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Surveillance of *Vibrio cholerae* serogroups (O1 and O139) from surface and ground water sources in the Vhembe district, Limpopo province, South Africa

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Introduction: *Vibrio cholera* is increasingly emerging as a significant public health concern in developing countries. Choleraenic *Vibrio cholerae* O1 and O139 has reported to cause devastating disease and economic burdens in developing countries. In rural areas of the Vhembe district, most rivers and several communal boreholes are polluted as a result of sanitation issues around these water sources. The aim of this study was to determine the presence of choleraenic *V. cholerae* O1 and O139 in rivers and communal boreholes.

Methods: The analysis of physicochemical parameters and molecular techniques was used to establish the adaptation and detect the serogroups of *V. cholerae* in the water samples.

Results and Discussion: The results reported that electrical conductivities (EC) ranged between 18.78 and 154 $\mu\text{S}/\text{cm}$, with rivers such as Madandze and Mvudi recording $>80 \mu\text{S}/\text{cm}$, and those of the communal boreholes were ranged between 23.4 and 295 $\mu\text{S}/\text{cm}$, which were above the acceptable South African water quality guidelines of 0–70 $\mu\text{S}/\text{cm}$ for rivers and communal boreholes. The results further revealed that most of the rivers detected positive for *V. cholerae*, except for the Mukhase river; the downstream points of Livuvhu and Nwedi rivers and the upstream point of the Nzhelele river; and several of the communal boreholes (Mak B1, B2, Kwe B3, 4, and 6) also tested positive for the presence of *V. cholerae*. The toxigenic *Vibrios* was also reported in Mutshundudi, Tshinane rivers, the upstream of Dzindi, Madanzhe, Nwedi, and the downstream of Sambandou rivers, as well as Mak B1, B2, and Kwe3 of communal boreholes. The serogroup O1 was detected on the Mutshundudi and Tshinane rivers, while serogroup O139 was detected upstream of the Dzindi, Madanzhe, Mutshundudi, and Tshinane rivers. There was also detection of the O1 serogroup in the communal boreholes (Mak B1 and Kwe B3), while O139 was only detected in one communal borehole (Mak B2). The development of robust policies,

including an integrated water and sanitation safety surveillance web tool for monitoring water resources and public health protection, is required to make sure that drinking water in rural communities is safe for consumption.

KEYWORDS

serotype group, *Vibrio cholerae* O1, surface water, communal borehole, water quality, waterborne transmission

1 Introduction

Access to safe, clean water and sanitation is a core human right, and it will also drive the achievement of sustainable development goals (SDGs), particularly Agendas 3 and 6 (Sikder et al., 2023; United Nations, 2024). According to the World Health Organisation (WHO), the common definition of safe drinking water and improved sanitation is access to piped water, protected wells, springs, rainwater, packaged water, boreholes, and sanitation facilities capable of effectively managing human excreta and urine (Bain et al., 2018).

The bacteria that cause cholera disease is known as *V. cholerae*, and it's transmitted either by food or water that is contaminated by this microorganism (Harris et al., 2012). *V. cholerae* has continued to ravage millions across the globe, especially in underdeveloped and developing countries such as South Africa and other Sub-Saharan, Asian, and Caribbean countries. This is because they have lagged in terms of inadequate access to safe, clean water and inadequate sanitation facilities, while these have been reduced or eradicated in developed countries (USA, United Kingdom, Canada, among other countries) with the development of an adequate safe water and sanitation infrastructure (Ali et al., 2015). Ozochi et al. (2024) further opined that the recent cholera cases around the world suggested that the outbreaks are due to *ctx*-positive *V. cholerae* O1. Currently, most of the pandemics or cases are not known, even with the World Health Organisation (WHO), which maintains a repository of cases or mortality through their weekly database, and they record less than 10% of cases due to inadequate reporting, especially in poor countries (WHO, 2014). According to Masuet et al. (2011) and Griffith et al. (2006), inadequate reporting of *V. cholerae* across the globe is a result of economic challenges, lack of political and social goodwill, inadequate capacity of both laboratories and epidemiological surveillance systems, and poor patient records. The outbreaks also arise from floods, conflicts, or extreme weather conditions that have triggered faecal contamination of water sources (Almagro-Moreno and Taylor, 2013).

There are approximately 200 serogroups of *Vibrio cholera* that are known, of which O1 and O139 are known for pathogenic symptoms and serious diarrhoea in humans (Almagro-Moreno and Taylor, 2013; Sharma et al., 2021). According to WHO (2005), choleraenic *V. cholerae* O1 and O139 are the only causative agents of cholera, a waterborne and foodborne disease with epidemic and pandemic potential. The most significant symptoms are watery diarrhoea, discomfort of the abdomen, and anorexia, which cause mild to severe gastrointestinal illness, dehydration, and death (WHO, 2005). The two serogroups are known for producing cholera toxins (CT) and toxin-coregulated pilus (TCP). There are also two non-O1 and non-O139 serogroups that are also associated with mild gastroenteritis and bloody diarrhoea to some extent and do not cause cholera (Yadava, Jain,

and Goel, 2013; Bhandari, 2023). The two toxins (CT and TCP) produced by choleraenic serogroups are major triggers of watery diarrhoea and colonisation factors in humans (Arteaga et al., 2020).

Kaper, Morris, and Levine (1995) further stated that the O1 serogroup is further divided into three serotypes, mainly Inaba, Ogawa, and Hikojima, which are further divided into two biotypes, which are classical and El Tor. According to Marin et al. (2013), the O1 serogroup is widespread across the globe, while O139 is only restricted to the Asian continent. Mondiale de la Santé and the World Health Organisation (WHO) (2017) also reported that El Tor survives longer in environments that are linked to mild cases, which are shredded in high numbers in the faeces.

Most of the rivers and communal boreholes in rural areas such as the Vhembe district of South Africa and other developing countries are the immediate sources of water for domestic, agricultural, and other activities due to inadequate access to clean water and sanitation, which are not or are partially treated by dysfunctional treatment facilities (Potgieter et al., 2020). The irony is that most of the surface and ground water sources are highly polluted, triggering contamination and cases of *V. cholerae* in these areas. Potgieter et al. (2020) further stated that to reverse pollution challenges, there is a need for effective, robust, and continuous surveillance of bacterial, microbiological, and physiochemical parameters to establish any possible risks that are associated with pollution of a given water source for the rural populace, leading to *V. cholerae* cases or epidemics. Given the extent of potential pathogenic cases of the O1 and O139 serogroups in rural areas of Vhembe district, there is a need for continuous surveillance, rapid detection, and identification because the devastation can be catastrophic, as reported by Keddy et al. (2013). This will not only revert the community's ill-health issues but also assist in developing mitigation measures in terms of an integrated water sanitation and hygiene system (Keddy et al., 2013; Tasnia, 2023). Therefore, the main aim of this study was to establish the presence of choleraenic serogroups O1 and O139 in rivers and communal boreholes in the Vhembe district of Limpopo Province.

2 Methods and materials

2.1 Study area description

This District Municipality of Vhembe (Figure 1) is found in the province of Limpopo, further north of South Africa, and occupies a landmass of approximately twenty thousand square kilometres, according to Vhembe (2013). This area is blessed with fertile soil, and agricultural activities are taking place across the region. According to Kachienga et al. (2024), this area has both seasonal

and permanent rivers. There are a few existing communal boreholes that are still found in some villages that lack access to municipal water supply. Permanent rivers ($n = 11$) and communal boreholes ($n = 12$) that were sampled for this study are indicated by the red dots in Figure 1.

2.2 Collection of water samples

Water samples were collected between the months of April and July 2024 in 2-L sterile plastic bottles using aseptic techniques from rivers at specific sites as described by Kachienga et al. (2024) and communal boreholes. The collection was done once off, and the samples were in duplicates from both river (down and downstream) and communal boreholes. The samples were transported to the laboratory at the University of Venda in a cooler box containing ice and analysed upon arrival (Rice et al., 2012).

2.3 Physicochemical analysis of river and communal borehole samples

Various parameters, such as total dissolved solids (TDS), temperature, electrical conductivity (EC), and pH, were determined on site using a pH probe (Hach Intellical™ PHC101). The dissolved oxygen (DO) was analysed using a DO probe (Hach LDO® Model 2). In addition, a Lovibond TB 211 turbidimeter (Lovibond, IR, Germany) was used to analyse turbidity on site.

2.4 Culturing/enrichment of isolated *Vibrio cholerae* colonies

The filtration of the samples was done according to the protocol of the National Center for Infectious Diseases, 1994; Momtaz et al. (2013), where a 47 mm cellulose acetate filter with a 0.45 μm pore size was used to filter the samples. The enrichment of filtrate that was

trapped in the filter paper after sample filtration was done in 100 mL of sterile alkaline peptone water (NutriSelect® Plus, Sigma Aldrich, SA); thereafter, the incubation was done at 37°C for one full day.

The enriched samples (10 μL) were transferred into the sterile thiosulphate citrate bile salt (TCBS) (Mass Group Ltd. Reinfeld, Germany) agar plates and then incubated for 24 h at 37°C. The analysis was done in triplicate for each sample. The presumptive positive *V. cholerae* and their colonies forming units were determined according to Kachienga et al. (2024). The preservation of the presumptive *Vibrio* colonies was done according to Huq et al. (2012), and we further tested using biochemical tests for the confirmation of presumptive positive *V. cholerae*. The presumptive *Vibrio* colonies obtained were further used to extract the genomic DNA, which was used for molecular analysis for confirmation of toxigenic and serogroups of cholerae *V. cholerae*.

2.5 API 20E technique (biochemical test) for analysing of the isolates

The presumptive colonies were tested with the API 20E system, and the interpretation of the isolates was based on the API 20E analytical profile index (Version 5, Biomerieux Industries, France). This technique was used to identify the presumptive positive *V. cholerae* obtained from other closely related Vibrionaceae species that might be present.

2.6 DNA extraction and PCR conditions

DNA extraction from samples was carried out using the ZymoBIOMICSTM DNA Miniprep Kit (Zymo Research, CA, USA) per the protocol of the manufacturer from the purified preserved presumptive *Vibrio* colonies. The NanoDrop™ 2000 spectrophotometer (Thermo Scientific, Johannesburg, South Africa) was used to quantify the extracted DNA. The PCR reactions were performed in a total volume of 25 μL , as previously reported (Kachienga et al., 2024). The PCR technique was necessary to confirm the presence of *V. cholerae*, toxigenic *V. cholerae*, and serogroups of cholerae *V. cholerae* in the samples using specific primers reported in Table 1. Amplification reactions were done in the following order: heat denaturation at 94°C for 2 min, followed by 35 cycles of heat denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and DNA extension at 72°C for 1 min (Momtaz et al., 2013) using a qPCR Eppendorf Master Cycler 5330 (Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany). The specific primers used included *OmpW*, *ctxA*, and *O1rfb/O139rfb* for detecting *V. cholerae*, toxigenic *V. cholerae*, and serogroups of cholerae *V. cholerae* (Nandi et al., 2000; Huang et al., 2009; Mehrabadi et al., 2012) (Table 1).

2.7 Statistical analysis

The correlation of physicochemical parameters based on different sampling sites (upstream and downstream rivers, communal boreholes) through principal component analysis (PCoA) was

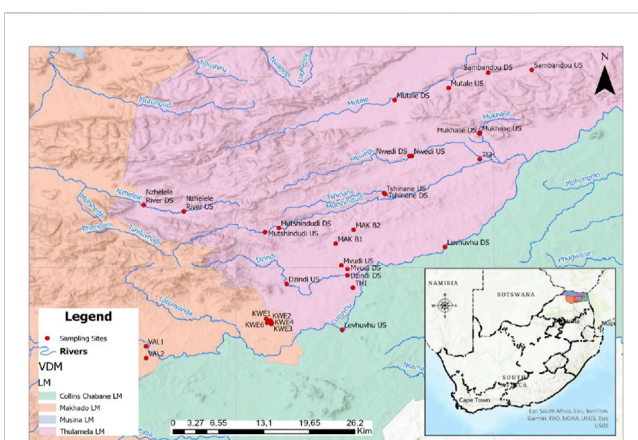


FIGURE 1
Map showing names of the rivers and communal boreholes (red dots) that were sampled in Vhembe District (developed by the department of environmental science, University of Venda).

TABLE 1 List of primers used for the detection of *Vibrio cholerae*.

Primer	Nucleotide sequences 5'-3'	Length (bp)	References
<i>OmpW</i>	F-CACCAAGAAGGTGACTTTATTGTG R-GAACTTATAACCAACCCCGG	588	Nandi et al. (2000)
<i>ctxA</i>	F-GGTCTTATGCCAGAGGACAG R-GTTGGGTGCAGTGGCTATAAC	219	Mehrabadi et al. (2012)
<i>O1rfb</i>	F- CCAGATTGTAAAGCAGGATGGA R- GGTCATCTGTAAGTACAAC	203	Huang et al. (2009)
<i>O139rfb</i>	F- CATAACCAACGCCCTTATCCATT R- GCATGACTGGCATCCCAAAAT	160	Huang et al. (2009)

done using ClustVis (Metsalu and Vilo, 2015). The clustering together of these parameters vis a vis their sampling sites based on the geographical areas and the surrounding activities.

3 Results and discussion

3.1 Activities around the rivers of study

The activities observed around these water sampling points were agricultural (subsistence and commercial farming), brickmaking, laundry, car washing, and open defecation by community members, amongst others. Various activities taking place around the water sources and occasional flooding trigger the presence of pathogenic microbes through runoff into the water sources. Yeung and Thorsen (2016) further reported that most of the urinal infections are also spread during recreational activities in and around rivers and communal boreholes.

3.2 Physicochemical parameters of the rivers

Polluted water is associated with a heavy burden on the population's overall health, and this has negatively impacted the life expectancy in developing countries such as South Africa (WHO, 2002). Cases of *V. cholera* have become a burden in poorer communities, especially in sub-Saharan countries where there is inadequate safe, clean water and sanitation infrastructure (Khouadja et al., 2014). According to Huq et al. (2012), assessment of vital parameters (temperature, turbidity, TDS, pH, DO, and EC) is necessary to establish the survival and adaptation of pathogenic and serogroups O1 and 139 and non-O1/O139. The results of the above parameters are captured in Table 2.

The pH counts of the rivers ranged from 7.03 to 8.21 for rivers and 6.88 to 8.21 for communal boreholes. They were both within the acceptable South African water quality guidelines of 6.5–8.5 for aquatic environments (rivers) and 6.0–9.0 for domestic usage (communal bore holes) (Department of Water Affairs and Forestry, 1996a). A pH between 7.5 and 9.0 normally influences the survival or adaptability of microorganisms; moreover, a pH > 8.5 is optimal for their survival in water (Grandjean et al., 2005). *V. cholerae*, especially serogroup O1, is extremely sensitive to acidic environments, and normachlorohydric people are not easily susceptible to cholera.

This type of *vibrio* is also sensitive to desiccation and heat (ICMSF, 1996).

The average electrical conductivities (EC) for rivers and communal boreholes ranged between 18.78 and 154 $\mu\text{S}/\text{cm}$ and 23.4 and 295 $\mu\text{S}/\text{cm}$ (Table 2), which were above the acceptable South African water quality guidelines of 0–70 $\mu\text{S}/\text{cm}$ for rivers and domestic use (Department of Water Affairs and Forestry, 1996a). The higher conductivity value recorded is directly proportional to an increase in ions and organic compounds that can conduct electric current (Holmes, 1996; Ferreira, 2011). Atekwana et al. (2004) further reported that the high levels of conductivity and TDS in water and the environment are due to the biodegradation of organic compounds by natural microorganisms. The presence of chitinase that is produced by *V. cholera* also assists them in adapting and surviving in extreme conditions such as saline and acidic environments, as reported by Huq et al. (2012).

The overall range for the TDS for the rivers in this study ranged between 10 and 90 mg/L, while for communal boreholes, it ranged between 70 and 320 mg/L. These data revealed that both rivers and communal boreholes were still within the acceptable South African water quality guidelines of 0–450 mg/L (Department of Water Affairs and Forestry, 1996a; Department of Water Affairs and Forestry, 1996b).

The average temperature obtained ranged between 18.05°C and 30.60°C for both rivers and communal boreholes. According to Franco et al. (1997), temperature is vital in triggering the formation of environmental biofilms and sediments that are supporting the survival of *V. cholera*, which will increase their counts and human illness. Huq et al. (2012) also state that a wider range of temperatures is critical for the heterogeneous nature of *V. cholera*. Temperature and other parameters, such as salinity, are the main drivers of the adaptability and survival of *V. cholerae* in any given environment, including contaminated water (Takahashi et al., 2023).

Most of the rivers ranged between 1.01 and 14.85 NTU, while the turbidity of communal boreholes ranged between 0.71 and 13.23 NTU. Despite all, most of the rivers were found to be within the acceptable set standards limit of <25 NTU for river water containing sediments and untreated aquatic water (Department of Water Affairs and Forestry, 1996a). However, only some communal boreholes (Val B1, Kwe B1, B3, and B4) were reported to be within the acceptable South African water quality guidelines of 0–1 NTU for domestic use (Department of Water Affairs and Forestry, 1996b). Turbidity is influenced by the formation of biofilms, sediments, and eutrophication by the presence of nitrates and phosphates because of the agricultural,

TABLE 2 Physicochemical parameters of the river and communal borehole samples.

Sample origin	Sample ID	pH	Temp (°C)	EC (µS/cm)	TDS (mg/L)	Turbidity (NTU)	DO (ppm)
Mvudi River	Upstream	7.85	22.90	83.65	53.45	9.613	16.8
	Downstream	7.71	22.65	81.10	66.55	9.525	15.0
Livuvhu River	Upstream	8.09	24.45	63.40	40.45	4.615	16.6
	Downstream	7.81	25.35	80.05	50.70	2.890	22.1
Dzindi River	Upstream	7.94	18.95	34.80	22.30	9.910	8.56
	Downstream	8.21	23.70	65.00	41.60	14.85	19.1
Nzhelele River	Upstream	7.98	19.65	23.05	14.78	3.085	9.23
	Downstream	7.69	18.00	30.90	19.84	4.210	9.91
Mutshundudi River (Phiphidi)	Upstream	8.20	18.90	18.78	12.02	2.240	8.61
	Downstream	8.09	19.30	19.38	12.42	2.870	8.37
Mutale River	Upstream	7.03	21.9	27.00	11.33	5.28	8.55
	Downstream	7.12	24.4	27.00	17.25	5.87	8.75
Sambandou River	Upstream	8.52	23.6	21.35	13.58	1.01	14.6
	Downstream	7.03	21.85	24.65	15.79	1.96	9.43
Nwedi River	Upstream	7.73	24.90	35.2	22.55	3.94	8.15
	Downstream	7.19	25.00	35.6	23.15	5.50	8.51
Tshinane River	Upstream	8.07	26.1	43.3	27.7	8.31	10.7
	Downstream	7.87	24.4	43.4	27.8	8.26	11.4
Mukhase River	Upstream	7.72	22.1	21.15	13.56	1.47	8.47
	Downstream	7.44	23.50	21.10	13.57	1.40	9.85
Madandze River	Upstream	8.70	20.00	150.5	51.9	12.70	2.09
	Downstream	8.05	20.25	153.55	88.45	11.55	1.97
South Africa water quality guidelines by Department of Water and Sanitation		6.5–8.5	NA	0–70	0–450	0–5 <25 for rivers	6.5–8.0
Communal boreholes							
Mak B1	Common tap	7.14	17.9	188.8	121.55	1.165	9.34
Mak B2	Common tap	7.79	18.05	23.35	15.25	1.740	9.10
Thi B1	Common tap	7.29	26.7	364	233	1.205	18.6
Val B1	Common tap	7.09	22.4	112.60	72.20	0.81	8.64
Val B2	Common tap	6.88	27.3	114	73.1	2.44	10.2
Kwe B1	Common tap	7.00	26.2	173.4	110.7	0.86	9.38
Kwe B2	Common tap	7.35	25.55	497	318	3.23	2.77
Kwe B3	Common tap	7.54	28.6	257	261	0.95	5.83
Kwe B4	Common tap	7.07	29.0	282.5	181	0.71	1.65
Kwe B5	Common tap	8.21	30.6	376	240	1.18	4.01
Kwe B6	Common tap	7.26	30.2	285	180	2.31	1.35
Tsh B1	Common tap	7.46	28.3	295	189.15	1.06	8.71
South Africa water quality guidelines by Department of Water and Sanitation		6.0–9.0	NA	0–70	0–450	0–1	NA

NA, no standards given by DWA.

TABLE 3 The biochemical test: API results for the identification of *Vibrio cholerae* in rivers and communal borehole samples.

River	Sample site	Biochemical test: API identification
Mvudi River	Upstream	presumptive <i>V. fluvialis</i> , <i>Erwina</i> spp
	Downstream	presumptive <i>V. fluvialis</i> , <i>Erwina</i> spp
Livuvhu River	Upstream	N/A
	Downstream	<i>Erwina</i> spp
Dzindi River	Upstream	<i>Erwina</i> spp, <i>Raoutella planticola</i> or <i>terrigena</i>
	Downstream	<i>Erwina</i> spp, <i>Raoutella terrigena</i>
Madanzhe River	Upstream	presumptive <i>V. fluvialis</i> , <i>Erwina</i> spp, <i>Raoutella planticola</i>
	Downstream	presumptive <i>V. cholerae</i> and <i>V. fluvialis</i> , <i>Erwina</i> spp, <i>Raoutella planticola</i> or <i>terrigena</i>
Nzhelele River	Upstream	presumptive <i>V. cholerae</i> , <i>Serratia</i> spp
	Downstream	presumptive <i>V. fluvialis</i> , <i>Serratia</i> spp
Mutshundudi River (Phiphidi)	Upstream	presumptive <i>V. fluvialis</i> , <i>Erwina</i> spp
	Downstream	presumptive <i>V. fluvialis</i> , <i>Klebsiella</i> spp., <i>Erwina</i> spp
Mutale River	Upstream	presumptive <i>V. fluvialis</i> , <i>Erwina</i> spp
	Downstream	presumptive <i>V. fluvialis</i> , <i>Erwina</i> spp
Sambandou River	Upstream	<i>Raoutella planticola</i> , <i>Erwina</i> spp
	Downstream	presumptive <i>V. cholerae</i>
Nwedi River	Upstream	<i>Erwina</i> spp, <i>Serratia</i> spp
	Downstream	<i>Aeromonas</i> spp, <i>Serratia</i> spp., <i>Raoutella planticola</i>
Mukhase River	Upstream	N/A
	Downstream	N/A
Tshinane River	Upstream	<i>Erwina</i> spp
	Downstream	<i>Raoutella planticola</i> , <i>Serratia</i> spp., <i>Proteus vulgaris</i> group
Communal boreholes		
Mak B1	Common tap	N/A
Mak B2	Common tap	N/A
Thi B1	Common tap	presumptive <i>V. fluvialis</i> , <i>Erwina</i> spp, <i>Raoutella planticola</i> or <i>terrigena</i>
Val B1	Common tap	N/A
Val B2	Common tap	N/A
Kwe B1	Common tap	presumptive <i>V. fluvialis</i> and/or <i>Aeromonas</i> spp
Kwe B2	Common tap	presumptive <i>V. fluvialis</i> , <i>Erwina</i> spp, <i>Raoutella planticola</i> or <i>terrigena</i>
Kwe B3	Common tap	presumptive <i>V. fluvialis</i> , <i>Erwina</i> spp
Kwe B4	Common tap	presumptive <i>V. fluvialis</i> , <i>Erwina</i> spp
Kwe B5	Common tap	presumptive <i>V. fluvialis</i> , <i>Erwina</i> spp
Kwe B6	Common tap	<i>Erwina</i> spp
Tsh B1	Common tap	<i>Erwina</i> spp

N/A-there were no yellow colonies analyzed using biochemical test due to no growth.

Livuvhu and Nwedi rivers, and the upstream Nzhelele river point (Table 4). Only Mak B1, B2, Kwe B3, 4 and 6 boreholes tested positive for *V. cholerae*, using the *OmpW* primer (Table 4), which specifically targets the outer membrane of *V. cholerae*, and

according to Fu et al. (2018), this gene acts as a passage of iron and other organic molecules that are pumped across and regulate survival in extreme environments. The detection of *V. cholerae* was also directly connected to the higher

TABLE 4 qPCR results for the detection of toxigenic *Vibrio* from river samples.

River	Sample site	Molecular test (qPCR) using the <i>OmpW</i> primer/probes	qPCR analysis for toxigenic <i>V. cholera</i> using <i>CtxA</i> primer	qPCR analysis for serogroups of the toxigenic <i>V. cholera</i> detected using O1 <i>rfb</i> and O139 <i>rfb</i> primers	
				O1 <i>rfb</i>	O139 <i>rfb</i>
Mvudi River	Upstream	+	—	—	—
	Downstream	+	—	—	—
Livuvhu River	Upstream	+	—	—	—
	Downstream	—	—	—	—
Dzindi River	Upstream	+	+	—	+
	Downstream	+	—	—	—
Madanzhe River	Upstream	+	+	—	+
	Downstream	+	—	—	—
Nzhelele River	Upstream	—	—	—	—
	Downstream	+	—	—	—
Mutshundudi River (Phiphidi)	Upstream	+	+	+	+
	Downstream	+	+	+	—
Mutale River	Upstream	+	—	—	—
	Downstream	+	—	—	—
Sambandou River	Upstream	—	—	—	—
	Downstream	+	+	—	—
Nwedi River	Upstream	+	+	—	—
	Downstream	—	—	—	—
Mukhase River	Upstream	—	—	—	—
	Downstream	—	—	—	—
Tshinane River	Upstream	+	+	+	+
	Downstream	+	+	+	+
Communal boreholes					
Mak B1	Common tap	+	+	+	+
Mak B2	Common tap	+	+	-	+
Thi B1	Common tap	—	—	—	—
Val B1	Common tap	—	—	—	—
Val B2	Common tap	—	—	—	—
Kwe B1	Common tap	—	—	—	—
Kwe B2	Common tap	—	—	—	—
Kwe B3	Common tap	+	+	+	+
Kwe B4	Common tap	+	—	—	—
Kwe B5	Common tap	—	—	—	—
Kwe B6	Common tap	+	—	—	—
Tsh B1	Common tap	—	—	—	—

physicochemical parameters such as EC, TDS, and turbidity that were reported in both river and communal boreholes. The higher values obtained trigger the survival and distribution of *V. cholerae* in these water sources.

From the rivers that tested positive for *V. cholerae*, the pathogenicity of these *Vibrio* isolates was assessed using the *ctxA* primer and Mutshundudi, Tshinane rivers, the upstream of Dzindi, Madanzhe, and Nwedi samples, and the downstream of Sambandou rivers reported positive for toxigenic *V. cholerae* (Table 4). Only Mak B1, B2, and Kwe 3 communal boreholes were found to have toxigenic *V. cholerae* (Table 4). Bina et al. (2003) reported that toxigenic *V. cholerae* produces a toxin-regulated pilus that facilitates colonisation of the intestine walls and is the main regulator of the *ctxA* gene, and this gene was targeted by the *ctxA* primer. According to Liang et al. (2007), colonisation results in the activation of the G protein, which stimulates the adenylate cyclase. This process leads to the loss of electrolytes from the cell membrane, resulting in vomiting and watery, loose stools into the large intestines (World Health Organization, 2017). It is imperative to understand that any mutation or mismatch in the *ctxA* gene sequence leads to either a positive *ctxA* or a non-toxin-producing strain (Hoshino et al., 1998; Takahashi et al., 2023). The presence of toxigenic *V. cholerae* in these water sources was driven by the conditions and activities around them, as shown in S1-3, where some of the water sources were closer to sewer lines, toilets, and backyard farming. For the contamination or pollution of communal boreholes, Barcelona et al. (1988) stipulated different routes such as infiltration, direct migration, inter-aquifer exchange, and recharge from surface water that are the major drivers. In the clinical context, pathogenic *V. cholerae* differs from the non-pathogenic ones based on toxin-co-regulated pilus (TCP) and cholera toxin (CTX), which allow colonisation of the gut encoded by the phage CTX ϕ , which uses the TCP to attach to the bacterium (Waldor and Mekalanos, 1996; Ja'afar et al., 2021). Toxigenic *V. cholerae* also utilises the commensal association, leading to the formation of biofilm with copepods on their chitinous surfaces, which further leads to the formation of algal (de Magny et al., 2011; Rita and Colwell, 2009).

Once the pathogenicity had been analysed using PCR, the serogroups of the detected toxigenic were determined using the O1 *rfb* and O139 *rfb* primers. Serogroup O1 was detected on the Mutshundudi and Tshinane rivers, while serogroup O139 was detected on the upstream of the Dzindi, Madanzhe, Mutshundudi, and Tshinane rivers (Table 4). There was detection of both the O1 and O139 serogroups in the communal boreholes Mak B1 and Kwe B3, while serogroup O139 was only detected in the communal borehole Mak B2 (Table 4). In a study done by Phetla (2021), there was a higher percentage of serogroup O1 from sewage sludge and the riverbed from the Limpopo region, and therefore, there was a potential for O1 circulation in water sources leading to sporadic cholera outbreaks. The study of Phetla (2021) also reported that the O139 *V. cholerae* serotype was not found, and it was similar to a study done by Ansaruzzaman et al. (2007), who reported that this serotype was not endemic to the African continent but more endemic to India, Bangladesh, and other parts of the Asian continent. However, in this study,

serogroup O139 was identified in both rivers and communal boreholes. This might be due to cross-border migration from cholera-prone countries/areas into South Africa through seeking medical treatment, tourism, traders, relatives, and refugees from neighbouring cholera-endemic countries or communities with ongoing epidemics like Haiti, Malawi, or Mozambique and Asian countries (Chin et al., 2011; Zarocostas, 2017). The serogroups detected from rivers and communal boreholes in the study were also due to the discharge of raw sewage, especially on the upstream side of the Madanzhe River, and the discharge of partially or untreated wastewater effluents into the rivers. The communal boreholes were also closer to the septic pit, leaking sewer line, and latrine, as depicted in S1-3.

Keddy et al. (2013) and Alaoui et al. (2021) stated that non-O1 or non-O139 strains of *V. cholerae* have been identified in birds, which might act as carriers/hosts into the environment. Therefore, continuous surveillance of rivers and communal boreholes, an integrated approach, and robust policies are needed to monitor these cases in a timely and reliable manner to prevent further discharge of raw sewage into rivers and closer to communal boreholes.

S1-3 depict images of rivers (upstream and downstream) and communal boreholes where serogroups (O1 and O139) were discovered. The majority of the rivers were contaminated, and the communal boreholes were closer to the pit latrine while the sewer line also discharged dirty effluent directly into the river catchment.

3.5 Limitation of this study

The study involved analysing water samples from rivers and communal boreholes, which could also be reservoirs for other organisms such as fish, snails, and others that are involved in commensal association with *V. cholerae*, including feeding, but were not considered in this study (You et al., 2019; Cho et al., 2021). Further studies using appropriate techniques targeting ecosystems that were not covered by this study should be conducted to provide more informed and reliable information on the presence of cholerae *V. cholerae* in both surface water sources and communal boreholes. This study was conducted for a period of 4 months only, and a longer study might be needed to establish the prevalence and distribution of *V. cholerae* O1 or O139 and the potential health risk to vulnerable communities in Vhembe district, which is predominantly in rural areas.

4 Conclusion

The findings of this investigation indicate that pathogenic serotypes (O1 and O139) of *V. cholerae* were found in rivers and several communal boreholes. The presence of all *V. cholerae* serotypes in these contaminated water sources should be closely monitored at all times because the communities that rely on them are at risk. As a result, continual surveillance of rivers and communal boreholes, an integrated approach, and strong policies are required to monitor these cases in a timely and consistent

manner. This will also help to improve cleanliness around these sources of water. Further research is also needed to identify the ecology, virulence characteristics, and antibiotic sensitivity of the various serogroups (O1 and O139) found in this location.

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.

Author contributions

LK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing. MR: Formal Analysis, Investigation, Methodology, Software, Writing—review and editing. AT: Investigation, Methodology, Supervision, Writing—review and editing. NP: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing—review and editing.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2024.1498893/full#supplementary-material>

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