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Anthropogenic impact and antibiotic resistance among the indicator and pathogenic bacteria from several industrial and sewage discharge points along the coast from Pydibhimavaram to Tuni, East Coast of India

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Abstract Increasing urbanisation and industrialisation of the Visakhapatnam region have brought domestic sewage and industrial wastewater discharge into the coastal ocean. This study examines the indicator and pathogenic bacteria's quantitative abundance and antibiotic susceptibility. This study collected surface and subsurface water samples from ten different regions (147 stations; 294 samples), including 12 industrial discharge points, surrounding stations and two harbours from the coast of Pydibheemavaram to Tuni. Physicochemical parameters like salinity, temperature, fluorescence, pH, total suspended matter, nutrients, chlorophyll-*a* and dissolved oxygen showed a difference between regions. We noticed the presence of indicator (*Escherichia coli* and *Enterococcus faecalis*) and pathogenic (*Aeromonas hydrophila*, *Klebsiella pneumoniae, Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella* and *Shigella*, *Vibrio cholera* and *Vibrio parahaemolyticus*) bacteria among the samples. Waters from the near harbour and Visakhapatnam steel plant showed lower bacterial load with no direct input from industries to the coastal water. Samples collected during the industrial discharge period had a higher bacterial load, including *E. coli*. Enteric bacteria were found in higher numbers at most stations. Some isolates were resistant to multiple antibiotics with higher antibiotic resistance and multiple antibiotic resistance indexes compared with the other coastal water habitats in the Bay of Bengal. The occurrence of these bacteria above the standard limits and with multiple antibiotic resistance in the study region may pose a potential threat to the local inhabitants. It can create an alarming situation in the coastal waters in the study region.

Keywords Effluent discharge · Faecal pollution · Inhibition zone · AR index · MAR index · Multi-drug resistance

Introduction

Water is considered one of the major carriers of various diseases. More than 5 million people have mortality from water-related diseases annually (Gleick, 2002). Among the water-borne diseases, cholera alone is responsible for 21,000 to 143,000 deaths worldwide caused by a pathogenic bacterium, *Vibrio cholerae* (Ali et al., 2015). Pathogenic bacteria-mediated diseases are widespread and often fatal after viruses.

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Antibiotic resistance in pathogenic bacteria makes them multidrug-resistant, contributing to the spread and difficulty in treating the diseases caused by them. Studies from coastal water, mariculture sites (Chen et al., 2017; Zou et al., 2011) and wastewater treatment, pharmaceutical plants (Obayiuwana et al., 2018) have shown the presence of both antibiotic-resistant bacteria and antibiotic residues in them. Furthermore, 40% of the world's population lives within 100 km of the coast (Farmasi & Dan, 2017). Therefore, these people are always at immediate risk of contracting waterborne diseases from consuming seafood and other water-related activities, including fishing and recreation (Fowsul Ameer, 2017; Majra & Gur, 2009; Ralston et al., 2011).

Developing countries like India, Bangladesh and Myanmar surround the Bay of Bengal region from three sides. The population living in this region is one-third of the world. Among these, India has a vast coastline of 7516.6 km. According to a census by the Center for International Earth Science Information Network (CIESIN, 2007) (http://www.ciesin.org), the lower elevation zones of the East coast of India are densely populated. In addition to the population pressure, the presence of industries, thermal power plants and activities of harbours add to the increasing pollution and diseases along coastal regions. Moreover, Indian water receives a considerable amount of discharge from pharmaceutical companies in terms of active pharmaceutical ingredients (Larsson, 2008). A report by Reddy and Kostenzer (2011) indicates that the East coastal population of India is more vulnerable and at higher risk of contracting diseases.

Visakhapatnam, one of the major cities on the East coast of India, also suffers from industrial-derived pollution. This city's industrial region falls under India's eight major industrial regions, with many industries related to maritime, petroleum chemicals, pharmaceuticals, etc. The presence of two ports, one primary and a minor, adds to the industrial activities of this region. The pollution levels and the resulting contaminants in the coastal water of the city have been studied in the past, indicating its rise in recent times (Dileep & Prameela, 2021; Sarma et al., 1996). Microbiological studies have reported that treated/untreated sewage and urban run-off carry disease-causing bacteria that impair the coastal waters the most compared to other contaminants (Naidoo & Olaniran, 2014; Pandey et al., 2014).

Studies by Clark et al. (2003) and Lakshmi et al. (2019) showed coastal waters of Visakhapatnam city have a high pollution index in terms of different indicator organisms such as total coliforms, faecal coliforms and protozoan cysts. The city's fishing harbour and shipyard are primarily targeted for microbiological pollution studies, which showed that the abundance of selected pathogenic bacteria is higher than that on other city beaches (Babu et al., 2014; Myla et al., 2015; Sailaja et al., 2013). Heterotrophic bacterial counts, total coliforms, *Enterococcus faecalis* and *Pseudomonas aeruginosa*-like organisms in off-Visakhapatnam coastal waters were shaped by physicochemical conditions (Sudha Rani et al., 2018). Salinity, temperature and suspended particulate matter among these parameters influenced the presence and abundance of a pathogenic bacterium *V. cholerae* (Khandeparker et al., 2020).

The city across its coast has many industrial outfalls into the ocean. There are no studies on bacterial abundance and pollution near these industrial discharge regions. However, a study on plankton community composition from this region suggests no impact from the discharge sites (Shaik et al., 2017). The current study targets the coastal waters of the Visakhapatnam district, where major industrial discharge points are spread across a 150-km coastal area. The study aims to determine whether the heterotrophic and pathogenic bacterial abundance varies across the coast, and whether physicochemical parameters of the region have any role to play with the bacterial load. It tries to determine the effect of industrial discharge on the selected pathogenic bacterial groups in the coastal waters. Also, it attempts to detect the antibiotic resistance potential in the bacteria isolated from different discharge points. As part of this study, various physical, chemical and microbiological parameters are measured and analysed from these coastal waters. The parameters are then compared in groups to understand their variability, dependence and underlying cause with support from statistical analyses. The current study gives a primary understanding of various pathogenic bacteria present in these waters and their resistance to the different antibiotics from this region. This kind of study from the industrial coastal belt of Visakhapatnam is crucial and first from this region.

Materials and methods

Study area

In this study, the coastline from Pydibhimabaram to Tuni in the district of Visakhapatnam was chosen. This region covers ten central sampling locations, including treated effluent discharge points from industries and harbours (Fig. 1). The details of the study sites, industries, type of discharge and the number of stations sampled from each station are listed in Table 1. Except for 12 major discharge points, nearby stations varying in numbers from 9 to 24 surrounding the discharge points are also sampled. A total of 147 stations were sampled as part of this study. All these stations are between 0.5 and 6.6 km from the coast. For easier understanding, the stations are broadly divided into three groups, i.e. north stations (NS), middle stations (MS) and south stations (SS), based on their geographic location and time of collection. The NS included PBM and TPM; the MS included CHP, VSH, GPH and VSP; and the SS included TVM, PDM, NKP and TUN.

Sample collection and analyses

Samples were collected during two time periods. During the 1st phase of sampling (8 to 11 Dec 2018), Water samples from all the stations from CHP, VSH, GPH and VSP were collected. In the 2nd phase of sampling (24 Dec 2018 to 1 Jan 2019), water samples from the rest of the stations were collected. The second sampling phase was conducted to collect water samples from the stations that included industrial discharge points during their respective discharge period. A Niskin bottle (10 l) was used for a sample collection from 147 stations along the study area's coast. Both surface (below 0.5 m from the surface) and bottom (above 1 m from the bottom) water samples were collected from each station and fixed/preserved accordingly for downstream analyses.

Physicochemical parameters

Vertical profiles of pressure, temperature (°C) and salinity (ppt) were measured using a portable conductivity, temperature and depth profiler (SBE 19 plus; Sea-Bird Electronics, USA). First, bubble-free water is collected for dissolved oxygen (DO) and immediately fixed with Winkler's reagents, which were analysed later by the Winkler titration method following Carritt and Carpenter (1966). Water samples were collected in cleaned plastic bottles for nutrient analysis and fixed by adding mercury chloride (for ammonia, nitrate, nitrite and phosphate). The concentrations of nutrients were measured following standard spectrophotometric procedures (Grasshoff et al., 1983). A 2-l water sample was filtered through a GF/F filter

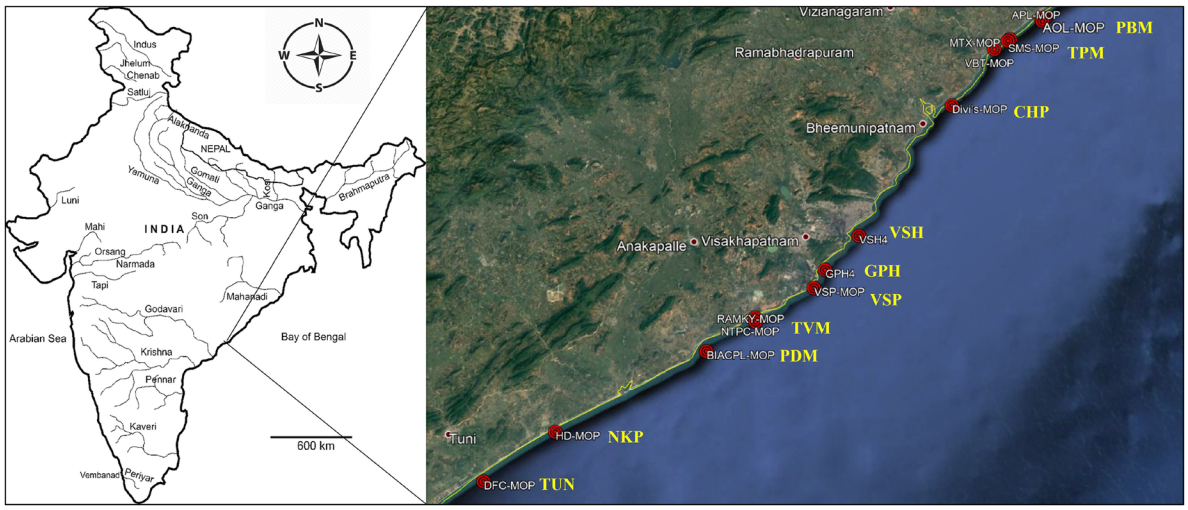

Fig. 1 Study area (PBM, Pydibheemabaram; TPM, Tammayyapalem; CHP, Chippada; VSH, Visakhapatnam harbour; GPH, Gangavaram; VSP, Visakhapatnam steel plant; TVM, Tikkavanipalem; PDM, Pudimadaka; NKP, Nakkapalli; TUN, Tuni)

Table 1 List of discharge points and their description

Place (abbr.)	Name of the industry	Nature of discharge	Date of stations sampled	No. of stations sampled
Pydibheemabaram (PBM)	Aurobindo Pharma Ltd. Andhra Organics Ltd. Lantech Pharmaceuticals Ltd.	Pharmaceutical	24 Dec 2018	03 discharge points 25 additional stations
Tammayyapalem (TPM)	M/s. Mylan Laboratories Ltd. SMS Pharmaceuticals Ltd. Vijayanagar Bio-Tech. Ltd.	Pharmaceutical, Industrial	25 Dec 2018	03 discharge points 22 additional stations
Chippada (CHP)	Divi's Laboratories Ltd.	Pharmaceutical	11 Dec 2018	01 discharge point, 11 additional stations
Visakhapatnam harbour (VSH)			8 Dec 2018	0 discharge point 09 stations
Gangavaram harbour (GPH)			9 Dec 2018	0 discharge point 09 stations
Visakhapatnam steel plant (VSP)	Rashtriya Ispat Nigam Ltd.	Industrial	10 Dec 2018	01 discharge point 10 additional stations
Tikkavanipalem (TVM)	Ramky's Pharma City	Pharmaceutical	27 Dec 2018	01 discharge point, 16 additional stations
Pudimadaka (PDM)	Brandex India Apparel City (Pvt.) Ltd.	Industrial	1 Jan 2019	01 discharge point 12 additional stations
Nakkapalli (NKP)	Drugs and Hetero Laboratories Ltd.	Pharmaceutical	28 Dec 2018	01 discharge point, 10 additional stations
Tuni (TUN)	Deccan Fine Chemicals India (Pvt.) Ltd.	Chemical, Pharmaceutical	30 Dec 2018	01 discharge point, 11 additional stations

(0.5-µm pore size, Whatman). Chlorophyll-*a* on the filter was extracted with 90% acetone at 4 °C in the dark for 12 h and then spectrofluorometrically analysed. Samples collected in acid-washed glass bottles were analysed for pH by potentiometric method (Dickson et al., 2007) using 835 Titrando (Metrohm, Switzerland). For total suspended matter (TSM), 1–2 l of water samples were filtered through 0.22-µm filter paper (MCE, 47 mm, Millipore), and they were dried, weighed and calculated in grammes per litre. The average values, along with standard deviations of the physicochemical parameters measured, are presented in Table 2.

Bacteriological parameters

About 100 ml of sample was taken into a pre-sterilised screw-capped bottle for bacterial analysis from the surface and bottom water from the Niskin water sampler's nozzle. All samples were collected after rinsing the sterile collected bottle with precautions required for microbiological analysis while wearing gloves. Samples were kept in an ice box for transportation to the lab, where it is analysed soon after or kept at 4 °C whenever necessary to stunt bacterial growth (APHA et al., 2012; Ramaiah et al., 2004). The bacteriological examinations were carried out following Ramaiah et al. (2004) and Prasad et al. (2015) to enumerate heterotrophic, indicator and few pathogenic bacteria. A 100-µl sample was taken, spread on various plates and incubated at 25 °C for 24–48 h. Sample dilution (10 times) was performed whenever the plate became overcrowded and re-plated. All the samples were plated in replicates. The number of colonies was then counted using a colony counter. The specific colonies (unique to the organism of interest) on the respective agar media (HIMEDIA) were quantified, and the results were expressed in colony-forming units (CFU ml^{-1}) after averaging out from the replicate plates according to Nagvenkar and Ramaiah (2009). Different selective, isolation and differential media were used for the specific bacterial groups. Heterotrophic bacterial counts were determined on R2A agar. Enumeration of coliforms on MacConkey agar, *Aeromonas hydrophila* on *Aeromonas* Isolation agar, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Staphylococcus aureus* on Universal differential

Table 2 Mean and standard deviation of physicochemical parameters

Parameter	Depth	PBM	TPM	CHP	VSH	GPH	VSP	TVM	PDM	NKP	TUN
Temp (°C)	Sur	24.50 ± 0.42	24.73 ± 0.11	26.33 ± 0.09	26.51 ± 0.14	26.45 ± 0.08	26.43 ± 0.11	24.16 ± 0.26	NA	24.22 ± 0.22	NA
	Bot	24.48 ± 0.16	24.63 ± 0.09	26.44 ± 0.28	27.13 ± 0.43	27.18 ± 0.48	27.18 ± 0.47	24.42 ± 0.41	NA	24.31 ± 0.07	NA
Sal (PPT)	Sur	28.48 ± 0.14	28.73 ± 0.07	27.83 ± 0.08	27.93 ± 0.12	27.82 ± 0.08	27.81 ± 0.03	29.25 ± 0.15	NA	29.46 ± 0.16	NA
	Bot	28.57 ± 0.09	28.81 ± 0.05	28.22 ± 0.68	30.15 ± 1.82	30.66 ± 2.05	30.54 ± 1.68	29.51 ± 0.41	NA	29.38 ± 0.10	NA
Flr (mg/m^3)	Sur	0.30 ± 0.09	0.89 ± 0.28	0.38 ± 0.14	0.24 ± 0.03	0.26 ± 0.03	0.34 ± 0.15	0.83 ± 0.52	NA	0.50 ± 0.49	NA
	Bot	0.56 ± 0.10	0.97 ± 0.22	0.64 ± 0.12	0.54 ± 0.17	0.41 ± 0.09	0.70 ± 0.24	1.51 ± 0.54	NA	0.50 ± 0.14	NA
PAR (µE/m^{2}s)	Sur	320.49 ± 227.28	271.53 ± 190.53	264.69 ± 168.10	264.42 ± 184.95	175.07 ± 194.51	262.11 ± 198.28	335.17 ± 320.22	NA	368.95 ± 334.92	NA
	Bot	6.86 ± 10.49	0.92 ± 0.87	31.28 ± 24.41	17.05 ± 14.61	24.51 ± 32.32	19.28 ± 19.43	14.87 ± 10.21	NA	27.05 ± 27.19	NA
pH	Sur	8.06 ± 0.57	8.08 ± 0.56	7.77 ± 0.46	7.76 ± 0.29	7.66 ± 0.12	7.65 ± 0.13	8.52 ± 0.15	8.56 ± 0.11	7.86 ± 0.48	8.41 ± 0.23
	Bot	8.17 ± 0.53	8.09 ± 0.53	7.79 ± 0.45	7.78 ± 0.33	7.60 ± 0.13	7.63 ± 0.08	8.45 ± 0.12	8.50 ± 0.19	7.89 ± 0.49	8.49 ± 0.08
TSM (mg/l)	Sur	10.87 ± 3.14	12.47 ± 5.32	13.32 ± 2.73	19.16 ± 4.12	31.66 ± 19.80	25.47 ± 5.07	11.88 ± 3.57	11.03 ± 5.24	10.21 ± 1.30	10.69 ± 3.03
	Bot	10.71 ± 2.57	12.27 ± 6.53	14.25 ± 2.41	20.93 ± 5.86	27.20 ± 5.26	26.95 ± 8.18	10.86 ± 3.46	8.53 ± 3.87	11.34 ± 2.83	12.26 ± 6.39
Chl-*a* (µg/l)	Sur	0.97 ± 0.57	1.61 ± 0.66	NA	0.41 ± 0.17	0.41 ± 0.17	NA	2.34 ± 1.34	1.18 ± 1.06	0.61 ± 0.29	0.48 ± 0.52
	Bot	0.86 ± 0.42	1.06 ± 0.52	NA	0.73 ± 0.33	0.73 ± 0.33	NA	1.73 ± 0.90	0.98 ± 0.80	0.60 ± 0.25	0.77 ± 0.56
DO (mg/ml)	Sur	7.74 ± 0.28	7.65 ± 0.37	6.95 ± 0.33	6.46 ± 0.34	7.03 ± 0.47	7.18 ± 0.32	7.08 ± 0.42	7.06 ± 0.40	7.36 ± 0.37	7.03 ± 0.46
	Bot	7.15 ± 0.26	7.03 ± 0.20	6.90 ± 0.34	6.54 ± 0.29	7.00 ± 0.34	7.08 ± 0.34	7.05 ± 0.34	6.97 ± 0.45	7.19 ± 0.37	7.02 ± 0.34
NH_4 (µM)	Sur	0.40 ± 1.12	0.40 ± 0.12	0.27 ± 0.09	0.39 ± 0.07	0.47 ± 0.13	0.36 ± 0.08	0.34 ± 0.14	0.35 ± 0.16	0.22 ± 0.10	0.56 ± 0.04
	Bot	0.40 ± 1.12	0.40 ± 0.13	0.30 ± 0.08	0.36 ± 0.05	0.48 ± 0.06	0.43 ± 0.08	0.41 ± 0.10	0.35 ± 0.17	0.27 ± 0.11	0.53 ± 0.06
NO_2 (µM)	Sur	0.23 ± 0.20	0.46 ± 0.33	0.31 ± 0.41	0.20 ± 0.07	0.27 ± 0.05	0.42 ± 0.38	0.47 ± 0.61	0.53 ± 0.62	0.51 ± 0.61	0.21 ± 0.03
	Bot	0.46 ± 0.47	0.56 ± 0.26	0.30 ± 0.25	0.29 ± 0.04	0.49 ± 0.65	1.77 ± 2.08	1.33 ± 1.91	1.70 ± 1.86	1.09 ± 0.29	0.29 ± 0.05
NO_3 (µM)	Sur	0.54 ± 0.48	1.58 ± 2.05	0.55 ± 0.35	0.81 ± 0.48	1.21 ± 0.33	1.38 ± 0.69	0.78 ± 0.84	1.23 ± 1.60	0.84 ± 0.82	0.82 ± 0.55
	Bot	0.62 ± 0.51	2.09 ± 2.29	1.00 ± 0.66	0.51 ± 0.23	1.99 ± 1.06	2.60 ± 1.57	0.82 ± 0.70	2.93 ± 3.01	1.13 ± 0.71	0.71 ± 0.69
PO_4 (µM)	Sur	0.54 ± 0.28	0.36 ± 0.39	0.13 ± 0.07	0.16 ± 0.07	0.24 ± 0.34	0.28 ± 0.24	0.41 ± 0.29	0.47 ± 0.51	0.29 ± 0.26	0.26 ± 0.17
	Bot	0.57 ± 0.28	0.70 ± 0.49	0.15 ± 0.08	0.31 ± 0.13	0.49 ± 0.58	0.50 ± 0.33	0.63 ± 0.36	0.47 ± 0.33	0.44 ± 0.50	0.50 ± 0.44

Temp temperature, *Sal* salinity, *PAR* photo active radiation, *DO* dissolved oxygen, *Chl-a* chlorophyll-*a*, *TSM* total suspended matter, *Flr* fluorescence, NH_4 ammonium, NO_3 nitrate, NO_2 nitrite, PO_4 phosphate, *NA* no data

medium, *Pseudomonas* sp. like *Pseudomonas aeruginosa* on Cetrimide agar, *Salmonella* spp. and *Shigella* spp. on xylose-lysine-deoxycholate agar, and *Vibrio* spp. like *Vibrio cholerae*, *Vibrio fluvialis*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* on thiosulphate citrate bile salts sucrose agar were determined.

Antibiogram

We isolated presumptive pathogenic bacterial colonies from respective agar plates using the streak plate method. Forty-six isolates from respective agar plates were transferred to 1 ml of Mueller Hinton broth and left to grow overnight. The concentrations of the cultures were adjusted to 0.5 McFarland (OD of 0.1 at 600 nm) before plating. Mueller Hinton agar plates were prepared, and new cultures from the tubes were spread evenly using sterilised cotton swabs covering the whole surface. A total of 21 antibiotic discs used for testing the sensitivity/resistance of *Enterobacteriaceae* isolates as per Clinical and Laboratory Standards Institute (CLSI) guidelines are cefuroxime, cefoxitin, ceftazidime, ceftriaxone, cefoperazone, cefotaxime, cefepime, piperacillin, piperacillin/tazobactam, ampicillin/sulbactam, nalidixic acid, norfloxacin, ciprofloxacin, gentamicin, amikacin, imipenem, meropenem, azithromycin, tetracycline, aztreonam and chloramphenicol (C). These antibiotic discs were placed on MH agar plates and left overnight to grow. The next day, the diameter of the inhibition zones was measured using a scale specifically for this purpose. Among them, 11 antibiotics are considered for *Pseudomonas aeruginosa* isolates as per CLSI guidelines in M100 (CLSI, 2017).

The resulting inhibition zones were compared with the inhibition zone diameter range given in the current CLSI guidelines that determine the inhibition zone diameter breakpoint where the bacterial strain is resistant, intermediate or susceptible to the given antibiotic, which follows the Kirby-Bauer disc diffusion method (Bauer et al., 1966). For the *Enterobacteriaceae* family, 21, and *Pseudomonas*, 11 antibiotics were taken for testing. The antibacterial resistance (AR) index for these samples based on the isolates was determined.

$$\text{AR index} = y/nx$$

where y is the number of resistant microbes in the sample, n is the population size and x is the total number of antibiotics used.

For isolates resistant to 3 or more antibiotics, multiple antibiotic resistance (MAR) index was calculated.

$$\text{MAR index} = a/b$$

where a is the no. of antibiotics, the isolate is resistant to; b is the no. of antibiotics, the isolate is exposed to/tested.

Statistical analyses

To compare the means of the parameters and whether they vary significantly spatially, pair-wise statistical analyses were performed between NS, MS and SS, combining both values in surface and sub-surface water samples. A parametric Tukey's honestly significant difference (HSD) test was performed for physicochemical parameters (Table 3). A non-parametric Dunn's post hoc test is carried out for bacteriological parameters (Table 4). Principal component analysis (PCA) was performed to infer the effect of physical (temperature, salinity, PAR and fluorescence) and chemical parameters (dissolved oxygen, chlorophyll-*a*, total suspended matter, pH, ammonium, nitrate, nitrite and phosphate) on bacteriological parameters (heterotrophic bacterial count, total coliforms, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Aeromonas hydrophila*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella*, *Shigella*, *Vibrio cholera* and *Vibrio parahaemolyticus*) for surface samples and bottom samples separately. All the statistical analyses were performed using PAST 4.03 software.

Results

Physicochemical parameters

The range of physicochemical parameters in both surface and bottom waters is summarised in Table 2 and Supplementary Figs. 2 and 3. The VSH and GPH stations were deeper compared to all other stations. They are also far from the coast (Supplementary Fig. 3). The mean temperature for the NS and SS groups varied between 24 and 25 °C. In contrast, the temperature of the MS group was 1 °C higher in both surface and subsurface waters (Table 2, Supplementary Figs. 2 and 3). The temperature in SS was found to be a little lower as compared to NS. In MS, the subsurface water temperature was more than the surface compared to

Table 3 Parametric Tukey's HSD (honestly significant difference) test of all the physicochemical parameters between three groups (*NS* north stations, *MS* middle stations, *SS* south stations)

Parameters	NS vs MS (*p*-value)	MS vs SS (*p*-value)	SS vs NS (*p*-value)
Temperature	< 0.005**	< 0.005**	< 0.05*
Salinity	NS	< 0.005**	< 0.005**
Fluorescence	< 0.005**	< 0.005**	< 0.005**
PAR	< 0.05*	NS	< 0.05*
pH	< 0.005**	< 0.005**	< 0.005**
Total suspended matter	< 0.005**	< 0.005**	NS
Chlorophyll-*a*	< 0.005**	< 0.005**	NS
Dissolved oxygen	< 0.005**	< 0.05*	< 0.005**
Ammonia	NS	NS	NS
Nitrite	NS	NS	< 0.05*
Nitrate	NS	NS	NS
Phosphate	< 0.005**	< 0.005**	NS

NS not significant (*p* value > 0.05)

*Significant

**Highly significant

NS and SS, where the temperature in the water column is more or less constant. Salinity showed an opposite trend as opposed to temperature. The salinity of MS was 1 ppt lower than SS and NS. The subsurface salinity of MS was 1 to 3 ppt higher than the surface water. Lower pH was found in MS compared to SS and NS (Table 2, Supplementary Figs. 2 and 3). Higher pH values were observed in TVM, PDM and TUN (Table 2, Supplementary Figs. 2 and 3). Stations of TVM showed a little higher chlorophyll-*a* than others (Table 2, Supplementary Figs. 2 and 3). TSM was higher in MS than SS and NS (Table 2, Figs. 2 and 3) and higher in surface and bottom waters. DO concentration was slightly higher on the surface than in the subsurface water. DO is less in both surface and bottom waters in the MS, especially in VSH (Table 2, Supplementary Figs. 2 and 3). Subsurface waters showed higher nitrate, nitrite and phosphate concentrations than surface waters, and ammonia concentration did not differ much. Nitrite concentration showed a higher trend towards SS (Table 2, Supplementary Figs. 2 and 3). Ammonia concentration was higher in TUN in surface and bottom waters (Table 2, Supplementary Figs. 2 and 3).

Table 4 Non-parametric Dunn's post hoc test (pairwise) of all the microbiological parameters between three groups (*NS* north stations, *MS* middle stations, *SS* south stations)

Parameters	NS vs MS (*p*-value)	MS vs SS (*p*-value)	SS vs NS (*p*-value)
HBC	< 0.005**	NS	NS
TC	< 0.005**	< 0.005**	NS
EC	< 0.005**	NS	< 0.005**
EF	< 0.005**	NS	< 0.005**
KP	< 0.05*	< 0.05*	< 0.005**
PM	< 0.005**	< 0.005**	NS
PA	< 0.05*	NS	NS
AH	< 0.005**	< 0.005**	< 0.05*
SS	< 0.005**	< 0.05*	< 0.005**
VC	< 0.005**	NS	< 0.005**
VP	< 0.005**	NS	< 0.005**

HBC heterotrophic bacterial count, *TC* total coliforms, *EC Escherichia coli*, *EF Enterococcus faecalis*, *KP Klebsiella pneumonia*, *AH Aeromonas hydrophila*, *PM Proteus mirabilis*, *PA Pseudomonas aeruginosa*, SS *Salmonella-* and *Shigella*-like organisms, *VC Vibrio cholera* and *VP Vibrio parahaemolyticus*, *NS* not significant (*p*-value > 0.05)

*Significant

**Highly significant

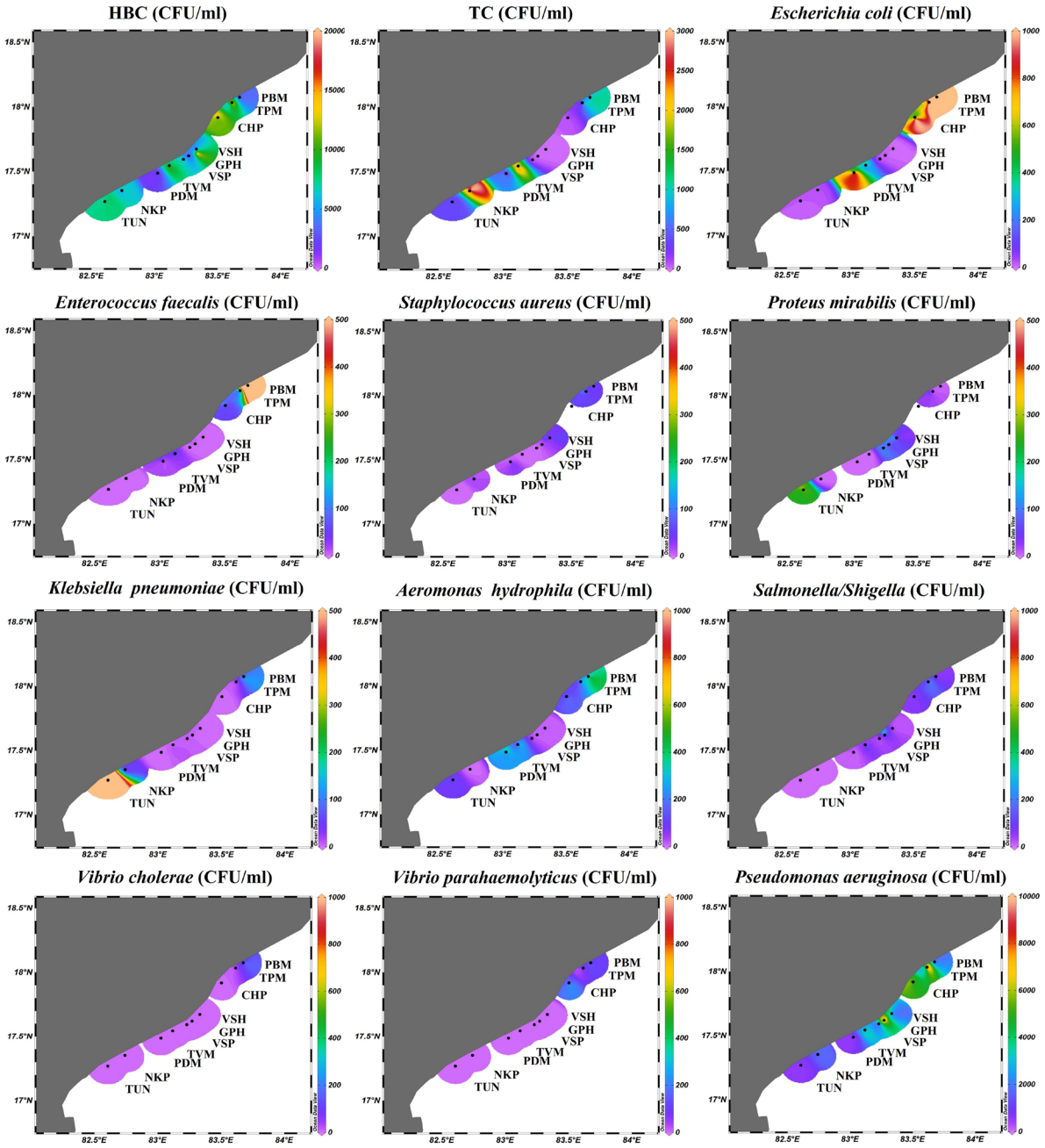

Fig. 2 Bacterial counts of surface water (HBC, TC, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Aeromonas hydrophila*, *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella*- and *Shigella*-like organisms, *Vibrio cholera* and *Vibrio parahaemolyticus*)

Heterotrophic, indicator and pathogenic bacteria

The range of all bacterial counts (CFU/ml) in both surface and bottom waters are summarised in Supplementary Table 1, and the trend is shown in Figs. 2 and 3. Surface water HBC was found to be higher in CHP stations, followed by VSP, NKP, GPH and TVM stations, lowest was found in VSH stations (Fig. 2). Similarly, CHP stations had the highest HBC in subsurface water (Fig. 3). HBC of subsurface waters

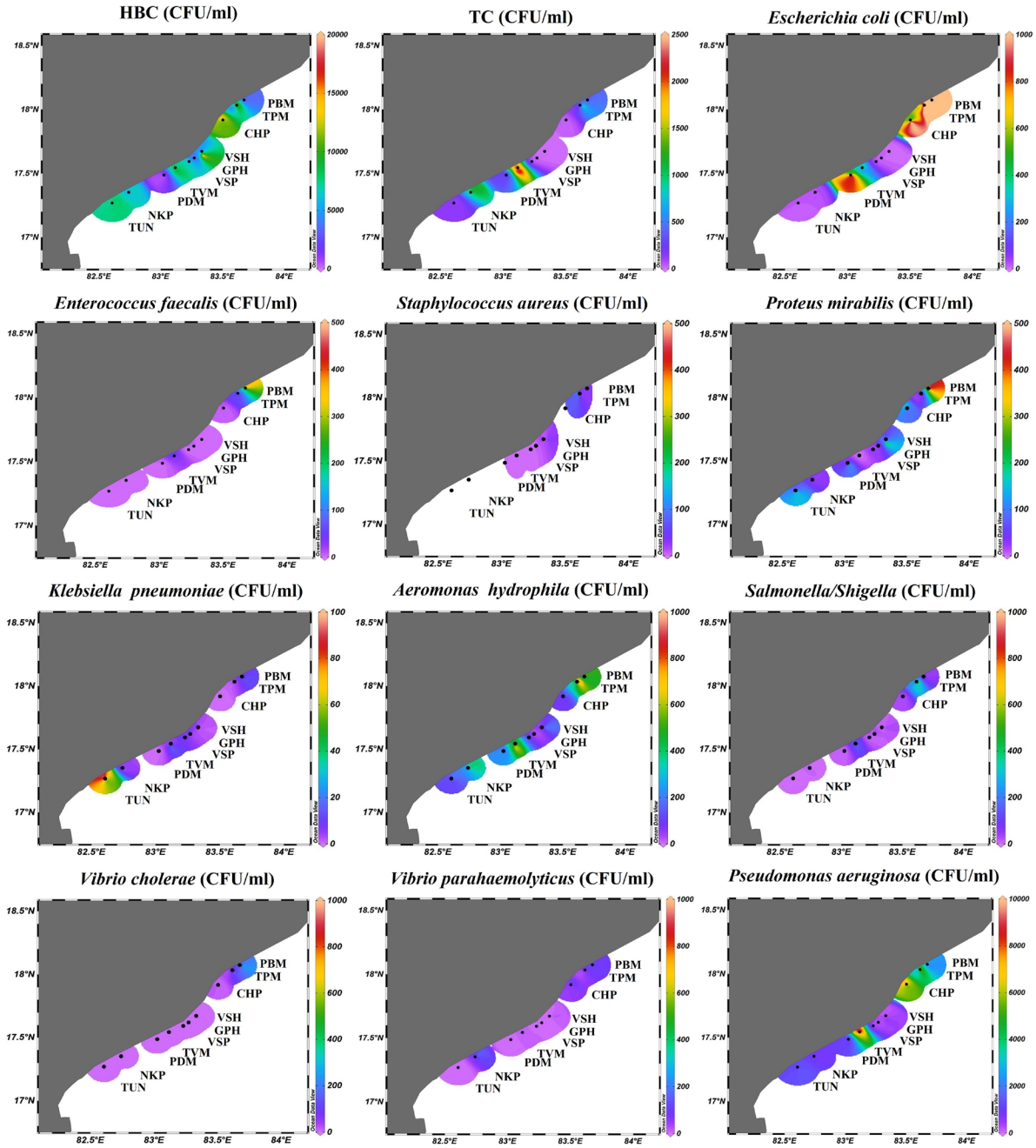

Fig. 3 Bacterial counts of subsurface water (HBC, TC, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Aeromonas hydrophila*, *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella*- and *Shigella*-like organisms, *Vibrio cholera* and *Vibrio parahaemolyticus*)

was more compared to surface in most of the stations. Surface water total coliform (TC) counts were highest in PDM stations and lowest in the MS group except for CHP. Subsurface TC was highest in TVM stations (Fig. 3). *Escherichia coli* (EC) showed a different pattern; surface EC numbers were found to be higher in the NS group and 2 stations of the SS group (PDM, NKP). Similarly, PBM, TPM and PDM stations had higher subsurface EC counts and CHP stations (Fig. 3). VSH, GPH and VSP stations had

the lowest EC numbers. Enterococcus faecalis (EF) was present only in PBM, TPM and CHP stations in surface and subsurface waters. *Klebsiella pneumoniae* (KP) was in relatively higher numbers in PBM and TUN stations (Figs. 2 and 3). KP counts in other stations are found to be low. *Proteus mirabilis* (PM) was higher in TUN stations, followed by VSH, GPH and VSP (Fig. 2) in surface waters. In subsurface waters, they were high in PBM (Fig. 3). Though the counts of *Staphylococcus aureus* were less across all the stations, some signatures were found in stations of PBM, TPM, CHP, VSP and TVM (Figs. 2 and 3). *Aeromonas hydrophila* (AH) was found in surface and subsurface waters NS and SS group and was less in MS (Figs. 2 and 3). *Vibrio cholera* (VC) and *Vibrio parahaemolyticus* (VP) were primarily found in the subsurface and surface waters of NS group stations (Figs. 2 and 3). VP was additionally found in CHP and some stations of NKP (Figs. 2 and 3). *Pseudomonas aeruginosa* (PA) was found in most stations in higher numbers than other pathogenic bacteria (Figs. 2 and 3). Their numbers were highest on the surface waters of TPM and GPH, followed by CHP and PBM stations. In the subsurface water, PA counts were highest in TVM, followed by CHP, TPM and TVM (Figs. 2 and 3).

Antibiotic susceptibility test

Among the 28 isolates belonging to the Enterobacteriaceae family, only 3 were sensitive to all antibiotics, and among the 18 *Pseudomonas* isolates, 5 were sensitive to all antibiotics. Out of all the 46 isolates, 38 were resistant to at least 1 antibiotic used in the study (Tables 5 and 6). Isolates from the *Enterobacteriaceae* family showed resistance to more antibiotics than *Pseudomonas* (Tables 5 and 6). Many *Enterobacteriaceae* isolates were resistant antibiotics such as aztreonam (24), cefuroxime (12), cefotaxime (12), ceftazidime (14), cefepime (12) and azithromycin (11). No *Enterobacteriaceae* isolates were resistant to chloramphenicol, norfloxacin and tetracycline. Among the *Enterobacteriaceae* isolates, 85% and among *Pseudomonas*, 66% were resistant to aztreonam, followed by ceftazidime (50%, 27%) and cefepime (42%, 22%) for *Enterobacteriaceae* and *Pseudomonas* respectively. In the case of *Enterobacteriaceae* isolates, 42.8%, 42.8% and 39.2% were resistant to cefuroxime, cefotaxime and azithromycin, respectively. Similar inference can be drawn in terms of AR indexes for each antibiotic, with aztreonam having an AR index of 0.04, followed by ceftazidime (0.023), cefuroxime (0.02), cefotaxime (0.02) and cefepime (0.02) (Table 5). Isolates belonging to the genera *Proteus* and *Escherichia* showed the most resistance to the antibiotics tested (Table 5). Aztreonam had the highest AR index of 0.06 among the *Pseudomonas* isolates, followed by 0.025 for ceftazidime and 0.02 for cefepime (Table 6). Among 28 isolates of the Enterobacteriaceae family, 13 had a MAR index more than or equal to 0.2, and among *Pseudomonas*, the number was 4 among the 18 isolates (Table 7).

Statistical analyses

Temperature, fluorescence, pH and DO concentrations showed significant variation in their means among the 3 stations when compared pair-wise (p-value < 0.05) at a 95% confidence interval (Tukey's HSD test) (Table 3). The salinity of the SS group showed a significant difference compared to NS and MS groups. Nutrients did not show significant variation among the 3 groups except for phosphate. The MS group was found to be different from the other two groups in terms of values of different physicochemical parameters. Dunn's post hoc test for the non-parametric distribution of bacteriological parameters suggested a significant difference between the 3 groups (Table 4). *Aeromonas hydrophila*, *Salmonella Shigella*-like organisms and *Klebsiella pneumoniae* numbers showed a significant difference in all three groups, as inferred by the pairwise test at a 95% significance level. The test suggested NS group differs from other groups regarding various bacterial counts.

The surface sample PCA (Fig. 4) has two significant clusters. The first cluster included stations of the MS group along the direction of higher temperature and TSM load and opposite to salinity. The other cluster contained mostly the SS group (TVM, PDM, NKP) and one from the NS group (TPM) in the direction of higher salinity, pH, fluorescence, chlorophyll-*a* and TC. PBM was separate from others and showed positive variation with dissolved oxygen concentration, EC, EF, AH, KP, VC and VP. The first two components explained the 22.82% variation in the data (PC1: 13.61%; PC2: 9.21%) in the PCA.

In the bottom samples, a similar grouping is seen (Fig. 5) but it is not that prominent compared to the

Table 5 Antibiotic resistance pattern among the isolates belonging to the Enterobacteriaceae family and AR indexes of each antibiotic tested

Antibiotic	Resistant (R)	Intermediate (I)	Susceptible (S)	AR Index
CTX	01 (*Escherichia*), 01 (*Enterococcus*), 05 (*Proteus*), 01 (*Staphylococcus*), 02 (*Klebsiella*), 02 (*Salmonella*)	01 (*Escherichia*), 01 (*Enterococcus*)	06 (*Escherichia*), 02 (*Enterococcus*), 02 (*Staphylococcus*), 03 (*Klebsiella*), 01 (*Salmonella*)	0.02
CAZ	03 (*Escherichia*), 01 (*Enterococcus*), 05 (*Proteus*), 02 (*Klebsiella*), 03 (*Salmonella*)	01 (*Escherichia*), 01 (*Enterococcus*)	04 (*Escherichia*), 02 (*Enterococcus*), 03 (*Staphylococcus*), 03 (*Klebsiella*),	0.023
PI	01 (*Escherichia*), 02 (*Enterococcus*), 02 (*Proteus*), 01 (*Klebsiella*), 01 (*Salmonella*)	01 (*Escherichia*), 01 (*Proteus*)	06 (*Escherichia*), 02 (*Enterococcus*), 03 (*Staphylococcus*), 04 (*Klebsiella*), 02 (*Proteus*), 02 (*Salmonella*)	0.011
IPM	01 (*Escherichia*), 01 (*Proteus*)	01 (*Enterococcus*)	07 (*Escherichia*), 03 (*Enterococcus*), 03 (*Staphylococcus*), 05 (*Klebsiella*), 04 (*Proteus*), 03 (*Salmonella*)	0.003
NA	01 (*Escherichia*), 01 (*Enterococcus*), 01 (*Staphylococcus*), 01 (*Klebsiella*), 01 (*Salmonella*)	02 (*Escherichia*)	05 (*Escherichia*), 03 (*Enterococcus*), 02 (*Staphylococcus*), 04 (*Klebsiella*), 05 (*Proteus*), 02 (*Salmonella*)	0.008
AT	07 (*Escherichia*), 03 (*Enterococcus*), 02 (*Staphylococcus*), 04 (*Klebsiella*), 05 (*Proteus*), 03 (*Salmonella*)	01 (*Enterococcus*)	01 (*Escherichia*), 01 (*Staphylococcus*), 01(*Klebsiella*),	0.04
CX	01 (*Enterococcus*), 03 (*Proteus*), 01 (*Salmonella*)	01 (*Escherichia*)	07 (*Escherichia*), 03 (*Enterococcus*), 03 (*Staphylococcus*), 05 (*Klebsiella*), 02 (*Proteus*), 02 (*Salmonella*)	0.008
AK	02 (*Escherichia*), 01 (*Enterococcus*), 01 (*Staphylococcus*), 01 (*Proteus*)	0	06 (*Escherichia*), 03 (*Enterococcus*), 02 (*Staphylococcus*), 05 (*Klebsiella*), 04 (*Proteus*), 03 (*Salmonella*)	0.008
AZM	04 (*Escherichia*), 01 (*Proteus*), 01 (*Enterococcus*), 01 (*Klebsiella*), 02 (*Staphylococcus*), 02 (*Salmonella*)	0	04 (*Escherichia*), 03 (*Enterococcus*), 01 (*Staphylococcus*), 04(*Klebsiella*), 04 (*Proteus*), 01 (*Salmonella*)	0.018
CPM	01 (*Escherichia*), 05 (*Proteus*), 01 (*Enterococcus*), 02 (*Klebsiella*), 01(*Staphylococcus*), 02 (*Salmonella*)	01 (*Escherichia*), 02 (*Enterococcus*)	06 (*Escherichia*), 01 (*Enterococcus*), 02 (*Staphylococcus*), 03 (*Klebsiella*), 01 (*Salmonella*)	0.02
CPZ	01 (*Proteus*), 01(*Klebsiella*)	02 (*Enterococcus*), 02 (*Proteus*), 01 (*Salmonella*)	08 (*Escherichia*), 02 (*Enterococcus*), 03 (*Staphylococcus*), 04(*Klebsiella*), 02 (*Proteus*), 02 (*Salmonella*)	0.003
CXM	02 (*Escherichia*), 05 (*Proteus*), 02(*Klebsiella*), 01(*Staphylococcus*), 01 (*Enterococcus*), 01 (*Salmonella*)	01 (*Enterococcus*), 01 (*Staphylococcus*)	06 (*Escherichia*), 02 (*Enterococcus*), 01 (*Staphylococcus*), 03 (*Klebsiella*), 02 (*Salmonella*)	0.02

Table 5 (continued)

Antibiotic	Resistant (R)	Intermediate (I)	Susceptible (S)	AR Index
CTR	01 (*Escherichia*), 01(*Klebsiella*), 02 (*Proteus*), 02 (*Salmonella*)	02 (*Enterococcus*), 01 (*Klebsiella*), 02 (*Proteus*)	07 (*Escherichia*), 02 (*Enterococcus*), 03 (*Staphylococcus*), 03 (*Klebsiella*), 01 (*Proteus*), 01 (*Salmonella*)	0.01
CIP	01 (*Proteus*)	03 (*Escherichia*), 02 (*Enterococcus*), 02 (*Staphylococcus*), 01(*Klebsiella*)	05 (*Escherichia*), 02 (*Enterococcus*), 01 (*Staphylococcus*), 04(*Klebsiella*), 04 (*Proteus*), 03 (*Salmonella*)	0.001
GEN	01(*Klebsiella*)	0	08 (*Escherichia*), 04 (*Enterococcus*), 03 (*Staphylococcus*), 04 (*Klebsiella*), 05 (*Proteus*), 03 (*Salmonella*)	0.001
PIT	01 (*Proteus*)	0	08 (*Escherichia*), 04 (*Enterococcus*), 03 (*Staphylococcus*), 05 (*Klebsiella*), 04 (*Proteus*), 03 (*Salmonella*)	0.001
A/S	01 (*Proteus*)	0	08 (*Escherichia*), 04 (*Enterococcus*), 03 (*Staphylococcus*), 05 (*Klebsiella*), 04 (*Proteus*), 03 (*Salmonella*)	0.001
MRP	01 (*Proteus*)	0	08 (*Escherichia*), 04 (*Enterococcus*), 03 (*Staphylococcus*), 05 (*Klebsiella*), 04 (*Proteus*), 03 (*Salmonella*)	0.001
C	0	01 (*Escherichia*), 01 (*Proteus*)	07 (*Escherichia*), 04 (*Enterococcus*), 03 (*Staphylococcus*), 05 (*Klebsiella*), 04 (*Proteus*), 03 (*Salmonella*)	0
NX	0	01 (*Staphylococcus*)	08 (*Escherichia*), 04 (*Enterococcus*), 02 (*Staphylococcus*), 05 (*Klebsiella*), 05 (*Proteus*), 03 (*Salmonella*)	0
TE	0	0	08 (*Escherichia*), 04 (*Enterococcus*), 03 (*Staphylococcus*), 05 (*Klebsiella*), 05 (*Proteus*), 03 (*Salmonella*)	0

CXM cefuroxime, *CX* cefoxitin, *CAZ* ceftazidime, *CTR* ceftriaxone, *CPZ* cefoperazone, *CTX* cefotaxime, *CPM* cefepime, *PI* piperacillin, *PIT* piperacillin/tazobactam, *A/S* ampicillin/sulbactam, *NA* nalidixic acid, *NX* norfloxacin, *CIP* ciprofloxacin, *GEN* gentamicin, *AK* amikacin, *IPM* imipenem, *MRP* meropenem, *AZM* azithromycin, *TE* tetracycline, *AT* aztreonam, *C* chloramphenicol

Table 6 Antibiotic resistance pattern among the isolates belonging to the *Pseudomonas* genus and AR indexes of each antibiotic tested

Antibiotic	Resistant (R)	Intermediate (I)	Susceptible (S)	AR index
CAZ	05 isolates	02 isolates	11 isolates	0.025
PIT	01 isolate	0	17 isolates	0.005
PI	02 isolates	03 isolates	13 isolates	0.01
IPM	01 isolate	0	17 isolates	0.005
MRP	01 isolate	0	17 isolates	0.005
NX	01 isolate	01 isolate	16 isolates	0.005
CIP	01 isolate	0	17 isolates	0.005
AT	12 isolates	01 isolate	05 isolates	0.06
GEN	01 isolate	0	17 isolates	0.005
AK	02 isolates	03 isolates	13 isolates	0.01
CPM	04 isolates	0	14 isolates	0.02

CAZ ceftazidime, *CPM* cefepime, *PI* piperacillin, *PIT* piperacillin/tazobactam, *NX* norfloxacin, *CIP* niprofloxacin, *GEN* gentamicin, *AK* amikacin, *IPM* imipenem, *MRP* meropenem, *AT* aztreonam

former arrangement of the surface samples. Again, MS group stations are clustered with CHP far from the main group. The grouping was in the direction of temperature, TSM and salinity. The second cluster was found to be present in the middle with the SS group (PDM, TUN, NKP) except TVM. TVM was present in the direction of TC, PA and SS counts. Station PDM fell in the direction of EC < EF, VC and PM, separated from other stations. The first two components in this PCA explained a 23.99% variation in the data (PC1: 14.63%; PC2: 9.36%).

Discussion

Effect of rainfall on physicochemical parameters

A temperature difference of 2 °C in the MS group station surface waters compared to other groups can be attributed to the time of collection. Water samples from these places were collected during the 1st phase of sampling, whereas other samples were collected during the 2nd phase. The water temperature corresponds to the air temperature recorded during these times, which was 23 to 30 °C during early December and decreased to 17 to 27 °C during late December. The lower salinity observed in the MS group is attributed to rainfall near these places before the period of sampling (Supplementary Fig. 1). Except for these variations, the usual trend of salinity towards the southern station is increasing because the northern Bay of Bengal is directly affected by freshwater influx from rivers that drain into the ocean. During December, East India Coastal Current is southwards (Shankar et al., 1996), which brings a gradual change in salinity which increases downwards to the South. The stations which have a 3–4-ppt increase in salinity from surface to subsurface waters of VSH, GPH and VSP have a depth of 25 m or more, whereas other stations where salinity has a maximum 1 ppt difference between the surface and subsurface waters have depths under 15 m. A little lower pH of these stations can also be attributed to the rainfall, as rainwater has a lower pH. These particular stations also had 2 to 2.5 times higher TSM than other stations, corresponding to lower chlorophyll-*a* contents, as higher TSM can reduce the availability of sunlight, reducing phytoplankton abundance (Sarma et al., 2010; Sudha Rani et al., 2018). This result is supported by the PCA (Figs. 4 and 5), where total suspended matter is

Table 7 MAR indexes of isolates

	Enterobacteriaceae	*Pseudomonas* sp.
Number of isolates with MAR index 0	03	05
Number of isolates with MAR index $0 > X < 0.2$	12	09
Number of isolates with MAR index 0.2–0.3	06	03
Number of isolates with MAR index > 0.3	07	01
The total number of isolates tested	28	18

MAR multiple antibiotic resistance (*X*)

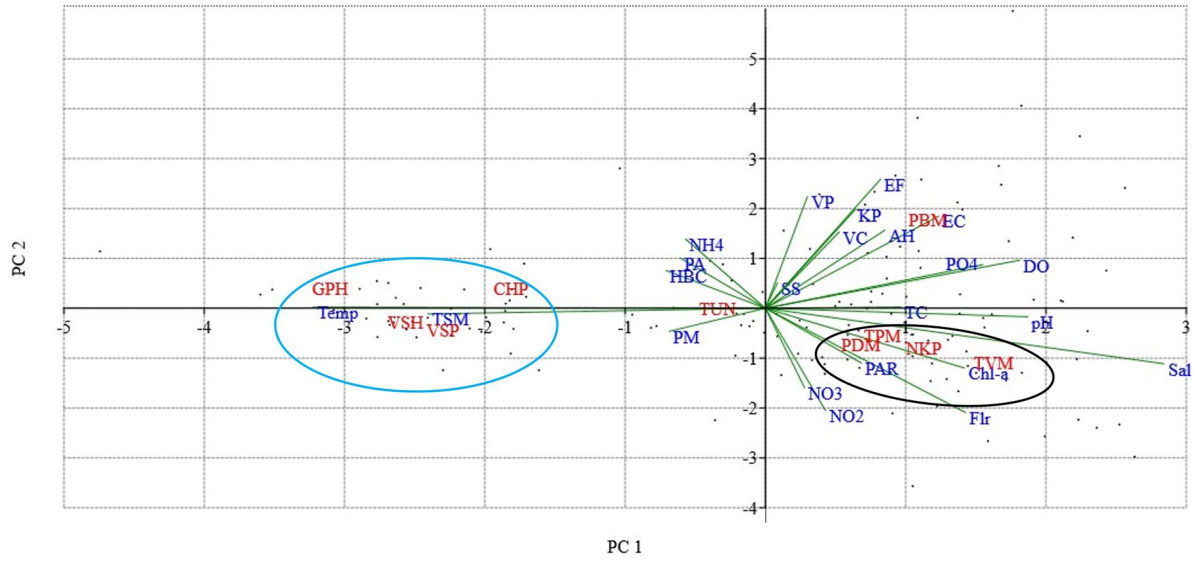

Fig. 4 Principal component analysis of surface samples (Temp, temperature; Sal, salinity; PAR; DO, dissolved oxygen; Chl-*a*, chlorophyll-*a*; TSM, total suspended matter; Frsn, fluorescence; pH; NH_4, ammonium; NO_3, nitrate; NO_2, nitrite; PO_4, phosphate; HBC, heterotrophic bacterial count; TC, total coliforms; EC, *Escherichia coli*; EF, *Enterococcus faecalis*; KP, *Klebsiella pneumonia*; AH, *Aeromonas hydrophila*; PM, *Proteus mirabilis*; PA, *Pseudomonas aeruginosa*; SS, *Salmonella-* and *Shigella*-like organisms; VC, *Vibrio cholera;* and VP; *Vibrio parahaemolyticus*)

grouped with stations VSH, GPH and VSP. Among these stations, the highest TSM load of GPH stations may depend on the port activity and dredging caused by the port authority, fishing nets and trawling. Nutrients like ammonium, nitrate, nitrite and phosphate did not differ greatly. Principal component analysis of surface and bottom waters shows the significant effect of rainfall on the stations where two different groups are formed, one including the MS group with higher temperature and low salinity, and another with other stations with lower temperature and higher salinity conditions (Figs. 4 and 5).

Spatial distribution of bacterial numbers

Sample properties of all the stations showed variation in two groups based on the time of collection, affecting the water's physical and chemical properties. During this sampling, changes in physical parameters are more prominent than chemical. The waters of MS group stations were warmer, less saline and less alkaline compared to other stations from which water samples were collected 13 days later. Heterotrophic bacteria use organic matter for energy production; their abundance in most samples indicates their presence is not affected considerably by the physical property change of the water column. While heterotrophic bacterial counts and total coliform counts are helpful in the prediction of the microbial processes that are going on in the water column, specific bacteria have been used as indicators of heavy metal pollution, faecal contamination, wastewater pollution and oil contamination (Sumampouw & Risjani, 2014). As enteric and faecal-origin bacteria like *Escherichia coli* and *Enterococcus faecalis* have a narrow host range of humans and other warm-blooded animals, they help determine the source of contamination and the extent of anthropogenic pollution (Brandt et al., 2017; Wheeler et al., 2002). Indicator bacteria like *E. coli* and *E. feacalis* counts showed a particular pattern in this study. Both were highest in PBM. *E. faecalis* was found in higher numbers in NS regions (TPM and CHP) and was mostly absent after that, except in a few stations. *E. coli* numbers were high in similar stations as *E. faecalis*, including PDM. These bacteria were present in fewer numbers in other stations and were very few in VSH and GPH stations. All these stations (PBM, TPM, CHP, PDM and TVM) are located near the coast where small villages, including fishing villages, are present. The

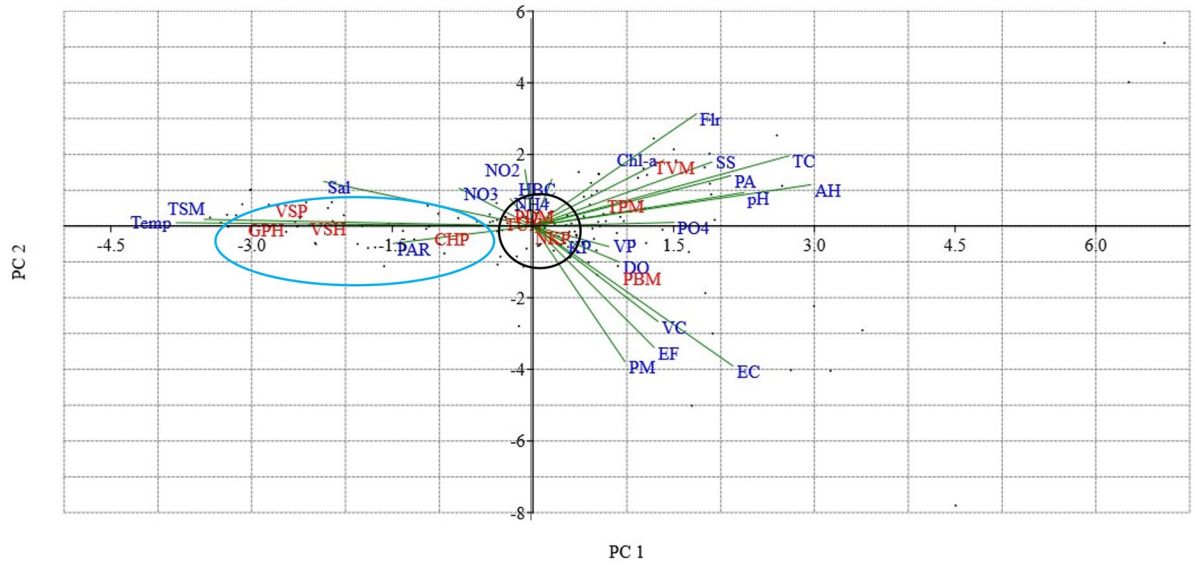

Fig. 5 Principal component analysis of bottom samples (Temp, temperature; Sal, salinity; PAR; DO, dissolved oxygen; Chl-*a*, chlorophyll-*a*; TSM, total suspended matter; Frsn, fluorescence; pH; NH_4, ammonium; NO_3, nitrate; NO_2, nitrite; PO_4, phosphate; HBC, heterotrophic bacterial count; TC, total coliforms; EC, *Escherichia coli*; EF, *Enterococcus faecalis*; KP, *Klebsiella pneumonia*; AH, *Aeromonas hydrophila*; PM, *Proteus mirabilis*; PA, *Pseudomonas aeruginosa*; SS, *Salmonella*- and *Shigella*-like organisms; VC, *Vibrio cholera* and VP, *Vibrio parahaemolyticus*)

sampling location PBM is close to the biggest village, among others. PCA of both surface and bottom water also indicates PBM stations have a higher abundance of *E. coli*, *E. faecalis*, *Aeromonas hydrophila*, *Klebsiella pneumoniae*, *Vibrio cholerae* and *Vibrio parahaemolyticus* as compared to others (Figs. 4 and 5). The presence of villages can affect the health of coastal water negatively. Land run-off into the ocean from these places can be affected by human and animal faeces via domestic sewage, open defecation and water from animal sheds. This water can harbour various enteric and pathogenic bacteria. Less commonly used indicator bacteria like *K. pneumoniae* were found in VSH and GPH along with northern stations. Another pathogenic bacterium, *Proteus mirabilis*, showed a reverse pattern in its presence. They were found in most of the stations of the MS group (VSH, GPH and VSP) and the bottom waters of a few other stations. Though these waters have few *E. coli* and *E. faecalis* and are present offshore, enteric bacteria like *P. mirabilis* show potential faecal contamination from the port region. The presence of opportunistic pathogenic bacteria like *Pseudomonas aeruginosa* in all of the stations in high abundance shows their adaptability to spatial and temporal variations of different parameters, as indicated by their genome study (Silby et al., 2011). Fish pathogenic bacteria like *A. hydrophila* counts were higher in bottom waters and did not show considerable variation spatially. *Vibrio* groups that included *V. cholerae* and *V. parahaemolyticus* were present primarily in the northern stations, including PBM, TPM and CHP. A study by Prasanthan et al. (2011) from the Kerala coast has shown that *V. cholerae* and *V. parahaemolyticus* have a positive correlation with each other similar to our study where these two are co-present in the stations. Increased counts of *E. coli*, *A. hydrophila* and *P. mirabilis* in the bottom waters of most of the stations indicate mixing activity near-bottom sediment and their mode of respiration (as these bacteria are facultative anaerobes). The difference between 24 days during samplings and rainfall during earlier sampling days effectively controlled these bacterial numbers. Additionally, bacterial abundance data directly from the discharge waters of PBM, CHP, TVM and NKP (from the industry before discharge into the sea) showed consistency with our data from the corresponding discharge points in the sea (Supplementary Table 2).

A study from a marine coastal zone in Italy found that the counts of *E. coli*, *Aeromonas* spp. and *Vibrio* are in the maximum range of 10^2 CFU/100 ml of sample (Maugeri et al., 2004). This bacterial range is lower than the present study's data. A study by Mudryk et al. (2014) from the coastal Baltic Sea showed the counts of *Aeromonas* to be higher compared to *Pseudomonas* and *E. coli*. In contrast, the highest numbers were seen for Pseudomonas counts in the present study. When studies from the Indian coast are concerned regarding indicator and pathogenic bacteria, the East Coast (off Chennai) showed a higher abundance of total viable counts and total coliforms as compared to the West Coast of India (off Kerala) (Sudhanandh et al., 2012; Vignesh et al., 2012). A study from Chennai coastal water showed TVC/heterotrophic bacterial numbers to be 10 times higher than this study from the coast of Andhra Pradesh (Vignesh et al., 2012). There have been many similar studies from Visakhapatnam coastal waters (primarily beaches and Visakhapatnam harbour) which show the water of this region with high pollution index concerning indicator bacteria (Clark et al., 2003; Khandeparker et al., 2020; Kumar et al., 2017). The current study compares how these bacteria are distributed along the coast from industrial discharge sites and harbours. Moreover, the abundance of indicator bacteria is higher than the standard limit in the study region (*Enterococcus faecalis* more than 35 CFU/100 ml set in recreational water). The coliform counts also far exceeded the faecal coliform limit set by the central pollution control board, India, i.e., 100 CFU/100 ml.

Occurrence of antibiotic-resistant bacterial isolates

Several Enterobacteriaceae isolates were resistant to antibiotics azithromycin, aztreonam, cefuroxime, cefotaxime, ceftazidime and cefepime. Except for azithromycin, other antibiotics mentioned have β-lactam rings in their structure. Many bacteria target these β-lactam rings and hydrolyse these antibiotics as part of their antibiotic resistance mechanism by producing β-lactamase (Paterson & Bonomo, 2005). Bacteria with extended-spectrum β-lactamase activity from Enterobacteriaceae and *Pseudomonas* can confer resistance against most β-lactam antibiotics such as penicillin, cephalosporins and aztreonam (Pang et al., 2019; Paterson & Bonomo, 2005). Resistance of many isolates to cefepime, a 4th generation of cephalosporin, is concerning. Azithromycin, which belongs to the macrolide class of antibiotics, was shown resistant by 39% of isolates belonging to *Enterobacteriaceae*. Surprisingly, only one *Pseudomonas* isolate showed resistance to an old broad-spectrum antibiotic like tetracycline. The same result was seen in the case of another antibiotic, chloramphenicol. Few isolates showed resistance to ampicillin/sulbactam, gentamicin and ciprofloxacin. Few isolates showed resistance to the carbapenem class of antibiotics used in the study (imipenem and meropenem). Carbapenems are generally used as last resort antibiotics, which are not frequently administered to help prevent antibiotic resistance against them. In our study, antibiotics belonging to cephalosporin family were shown most resistance from the bacterial isolates. As discovered by Farooqui et al. (2019) from medical audit data, β-lactam cephalosporins had the highest prescription rate, followed by β-lactam penicillin in India. This could be a reason for the earlier result as antibiotic resistance in bacteria is dependent on uses of antibiotics in those areas (Alipour et al., 2014). The current study deduced 23 out of 46 isolates (50%) to have a MAR index of more than 0.2. A MAR index greater than 0.2 suggests contamination from a source with higher use of antibiotics (Ayandele et al., 2020). There are various sources from which antibiotic-resistant bacteria can be originated. These bacteria can enter the natural environment by various means, from human faeces, livestock manure slurry, agricultural land run-off, sewage water, wild bird and animals, and pharmaceutical industry discharge during manufacturing process of antibiotics (Wellington et al., 2013).

A study by Obayiuwana et al. (2018) pointed out that pharmaceutical wastewater discharge can be a hot spot for genetic determinants for antibiotic resistance in bacteria. In the current study, antibiotic-resistant isolates can be attributed to the widespread open-defecation practices near the coastal villages. As there are several pharmaceutical companies' discharge points along the study area, they can act as a determinant too. Compared to the data obtained from isolates from different port regions along the east coast of India, these antibiotic susceptibility data in terms of AR and MAR indexes showed a considerable variation (Unpublished data). Data from the abovementioned port regions were collected during monsoon seasons (August 2019) and pre-monsoon (June 2020).

Table 8 Summary and comparison of antibiotic susceptibility studies around the world with the current study

Article	Study area	Antibiotics		Inference
		Most resistance	Least resistance	
Baya et al. (1986)	Sewage effluents and open ocean Puerto Rico, Atlantic Ocean	Penicillin, erythromycin, nalidixic acid, ampicillin	Chloramphenicol, tetracycline, kanamycin	Streptomycin resistance only from sewage samples
Sabry et al. (1997)	Eastern harbour, Alexandria, Egypt	Trimethoprim-sulphamethoxazole, streptomycin, tetracycline	Ampicillin, penicillin	70.38% of isolates showed MAR
De Souza et al. (2006)	Antarctic marine waters	Ampicillin, chloramphenicol, streptomycin		70.4% of isolates showed MAR
Moore et al. (2008)	Coastal urban area, Southern California	Erythromycin, rifampin and tetracycline	Vancomycin, ampicillin, gentamycin	Multi-drug resistance in 22% of isolates
Matyar (2012)	Eastern Mediterranean Sea coast	Ampicillin, streptomycin, cefazolin	Tetracycline, imipenem, meropenem, cefazolin, ceftizoxime	MAR ranged from 0.2 to 0.75
You et al. (2012)	Coastal water, Malaysia	Tetracycline, sulfonamide, mecillinam, sulfamethoxazole, erythromycin, streptomycin	Chloramphenicol, ampicillin, vancomycin, norfloxacin, ofloxacin, trimethoprim/sulfamethoxazole	70% of isolates resistant to 2 or more classes of Abs
Alipour et al. (2014)	Coastal waters, Northern Iran	Chloramphenicol, ciprofloxacin and tetracycline	Vancomycin, gentamycin	Resistance depends on the use of antibiotics in the area
Maloo et al. (2014)	Veraval coast, India	Bacitracin, oxacillin, ampicillin, vancomycin	Polymyxin, gentamicin, ofloxacin, cefpodoxime, cefotaxime	100% *Enterobacter* isolates showed MAR
Belding and Boopathy (2018)	Coastal recreational water, USA	Sulfamethizole, piperacillin	Imipenem, meropenem, ceftazidime/clavulanic acid, aztreonam	Some are resistant to 2 or more Abs
Divyashree et al. (2019)	Fish processing plants, Mangalore	Ampicillin, tetracycline	Nalidixic acid, cefotaxime	23.46% of isolates were multi-drug resistant
Adeniji et al. (2020)	Eastern Cape Province, South Africa	Rifampicin, erythromycin, tetracycline	Chloramphenicol, norfloxacin, levofloxacin	96% of *Enterococcus* spp. Multi-drug resistant
Gambino et al. (2022)	Mediterranean Sea	Cefazolin, amoxicillin/clavulanic, streptomycin	Enrofloxacin, colistin, tetracycline	No MAR
Current study	**Industrial outfalls, Visakhapatnam, North East coast, India**	**Aztreonam, Ceftazidime, cefepime, cefuroxime**	**Chloramphenicol, norfloxacin, tetracycline**	**46.42% MAR (Enterobacteriaceae), 22.22% MAR (*Pseudomonas* spp.), MAR ranged (0.2–0.66)**

When the percentage of bacterial isolates with a MAR index of more than 0.2 were considered, the current study has a higher (0.46) percentage value than 0.36% and 0.26% of the sampling mentioned above (Supplementary Table 3). The higher values of the indexes in the present study can be attributed to the closeness of the sampling sites to human settlements and the sampling time which is December, when dilution by fresh water is at the lowest. However, antibiotic susceptibility alone cannot correctly provide a whole understanding of the antibiotic resistance phenomenon. Chemical analysis of the water concerning the measurement of antibiotic residues is a better way to lay the foundation for determining how much quantity of these antibiotics are discharged into the coastal water in the first place. Furthermore, assessing the isolates for the presence of specific antibiotic resistance genes will be beneficial in the future.

A comparative analysis of antibiotic susceptibility studies from various coastal sites is presented in Table 8. In the present study, bacterial isolates showed more resistance to the antibiotics such as aztreonam, ceftazidime, cefepime and cefuroxime. This pattern is very different from other studies. The case of antibiotics that have less resistance from isolates is similar to other studies. Antibiotic susceptibility test from the Enterobacteriaceae family from Veraval, West Coast of India, showed 80% resistance to ampicillin (Maloo et al., 2014). The present study shows less than 10% for the same antibiotic. The same study also reported no resistance to antibiotics of the cephalosporin family, whereas in the present study, 10–50% resistance is seen for antibiotics of the same family. In the case of MAR, the percentage of isolates from other regions is 23.46 to 100%. The isolates' percentage in current study is in between these values. A study from Veraval, on the West coast of India, found 100% resistance in case of *Enterobacter*, which is high compared to the current study from the East coast of India.

Antibiotic resistance of *Pseudomonas* spp. in the present study is low compared to the Enterobacteriaceae family. However, there are no similar studies where the comparison can be performed for the same. The source of antibiotic resistance in the region can be anything, which can come from pharmaceutical companies or faecal matter from nearby coastal villages. Some studies suggest discharge from antibiotic manufacturing/pharmaceutical sites leads to the development and dissemination of multidrug-resistant bacteria (González-Plaza et al., 2019; Hubeny et al., 2021). Sewage contamination also affects marine life, including fish, as Al-Bahry et al. (2009) studied when antibiotic tests were performed on bacteria isolated from fish. The multi-drug-resistant bacteria in this study show this region's risk factors.

Conclusion

Indicator (*Escherichia coli* and *Enterococcus faecalis*) and pathogenic (*Aeromonas hydrophila*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella* and *Shigella*, *Vibrio cholera* and *Vibrio parahaemolyticus*) bacteria are found to be present in high numbers in the study region. Heterotrophic bacterial abundance was comparable in all the stations except a few. However, enteric and pathogenic bacteria showed spatial variation in the studied region, highest in the northern stations, followed by south stations and least in the middle stations. Counts of *E. faecalis* were found to be 10^3 to 10^4 times higher in Pydibhimavaram and Tammayyapalem stations than the standard for marine recreational water. It is found that the rainfall affects the physiochemical characteristics of the middle stations, which in turn impact the bacterial load. The variation in the bacterial abundance in the study sites can be attributed to on the presence of coastal villages and industrial discharge points. Antibiotic susceptibility tests of the isolates also revealed multidrug-resistant bacteria in these waters, which is of great concern. Continuous monitoring of these bacteria, their resistance potential and presence of antibiotic residues, their dissemination, persistence in natural waters and their source determination can lead to a clearer understanding of this grave situation.

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Author contribution Srinivas T. N. R.: conceptualisation, project administration, methodology, writing—review and editing. Swarnaprava Behera: methodology; writing—original draft, review and editing, visualisation; software; Sri Rama Krishna Moturi: writing—review and editing; Geethika Gudapati, Sravani, M., Satyanarayana Reddy, T.: formal analysis, investigation, data curation.

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Data availability The datasets generated during and analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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