1. *Trends Microbiol.* 18, 46–54. doi: 10.1016/j.tim.2009.10.003
- Sarkar, B. L., Nair, G. B., Banerjee, A. K., and Pal, S. C. (1985). Seasonal distribution of *Vibrio parahaemolyticus* in freshwater environs and in association with freshwater fishes in Calcutta. *Appl. Environ. Microbiol.* 49, 132–136. doi: 10.1128/AEM.49.1.132-136.1985
- Shaheen, A., Saeed Baig, H., Shahana, A., and Kazmi, U. (2016). Microbial flora isolated from polluted and non-polluted coastal waters of Karachi. *Pak. J. Bot.* 48, 1703–1708.
- Sharma, A., and Chaturvedi, A. N. (2007). Population dynamics of *Vibrio* species in the river Narmada at Jabalpur. *J. Environ. Biol.* 28, 747–751.
- Sharma, C., Thungapathra, M., Ghosh, A. K., Mukhopadhyay, A. K., Basu, A., Mitra, R., et al. (1998). Molecular analysis of non-O1, non-O139 *Vibrio cholerae* associated with an unusual upsurge in the incidence of cholera-like disease in Calcutta, India. *J. Clin. Microbiol.* 36, 756–763.
- Shinoda, S., Nakagawa, T., Shi, L., Bi, K., Kanoh, Y., Tomochika, K., et al. (2004). Distribution of virulence-associated genes in *Vibrio mimicus* isolates from clinical and environmental origins. *Microbiol. Immunol.* 48, 547–551. doi: 10.1111/j.1348-0421.2004.tb03551.x
- Skall, H. F., and Olesen, N. J. (2011). Treatment of wastewater from fish slaughterhouses. Evaluation and recommendations for hygienisation methods, National Veterinary Institute, Technical University of Denmark.
- Sultan, Z., Mizuno, T., Sakurai, A., Takata, N., Okamoto, K., and Miyoshi, S.-I. (2007). Growth phase dependant activation of the precursor of *Vibrio mimicus* Hemolysin (Pro-VMH). *J. Health Sci.* 53, 430–434. doi: 10.1248/jhs.53.430
- Takemura, A. F., Chien, D. M., and Polz, M. F. (2014). Associations and dynamics of vibronaceae in the environment, from the genus to the population level. *Front. Microbiol.* 5:38. doi: 10.3389/fmicb.2014.00038
- Tantillo, G. M., Fontanarosa, M., Pinto, A. Di, and Musti, M. (2004). Updated perspectives on emerging vibrios associated with human infections. *Lett. Appl. Microbiol.* 39, 117–126. doi: 10.1111/j.1472-765X.2004.01568.x
- Tarr, C. L., Patel, J. S., Puh, N. D., Sowers, E. G., Bopp, C. A., and Strockbine, N. A. (2007). Identification of *Vibrio* isolates by a multiplex PCR assay and rpoB sequence determination. *J. Clin. Microbiol.* 45, 134–140. doi: 10.1128/JCM.01544-06
- Topalcengiz, Z., Strawn, L. K., and Danyluk, M. D. (2017). Microbial quality of agricultural water in Central Florida. *PLoS One* 12:e0174889. doi: 10.1371/journal.pone.0174889
- Turner, J. W. (1997). Environmental factors and reservoir shifts contribute to the seasonality of pathogenic.
- Urquhart, E. A., Jones, S. H., Yu, J. W., Schuster, B. M., Marcinkiewicz, A. L., Whistler, C. A., et al. (2016). Environmental conditions associated with elevated *Vibrio parahaemolyticus* concentrations in Great Bay Estuary, New Hampshire. *PLoS One* 11:e0155018. doi: 10.1371/journal.pone.0155018

- USEPA (2001). Source Water Protection Practices Bulletin Managing Septic Systems to Prevent Contamination of Drinking Water. United States Environ. Prot. Agency i, July.
- Venkateswaran, K., Kiiyukia, C., Takaki, M., Nakano, H., Matsuda, H., Kawakami, H., et al. (1989). Characterization of toxigenic vibrios isolated from the freshwater environment of Hiroshima, Japan. *Appl. Environ. Microbiol.* 55, 2613–2618. doi: 10.1128/AEM.55.10.2613-2618.1989
- Vinothkumar, K., Kushwaha, A., and Ramamurthy, T. (2013). Triplex PCR assay for the rapid identification of 3 major *Vibrio* species, *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio fluvialis*. *Diagn. Microbiol. Infect. Dis.* 76, 526–528. doi: 10.1016/j.diagmicrobio.2013.04.005
- Watkins, W. D., and Cabelli, V. J. (1985). Effect of Fecal pollution on *Vibrio parahaemolyticus* densities in an estuarine environment. *Appl. Environ. Microbiol.* 49, 1307–1313. doi: 10.1128/AEM.49.5.1307-1313.1985
- Wei, S., Zhao, H., Xian, Y., Hussain, M. A., and Wu, X. (2014). Multiplex PCR assays for the detection of *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio cholerae* with an internal amplification control. *Diagn. Microbiol. Infect. Dis.* 79, 115–118. doi: 10.1016/j.diagmicrobio.2014.03.012
- Wong, R. S. Y., and Chow, A. W. (2002). Identification of enteric pathogens by heat shock protein 60 kDa (HSP60) gene sequences. *FEMS Microbiol. Lett.* 206, 107–113. doi: 10.1111/j.1574-6968.2002.tb10994.x
- Zhou, S., Hou, Z., Li, N., and Qin, Q. (2007). Development of a SYBR green I real-time PCR for quantitative detection of *Vibrio alginolyticus* in seawater and seafood. *J. Appl. Microbiol.* 103, 1897–1906. doi: 10.1111/j.1365-2672.2007.03420.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Abioye, Osunla and Okoh. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.