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Research Article

The Prevalence of Bacteria Commonly Related to the Production of Mussels and Oysters in Saldanha Bay

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Bivalve molluscs are a good source of high quality protein and perform important ecological functions. Their ability to bioaccumulate materials in their soft tissues makes them suitable aquatic species for biomonitoring of environmental conditions. The discharge of treated and untreated sewage into the bivalve-growing areas is a concern. The aim of this study was to investigate the prevalence of bacterial microbiota in shellfish farms in Saldanha Bay harbour using pathogens commonly associated with shellfish-related foodborne disease outbreaks. Seawater and mussel samples were collected from five sampling points located in three sampling locations. Oyster samples were collected from the harbour deck immediately after harvesting by the farmers. The most probable number (MPN) method was used to enumerate *E. coli* and faecal coliforms. Cultural methods were used for the detection of *Salmonella* and *Vibrio* spp. The *E. coli* concentrations for 15 March and 14 July are <0.18 MPN/100 ml at all sampling sites and for 25 August, <0.18 MPN/100 ml for all sampling sites except sampling site SP2 (0.2 MPN/100 ml). Spikes were observed on the total MPN counts in winter. *Salmonella* and *Vibrio* spp. were not detected. However, other bacterial species were identified through their phenotypic profile using the VITEK 2 system. Based on the low *E. coli*-MPN concentrations, the study concluded that the molluscs were safe for human consumption. Further studies need to be conducted on the bacterial species identified.

1. Introduction

Shellfish farming is becoming an important sector for the South African government as it creates much-needed job opportunities for the coastal communities. The sustainability and safety of shellfish growing areas are essential in terms of protection from contaminants and preventing contaminants from reaching the bivalves produced [1]. Bivalve molluscs play important ecological functions in aquatic ecosystems as well as being highly nutritious. They can be found at the bottom of the sea or attached to hard surfaces or on one another. Their filter-feeding nature assists in purifying the surrounding waters and increases the penetration of sunlight [2].

Furthermore, they provide micronutrients to other marine organisms increasing primary production and nutrient recycling, coastal habitat conservation, and restoration [3, 4]. These characteristics and ability to bioaccumulate materials in their soft tissues make the bivalve molluscs suitable aquatic species for biomonitoring of environmental conditions. Bioaccumulation of materials is not selective, as both beneficial and harmful materials are equally accrued [5]. Besides being highly nutritious compared to beef, chicken, and pork, bivalves are highly perishable and require proper handling from farm to fork. Failure to adhere to food safety best practices could lead to an increased risk of illness from pathogens, including bacteria, viruses, and protozoa [6, 7].

Aquatic environments are home to various microbiota, some indigenous and some introduced through anthropogenic activities around these environments. The presence of pathogens in aquatic ecosystems is a risk to the shellfish production industry and poses a public health threat. Several foodborne outbreaks have been reported globally due to the consumption of contaminated shellfish [8–10]. Zgouridou et al. [11] in their study indicated that mussels of the genus *Mytilus* are primarily the bivalve species that are a public health risk to consumers, as well as oysters (*Ostrea edulis*) and clams (*Venus verrucosa*). Bioaccumulation and bioconcentration of pathogens vary according to host species and seasonality. Both mussels and oysters can concentrate pathogens in their body tissues. However, oysters are an important medium for infecting humans with these pathogens as they are eaten raw or partially cooked [12]. Several studies have been conducted worldwide to determine the bacterial prevalence in shellfish-growing waters [13–15]. To date, similar studies have not been conducted in Saldanha Bay except for the microbiological monitoring undertaken by the South African Department of Forestry, Fisheries, and Environment. This created a need to investigate the bacterial communities, especially the disease-causing ones that may be present in this Bay. This study investigated pathogens commonly associated with shellfish-related foodborne disease outbreaks, such as *Salmonella, Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio cholera,* and the prevalence of *Escherichia coli* as an indicator species. The results did not conform to prior expectations, as bacteria such as the *Enterobacter cloacae complex, Citrobacter freundii, Klebsiella pneumoniae* spp. *pneumoniae, Aeromonas sobria, Vibrio alginolyticus*, and *Sphingomonas paucimobilis* were confirmed through biochemical characterisation.

The study applied an experimental design, and interpretations were formed using a multimethod, quantitative strategy for three sampling occasions. The researcher collected samples during warm, cold, and rainy periods as informed by the obtained literature. Data collection and analysis techniques are detailed under materials and methods [16].

2. Materials and Methods

2.1. Study Area. Saldanha Bay harbour on the West Coast of South Africa (latitude: −33.027699, longitude: 17.917631) houses the biggest port in Southern Africa operating as an international port for the export of iron ore. The Bay's water depth is approximately 23.7 m. Construction of a 4 km long iron ore jetty has divided the Inner bay into Small Bay and Big Bay [17], and a 1.7 km long breakwater separated the Inner Bay from the Outer Bay. Small Bay is sheltered from offshore swells and has constrained water circulation, while Big Bay is semiexposed to wave energy with better circulation compared to Small Bay. The Outer Bay, which is located at the mouth of the Bay, is regarded as the less polluted site [18]. The Bay is exposed to the disposal of treated and untreated sewage from the nearby wastewater treatment plant, which discharges into the Bok river. Several sewage pumps, ballast water, dredging, stormwater discharge, and ship traffic are some of the pollutants sources close to Small Bay. Two mussels species are farmed, the indigenous black mussels (*Choromytilus meridionalis*), which are not a preferred species for farming due to the dark flesh colour of the female species. The exotic Mediterranean mussels (*Mytilus galloprovincialis*) and the Pacific oysters (*Crassostrea gigas*) are also farmed. Figure 1 shows five sampling points where mussels and seawater were collected.

2.2. Sample Collection. A total of 27 shellfish and seawater (mussels ($n = 12$) and seawater ($n = 13$) and oysters ($n = 2$)) samples were collected from various sampling sites. Samples were collected in the morning between 8:00 am and 11:00 am during low tides in order to reach all sites especially the offshore ones. Oysters were collected from the harbour deck immediately after harvesting by the farmers. Five sampling points were used for the collection of seawater and mussels. Three of them were located in Small Bay (SP1, SP2, and SP3), one in Big Bay (SP4), and the last one in Outer Bay (SP5) (Figure 1). Samples were collected in March (warm period), July (winter-before heavy rainfall), and August (winter-after heavy rainfall). During sampling, 30 oysters and 30 mussels were hand-picked and stored in sterile whirl-pack bags (Nasco, US). Seawater samples were collected (2 meters below the surface) in 1 liter sterile Schott bottles (Schott, UK) and mussels from a hanging rope. Physicochemical parameters (i.e., water temperature (°C), salinity (psu), and dissolved oxygen (ppm)) were measured during sampling at each sampling point using a Hanna HI9810-6 multimeter. Samples were transported to the laboratory, in a cooler box packed with ice packs maintaining a temperature between 2 and 8°C, within 2 hours, and microbiological analyses were performed immediately.

2.3. Sample Preparation. Upon arrival at the laboratory, the mussel and oyster samples were scrubbed under running tap water to remove shell debris, and attached algae and the shells were opened aseptically with a sterile chucking knife. Approximately, 300 g of flesh and intravalvular liquid of mussels and oysters were stored in 500 g sterile beakers and then transferred aseptically into stomacher bags (circulator 400, Seward, Worthing, UK). Samples were homogenised with 200 ml sterile phosphate water at 230 rpm speed for 2 minutes.

2.4. Most Probable Number (MPN) (Mussel, Oyster, and Seawater Samples), Escherichia coli. Lauryl Tryptose Broth (LTB) (Merck, Germany), Brilliant Green Broth (BGBB) (Merck, Germany), and Tryptone Water (TW) (Merck, Germany) were prepared following the manufacturer's instructions. The method was conducted using the method described by Leuta [19] (Figures 2(a)–2(c)).

Concentrated mussel and oyster homogenate extracted from the mussel samples was used as stock to conduct the five-tube MPN technique. Serial dilutions of 10^{-1} to 10^{-5} of the mussel and oyster homogenate and seawater samples, respectively, was performed before inoculation of 1 ml of each diluted sample into LTB tubes containing Durham tubes. Durham tubes provide a visual indication of gas

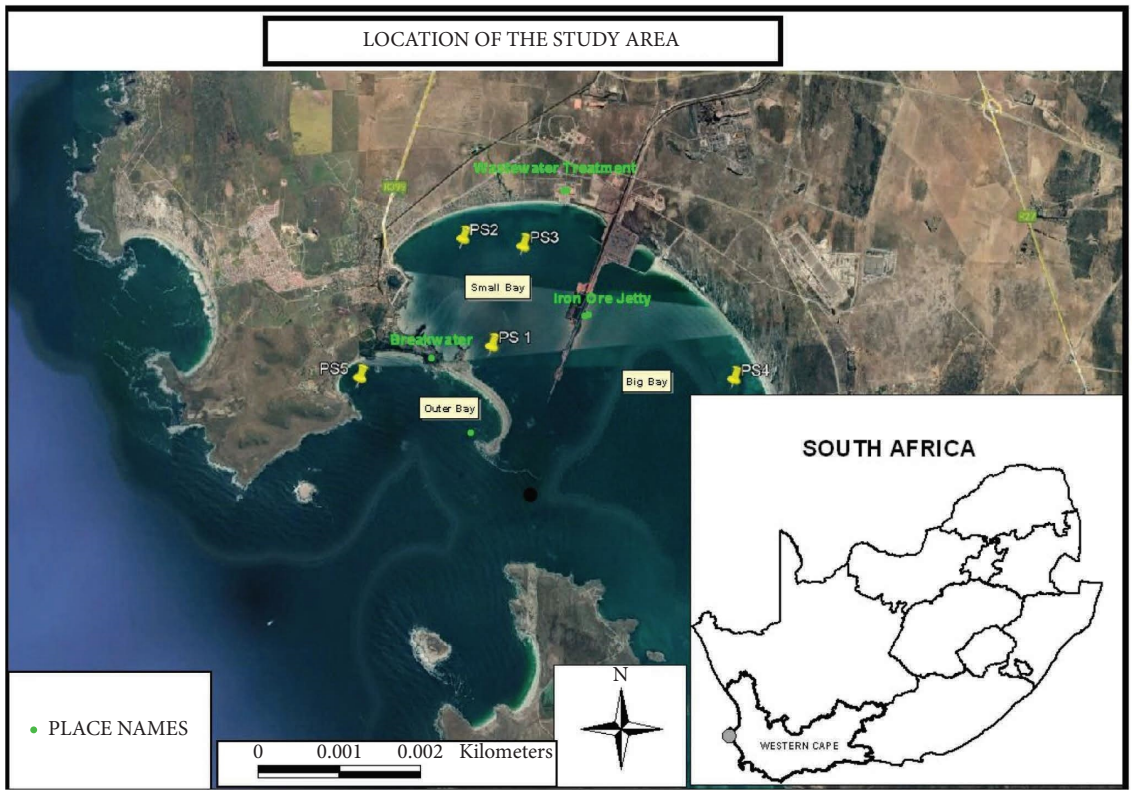

Figure 1: Aerial photograph depicting Saldanha Bay harbour and sampling points.

production. The inoculated test tubes were incubated for 48 hours at 37°C (indicating all gas-producing organisms). All tubes showing gas formation after a 48-hour incubation period were regarded as a positive presumptive test, and the presumptive total MPN count was read off De Man's tables [20]. For each positive presumptive LTB tube, a 10 ml Brilliant Green Bile Broth (BGBB) tube and 10 ml Tryptone Water (TW) tubes were prepared. One hundred microliters (μl) of the sample from each positive LTB tube were reinoculated into BGBB and TW tubes, respectively, according to the guidelines set out by the South African Bureau of Standards [21]. These guidelines also incorporate the standard methods set out by the American Public Health Association, for the examination of seawater and shellfish as well as the methods for the examination of water and wastewater (American Society for [22]; American Society for [23]. These tubes were incubated in a 44.5°C waterbath for 24 hours (44°C–44.5°C) and has the specific advantage of detecting *E. coli*, as it is the only faecal coliform present in water capable of producing indole at this temperature). With the observation of positive gas production in the BGBB tubes (indicating faecal coliforms (FC)) after 24 hours, a few drops of Ehrlich's reagent (LabChem, USA) were added to the corresponding TW tubes. The presence of *E. coli* was confirmed with a colour change from clear to pink or red in the Tryptone Water tubes.

2.5. Detection and Isolation of Salmonella. *Salmonella* spp. was detected according to the protocol based on ISO 6579-1:2007 [24]. Buffered Peptone Water (BPW) (Merck, Germany), Selenite Cysteine broth (SCB) (Merck, Germany), and *Salmonella shigella* agar (SS agar) (Oxoid, UK) were prepared according to the manufacturer's instructions. Twenty-five grams (25 g) of mussels and oysters homogenate were aseptically weighed and placed into a 225 ml sterile BPW to prepare a pre-enrichment culture. The mixture was then incubated at 37°C for 16–20 h. After incubation, the sample was gently mixed, and 1 ml of the BPW was added into a sterile McCartney bottle. Ten milliliters (10 ml) of SCB were added to the sample to prepare an enrichment culture and incubated at 37°C for 24 h. After the 24 h incubation period, the enriched culture was streaked onto SS agar and incubated inverted at 37°C for 24 h. The plates were examined (typical pinkish-red colonies) for the absence or presence of *Salmonella* spp. Subsequently, Gram stains were performed on the obtained colonies. The observation of Gram-negative, nonspore-forming colonies confirmed the presence of *Salmonella*, while biochemical identification was carried out using VITEK 2 compact Gram-negative (GN) ID cards (bio Mérieux, France). *Salmonella typhimurium* (NCTC 12023) strain was used as a positive control.

2.6. Detection and Isolation of Vibrio cholerae and Vibrio parahaemolyticus. *Vibrio* spp. were detected according to the protocol based on ISO/TS 21872-1:2007 [25]. Alkaline Salt Peptone Water (ASPW) (Oxoid, UK), Thiosulphate Citrate Bile Sucrose agar (TCBS) (Oxoid, UK), and saline nutrient agar (SNA) (Oxoid, UK) were prepared according to the manufacturer's instructions. Twenty-five grams (25 g)

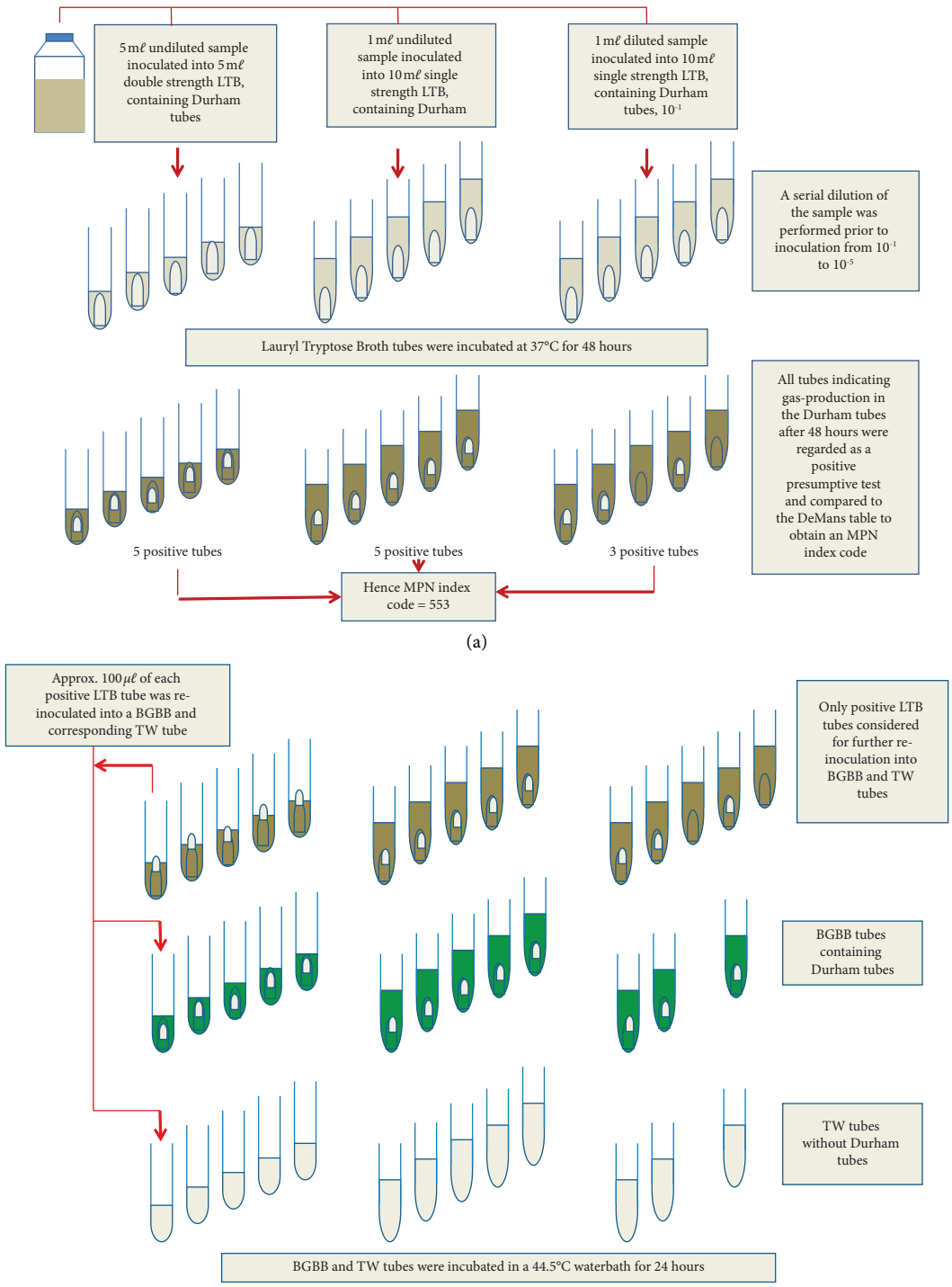

Figure 2: Continued.

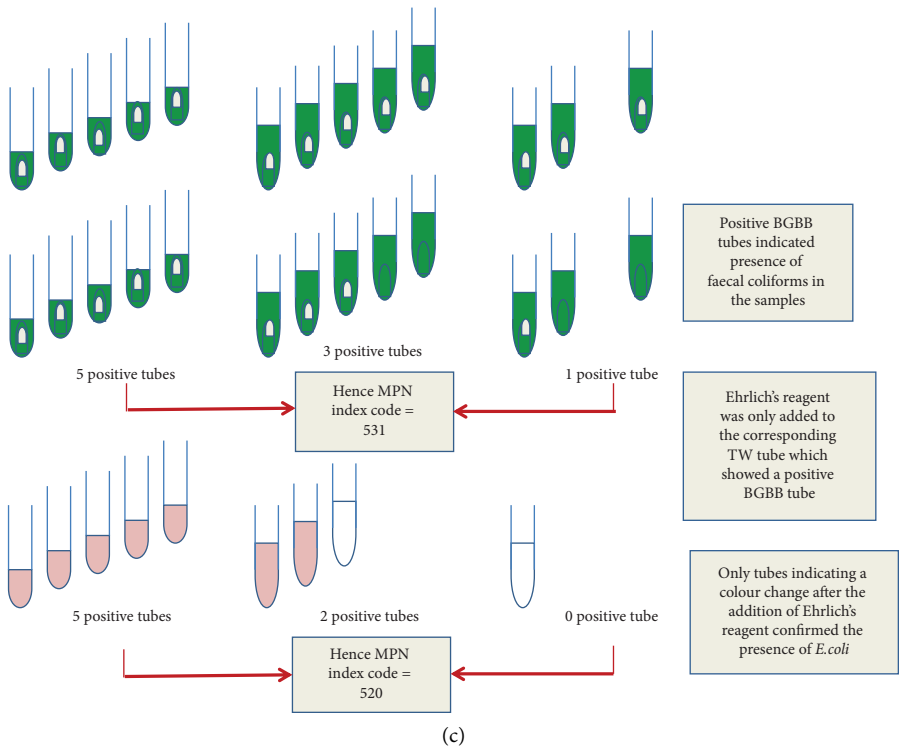

Figure 2: (a) Inoculation of undiluted and diluted samples into LTB tubes to obtain the positive presumptive test (adopted and adapted from [19]). (b) Reinoculation of positive LTB tubes into BGBB and TW tubes (adopted and adapted from [19]). (c) Enumeration of faecal coliforms (from BGBB tubes) and *E. coli* (from TW tubes) in water samples (adopted and adapted from [19]).

of mussel and oyster homogenate were aseptically weighed and placed into a 225 ml sterile ASPW to prepare a pre-enrichment culture. The mixture was then incubated at 41.5°C for 6 h (41.5°C is recommended for fresh products). After incubation, a loopful (1 μl) of the enriched sample was inoculated into fresh 10 ml ASPW (secondary enrichment) and incubated at 41.5°C for 18 h. After 18 h of incubation, the enriched culture was streaked onto a selective plating medium (TCBS) and incubated inverted at 37°C for 18–24 h. The plates were examined (suspected *V. cholerae* colonies would appear yellow, flat, and 2-3 mm in diameter; suspected *V. parahaemolyticus* colonies would appear blue/green and 2–5 mm in diameter) for the absence or presence of *Vibrio* spp. Five representative colonies were streaked from each plate onto SNA and incubated at 37°C for 24 h, after which Gram stains and oxidase tests were performed on the colonies isolated onto SNA. Typical *Vibrio* colonies were oxidase-positive, and isolates were identified using VITEK 2 compact Gram-negative (GN) ID cards (bio Mérieux, France). *Vibrio furnissii* (NCTC 11218) was used as a positive control for *Vibrio cholerae* and *V. parahaemolyticus* (NCTC 109030) as a positive control for *V. parahaemolyticus*.

2.7. Statistical Analysis. A Pearson correlation was conducted to determine the relationship between seawater samples, shellfish samples, and physicochemical parameters (temperature, salinity, and dissolved oxygen). For all the tests, the criterion for statistical significance was $p < 0.05$. The researcher performed statistical analysis using the statistical package IBM SPSS v28.0.0.0 (190).

3. Results and Discussion

3.1. Physicochemical Parameters. Physicochemical parameters recorded in Table 1 did not show a significant variation, where the recorded temperature ranged between 12°C and 19°C, salinity ranged between 33.91 psu to 35.45 psu, and dissolved oxygen between 0.71 and 2.96 ppm showing prevailing hypoxic conditions, which are often associated with pollution due to anthropogenic activities [26]. Seawater temperature, salinity, pH, dissolved oxygen, turbidity, and organic matter become water quality stressors when available in excessive amounts [27]. These stressors may influence the survival, health, and growth of shellfish, which depend on the water quality of their growing environments. Poor water quality increases the risk of shellfish contamination with disease-causing pathogens [28].

An increase in salinity during warm periods and a decrease during cold periods were observed throughout the study and correlated with similar findings reported by Lamine et al. [29]. The lowest rainfall was observed in July and the highest rainfall in August. Colaiuda et al. [30] found in their study that the amount of rainfall and the increased *E. coli* concentrations in shellfish depend on the specific area where the samples were collected. Chahouri et al. [31] and Padovan et al. [32] found that high precipitation increases levels of faecal coliform. In this study, no clear indication of the influence of rainfall on *E. coli* levels was detected. Similar

Table 1: Physicochemical parameters of shellfish production areas and rainfall in Saldanha Bay.

Sampling site	Sampling date	Temperature (degree Celsius)°C	Salinity (PSU)	Dissolved oxygen (ppm)	Monthly average rainfall (mm)	Samples
Sp1	15 March 2021	17	35	2.2	54.4	Mussels
Sp2	15 March 2021	19	35.45	2.90	54.4	Mussels
Sp3	15 March 2021	18	35.36	2.96	54.4	Mussels
Sp1	14 July 2021	18	35.38	0.85	5.6	Mussels
Sp2	14 July 2021	12	35.23	0.73	5.6	Mussels
Sp3	14 July 2021	12.5	35.30	0.71	5.6	Mussels
Sp4	14 July 2021	12.5	35.36	0.82	5.6	Water
Harbour Deck	14 July 2021	—	—	—	5.6	Oysters
Sp1	25 August 2021	14.25	34.04	0.85	61.4	Mussels
Sp2	25 August 2021	14.74	33.91	1.59	61.4	Mussels
Sp3	25 August 2021	14.97	33.95	1.5	61.4	Mussels
Sp4	25 August 2021	14.40	33.64	1.71	61.4	Water
Sp5	25 August 2021	13.90	34.11	0.95	61.4	Mussels
Harbour Deck	25 August 2021	—	—	—	61.4	Oysters

results were observed by Sampson et al. [33], where no association was found between precipitation and bacterial concentrations. Tabanelli et al. [34] included the influence of the flow rate of the river feeding into the coastal area of their study and concluded that meteorological events could bring a substantial amount of contaminated fresh water into coastal water. This could be the case with the Bok river, which feeds into Saldanha Bay. During heavy rainfall, the Bok river flow rate increase is suspected, which could wash down all the runoff from upstream agricultural areas and runoff from roads and residential areas [31].

3.2. Prevalence of Faecal Coliforms and Escherichia coli in Mussels and Oysters. Oyster harvesting did not take place during the March sampling occasion. Sampling sites SP4 (during March and July) and SP5 (during March) could not be reached due to high tides. In addition, no mussels were available during August at the SP4 site (Table 2). The total MPN count per 100 ml of mussel samples were between 4.9 and 4700 microorganisms/100 ml and for oysters were 18 and 1000 microorganisms/100 ml in (July and August), respectively. Increased total MPN counts of 400 microorganisms/100 ml were observed in mussel samples collected at SP1 in July. Of the recorded total MPN count at this site, FC and *E. coli* counts of <0.18 microorganisms/100 ml, respectively, were observed. In the August sampling run, mussels collected at SP2 recorded a total MPN count of 4700 microorganisms/100 ml, while a total MPN count of 1000 microorganisms/100 ml) were recorded in oyster samples at the Harbour Deck site. In comparison, the FC and *E. coli* concentrations at these respective sites were 0.2 microorganisms/100 ml, respectively (mussels), and <0.18 microorganisms/100 ml, respectively (oysters).

Mussels and oysters can accumulate and retain suspended particles of phytoplankton size and pathogenic microorganisms in their bodies due to their filter-feeding nature [35, 36]. This creates a public health concern, especially for oysters, as oysters are consumed raw or partially cooked [37, 38]. The spikes observed in mussels and oysters suggest possible contamination due to heavy rainfall or pollution sources including the sewage pump stations, stormwater drains, and a sewage discharge point that is located in close proximity to the affected sampling sites [39, 40]. Saldanha Bay Municipality recently made remarkable improvements to their sewage treatment plants and diverted the majority of treated effluent for the irrigation of sports grounds and use by interested local businesses. However, the little that is being discharged together with effluent from fish factory industries, untreated stormwater discharge, and ballast water should not be underestimated. According to Clark et al. [41], the shipping traffic has increased in the harbour, which brings large volumes of ballast discharge. All of these need to be monitored closely. Several studies in various parts of the world seem to agree on the fact that microbial contaminants are the results of treated and untreated sewage being discharged into shellfish growing waters, sewage overflow during rainfall periods, and runoff from agricultural areas [42, 43]. Sewage is loaded with nutrients that, in excessive amounts, could stimulate microbial growth, production of harmful algal blooms, and eutrophication, ultimately affecting the viability of shellfish mariculture [44]. Even though oyster samples were not taken from the farm but at the loading area of the harbour, i.e., the Harbour Deck, the samples came from the same farming area as the mussels.

3.3. Prevalence of Faecal Coliforms and Escherichia coli in Seawater. Sampling site SP5 could not be reached due to high tides in March and July (Table 3). The total MPN count/100 ml in seawater ranged from <0.18 to 1.3 microorganisms/100 ml, with a high spike recorded at SP2 in August (2400 microorganisms/100 ml). Faecal coliforms and *E. coli* concentrations were the same (<0.18 microorganisms/100 ml) at all sampling sites. The high increase in the total MPN count observed in SP2 in the seawater sample correlates with a spike in mussels collected during the same period. This could be attributed to heavy rainfall, stormwater drain discharges and sewage discharges, and the location and proximity of the sampling site to pollution sources. Sampling site SP2 is located in Small Bay, which is subjected to various sources of pollution including a sewage discharge outfall. Understanding the causes of faecal contamination in

Table 2: Faecal coliforms and *Escherichia coli* in mussels and oysters homogenate.

Sample date	Sample point	Total MPN count (microorganisms/100 ml)	Faecal coliforms (microorganisms/100 ml)	*E. coli* (microorganisms/100 ml)
15 March 2021	SP1	4.9	<0.18	<0.18
15 March 2021	SP2	4.9	<0.18	<0.18
15 March 2021	SP3	33	<0.18	<0.18
14 July 2021	SP1	400	<0.18	<0.18
14 July 2021	SP2	13	<0.18	<0.18
14 July 2021	SP3	24	<0.18	<0.18
14 July 2021	SP4	13	<0.18	<0.18
14 July 2021	Harbour Deck (oyster)	18	<0.18	<0.18
25 August 2021	SP1	7.9	<0.18	<0.18
25 August 2021	SP2	4700	0.2	0.2
25 August 2021	SP3	40	<0.18	<0.18
25 August 2021	SP5	60	<0.18	<0.18
25 August 2021	Harbour Deck (oyster)	1000	<0.18	<0.18

Table 3: Prevalence of faecal coliforms and *Escherichia coli* in seawater samples.

Sample date	Sample point	Total MPN count (microorganisms/100 ml)	Faecal coliforms (microorganisms/100 ml)	*E. coli* (microorganisms/100 ml)
15 March 2021	SP1	0.2	<0.18	<0.18
15 March 2021	SP2	0.2	<0.18	<0.18
15 March 2021	SP3	1.3	<0.18	<0.18
15 March 2021	SP4	0.2	<0.18	<0.18
14 July 2021	SP1	0.2	<0.18	<0.18
14 July 2021	SP2	0.2	<0.18	<0.18
14 July 2021	SP3	0.2	<0.18	<0.18
14 July 2021	SP4	<0.18	<0.18	<0.18
25 August 2021	SP1	<0.18	<0.18	<0.18
25 August 2021	SP2	2400	<0.18	<0.18
25 August 2021	SP3	<0.18	<0.18	<0.18
25 August 2021	SP4	<0.18	<0.18	<0.18
25 August 2021	SP5	<0.18	<0.18	<0.18

areas where shellfish are grown is essential for assessing the associated health risks and determining the way forward to address the problem [45].

During high tide episodes, pollutants can be transported rapidly from the areas where they are highly concentrated through advection, mixing, dispersion, and dilution of sewage [2]. The sampling sites in Small Bay, sheltered from the sea swells and close to the sewage discharge point, sewage pump stations, and stormwater drains may not benefit from this natural process and therefore presented higher contamination levels. These natural processes are also evident in the analysis results of the Big Bay and Outer Bay sampling sites where lower contamination levels were observed. Both sites are semiexposed to the sea swells, explaining the relative improvement in water quality. In other words, the possibility of having shellfish farms far away from sewage discharge points could eliminate the microbial contamination problem. Similarly, Florini et al. [45] reported a decrease in the concentrations of faecal indicator species with an increase in distance from sewage discharge points. The low concentration results were ascribed to possible dilution and die-off effects.

Contamination of water bodies by wastewater is a fundamental problem worldwide. The bacteria, parasites, and viruses from animals and humans reach the oceans through runoff from roads, agricultural areas, and sewage discharges [46]. In addition, heavy rainfall may cause sewage overflows and drain leakages [47]. As mentioned, faecal coliform and *Escherichia coli* are indicators of water quality. The presence of these organisms is undesirable in areas used for shellfish farming.

No correlation could be drawn between the total MPN count in water (microorganisms/100 ml) and shellfish (microorganisms/100 g) samples, physicochemical parameters, as well as between rainfall patterns and MPN counts in water and shellfish ($p > 0.05$) (Table 4). As the total MPN count in water samples increased, the total MPN count in shellfish samples increased ($r = 0.997$, $n = 11$, $p \leq 0.001$).

3.4. Bacterial Species Isolated from Selected Sample Sites. *Salmonella* spp., *Vibrio cholerae,* and *Vibrio parahaemolyticus* were not detected. Bacterial species identified included the *Enterobacter cloacae complex, Citrobacter freundii, Klebsiella pneumoniae* spp. *pneumoniae, Aeromonas sobria, Vibrio alginolyticus,* and *Sphingomonas paucimobilis* (Table 5). These microorganisms may be grouped into pathogens that are often present in aquatic

Table 4: Correlations between physicochemical parameters, rainfall, and total MPN count in seawater and shellfish.

		Shellfish (MPN count 100 ml)	Water (MPN count 100 ml)
Shellfish (MPN count 100 ml)	Pearson correlation	1	0.997**
	Sig. (2-tailed)		<0.001
	N	14	11
Water (MPN count 100 ml)	Pearson correlation	0.9977**	1
	Sig. (2-tailed)	<0.001	
	N	11	13
Temperature (°C)	Pearson correlation	−0.029	−0.048
	Sig. (2-tailed)	0.933	0.881
	N	11	12
Dissolved oxygen (ppm)	Pearson correlation	0.030	0.042
	Sig. (2-tailed)	0.931	0.897
	N	11	12
Salinity (psu)	Pearson correlation	−0.442	−0.357
	Sig. (2-tailed)	0.174	0.254
	N	11	12
Average monthly rainfall (mm)	Pearson correlation	0.245	0.243
	Sig. (2-tailed)	0.467	0.446
	N	11	12

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Table 5: Bacterial species isolated from mussels and oysters sampling points.

Samples and sampling point	Sampling date	Growth media	Isolated organisms	VITEK probability (%)
SP1-mussel	14 July 2021	SS agar	*Enterobacter cloacae complex*	99
Sp2-mussel	14 July 2021	SS agar	*Enterobacter cloacae complex*	99
Sp3-mussel	14 July 2021	SS agar	*Enterobacter cloacae complex*	99
HD-oyster	14 July 2021	TCBS	*Citrobacter freundii*	95
HD-oyster	14 July 2021	SS agar	*Klebsiella pneumoniae* spp.	95
Sp1-mussel	25 August 2021	SS agar	*Aeromonas sobria*	87
Sp2-mussel	25 August 2021	TCBS	*Vibrio alginolyticus*	90
Sp3-mussel	25 August 2021	TCBS	*Sphingomonas paucimobilis*	86
Sp5-mussel	25 August 2021	SS agar	*Aeromonas sobria*	89
HD-oyster	25 August 2021	SS agar	*Klebsiella pneumoniae* spp.	91

*SS agar, *Salmonella shigella* agar; TCBS, thiosulphate citrate bile sucrose agar; HD, Harbour Deck.

environments (e.g., *Klebsiella pneumoniae spp., Aeromonas sobria, Vibrio alginolyticus,* and *Sphingomonas paucimobilis)*. These microorganisms are pathogens of high priority as some are antimicrobial resistant and may cause illnesses in humans. In addition, pathogens naturally present in human beings and animals *(e.g., Citrobacter freundii* and *Enterobacter cloacae complex*) are also high priority pathogens and their presence should not be taken lightly [48]. The *Enterobacter cloacae complex* has also proved to be abundant in aquatic environments [49].

4. Conclusion

The study used conventional culture methods to isolate *Salmonella* and *Vibrio* spp.in mussels, oysters, and seawater samples obtained from the Saldanha Bay Harbour. The most probable number (MPN) analysis technique was used for detecting and enumerating faecal coliforms and *E. coli* in the obtained samples. The identification of species was conducted using the VITEK 2 automated system, which successfully identified species such as *Enterobacter cloacae complex, Citrobacter freundii, Klebsiella pneumoniae* spp. *pneumoniae, Aeromonas sobria, Vibrio alginolyticus,* and *Sphingomonas paucimobilis*. The findings observed with correlations between MPN counts in seawater, mussels, and oysters, and correlations between physicochemical parameters and rainfall, did not show any significant difference. However, total MPN count spikes were observed in mussels, oysters, and seawater, which could be ascribed to the rainfall period and winter season, although the spikes did not have a significant impact on the *E. coli* concentrations, as the concentrations were below the permissible limits. This information may be used as a basis to conduct an in-depth investigation of sources of pollutants. Further studies need to be conducted on the bacterial species

identified to determine their prevalence and assess the probability of their present becoming a public health threat to shellfish consumers.

Data Availability

The data used to support the findings are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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