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RESEARCH ARTICLE

Water quality and the presence of antibiotic resistant bacteria in the bayous of southeastern Louisiana, USA

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Abstract

Antibiotic resistance has become a significant public health concern due to the improper consumption and disposal of antibiotics. Improper handling often leads to selective pressure among bacteria that can lead to antibiotic resistance. Terrebonne parish in Louisiana being heavily populated, is dependent on Bayou for its seafood, recreational activities, transportation, and source of living. The water quality of Bayou Terrebonne and Bayou Petite Calliou has never been monitored before. The purpose of this study was to monitor the water quality of Bayou Terrebonne and Bayou Petite Calliou. Four sites along the bayous were monitored for water chemistry, total and fecal coliforms, and antibiotic resistance. The water samples were collected in triplicate and checked for the presence of antibiotic-resistant bacteria, coliforms bacteria, and the levels of organic carbon, nitrogen, ammonia, sulfate, and phosphate. The antibiotics tested on samples includes Tetracycline, Penicillin, Amoxicillin, Sulfamethoxazole-Trimethoprim, Bacitracin, Streptomycin Meropenem, and Erythromycin. The results showed that the bayou meets the criteria set by the Louisiana Department of Environmental Quality (LDEQ) for water chemistry except for sulfate concentration. The amount of total and fecal coliform exceeded the water quality standard due to fecal contamination from the discharge of untreated and/or under treated domestic wastewater from failing septic tanks and individual household sewage treatment systems along the bayous. Many antibiotic resistant bacteria and resistance genes were isolated from the water samples. The water is heavily impaired by the fecal contamination and the antibiotic resistant bacteria due to improper disposal of individual sewage treatment plant from households along the bayous.

Keywords: Water Quality, Bayous, Antibiotic Resistant Bacteria, Antibiotic Resistance Genes, Multidrug Resistant, Fecal Coliform.

INTRODUCTION

Antibiotic resistance has become a significant public health concern due to the improper consumption and disposal of antibiotics. Improper handling often leads to selective pressure among

bacteria, leading to antibiotic resistance. These antibiotic resistant bacteria can cause life-threatening infections that are difficult to treat with limited treatment options. According to the CDC, 2019 Report, the death rates due to antibiotic-resistant infections have increased to 35,000 in the USA (CDC, 2019) compared to the 2013 report, which reported 23,000 deaths (CDC, 2013). Thus, there is a drastic increase in the prevalence of drug-resistant pathogens while anti-infective agents are only being discovered or developed gradually. Consequently, the concern of untreatable infections will be a public health problem in the future.

Antibiotic resistance in bacteria emerge in response to the selective antibiotic pressure thus allowing the organism to survive and proliferate. Antibiotics function in our body by inhibiting the cell wall synthesis of bacteria, interfering with the cell membrane structure, inhibition of protein synthesis and nucleic acid function or by blocking and inhibiting metabolism. Bacteria can acquire antibiotic resistance through genetic transformation, conjugation, and transduction. Antibiotic resistant bacteria transfer their genes to environmental bacteria through horizontal gene transfer (HGT) while in vertical gene transfer the genes are transferred between generations. Plasmids, a circular piece of DNA, can carry multiple resistance genes which can get integrated into other bacterial genes. Bacteriophages also transfer resistance genes, especially in the case of Staphylococci (Hawkey, 1998). The four basic mechanisms to protect them from the antibiotics are the synthesis of enzymes by the cell that inactivate microbial drugs, decreased cell permeability and decreased uptake of a drug due to low enzyme affinity for the drug, altered site of the drug receptor in the cell, and modification of essential metabolic pathway for the antibiotics.

At least 30% of prescribed antibiotics are unnecessary (CDC, 2016). Most of the antibiotics are prescribed for respiratory conditions caused by the virus. Incorrectly prescribed antibiotics that are subinhibitory can promote the development of antibiotic resistance. Also, human body is not capable to break down antibiotic completely and therefore end up in sewage effluent and recreational waterways. Low levels of antibiotics also lead to strain diversification, as in Pseudomonas aeruginosa. About 80% of antibiotics sold in the US are utilized on animals to promote their growth and prevent infection (Ventola, 2015). Humans ingest the antibiotics used in livestock that can result in the transfer of resistance genes. In 2016, 50,000 Americans died due to Healthcare-Associated Infections. Improper usage and disposal of antibiotics by consumers, hospitals, industries, and sewage treatment plants has increased the emergence of antibiotic resistance in the waterways of Southeast Louisiana (Bird et al., 2019; Everage et al., 2014; Bergeron et al., 2016; Andrzejak et al., 2023; Bernharthorton et al., 2023). Bayou Terrebonne starts near Thibodaux, Louisiana, and goes through the city of Houma, ending in the Gulf of Mexico with a total distance of over 50 miles and Bayou Petit Caillou branches off Bayou Terrebonne. Terrebonne parish being heavily populated is dependent on its bayou for its seafood, recreational activities, transportation, and source of living. More than 100,000 people live in the vicinity of the Bayou Terrebonne watershed. The watershed is heavily impaired as a result of no inflow of water to the bayou other than the sewage outfall from the individual home sewage treatment systems and runoff from rainfall events (Belding and Boopathy, 2018). Water quality of this watershed has never been studied and therefore, we conducted this study to monitor the watershed's water quality. The purpose of this study was to monitor the water quality of the Bayou Terrebonne and Bayou Petit Caillou in southeast Louisiana at four different sites for a year and report the health of the bayous. Water quality parameters include, water chemistry, coliform bacteria, antibiotic resistant bacteria, and antibiotic resistance genes in the bayou water samples.

MATERIALS AND METHODS

Sample Collection

The water samples were collected at four sites along the Bayou Terrebonne once a month over a year in triplicates. Site 1 (Figure 1) is in Gray, LA near a veterinary clinic (GPS 29.692664 N, -90.785866 W); Site 2 in Houma, LA near Terrebonne Medical Center (GPS 29.598989 N, -90712484

W); Site 3 is in Chauvin, LA near the Chauvin Sculpture Garden (GPS 29.443926 N, -90.593522 W) and Site 4 location runs off to the Gulf of Mexico in Houma, LA (GPS 29.387014 N, -90621308 W). Site 3 and 4 are along the Bayou Petit Caillou which branches off from Bayou Terrebonne in Houma, Louisiana. The samples were collected in triplicates each month and transported in ice and stored in the fridge until processed. The samples were collected over a year from October 2021 to September 2022. The data for processed for each season.

Figure 1. Sites of sample collection in the Bayous of Southeast Louisiana

Water Chemistry Analysis

The dissolved oxygen, salinity, and temperature were measured on-site using a YSI portable Dissolved Oxygen Meter. The pH of the sample was measured using Denver Instrument Ultrabasic pH benchtop Meter. The organic carbon in the water was analyzed in the form of chemical oxygen demand (COD). COD was measured according to Hach method using calorimetric reaction digestion method as per instructions given in Hach Manual (Hach, 1999). Nitrate in four sites were measured using Cadmium Reduction Method (Hach, 1999). With 25 ml sample being the blank, a NitraVer 5 reagent was added to 25 ml of each sample and shaken for 1 minute. After 5 minutes of reaction time, the nitrate was measured in the HACH DR6000 spectrophotometer at a wavelength of 400nm. Ammonia was analyzed using Nessler's Method (Hach, 1999). The sulfate was measured using SulfaVer 4 method (Hach, 1999). The phosphate was monitored using Ascorbic Acid Method (Hach, 1999). PhosVer 3 reagent was added to each water sample, and a 25ml sample was used as a blank without the reagent. After two minutes, the samples were measured in the HACH DR6000 spectrometer at a wavelength of 880nm. Antibiotic contents were analyzed using high-performance liquid chromatography (HPLC) as per the method described by Bird et al., 2019.

Bacterial Analysis

The total and fecal coliforms bacteria were monitored by the most portable number (MPN) method (Clesceri et al., 1998). The triplicate of each sample was tested with three different concentrations of 10mL, 1mL, and 0.1mL. Ten milliliters of each sample were inoculated into A-1 2X media in triplicates. One milliliter and 0.1 milliliters were inoculated into A-1 1X media in triplicates. The total coliform tubes were incubated for 48 hours at 35°C and fecal coliform tubes were incubated for 24 hours at 44.5°C. Production of gas indicated a positive result. The MPN chart was used to get the estimation of bacteria based on the number of positive tubes for each test.

Coliform tubes from the fecal and total coliform tests were selected for identification of *Escherichia coli*, *Klebsiella pneumonia*, and *Enterobacter spp.* by isolation techniques using Eosin Methylene Blue (EMB) media plates. The EMB plates were incubated for 24 hours at 37°C. A dark brown or blackish with a metallic green sheen indicated the presence of *Escherichia coli*. K. *pneumonia*, if detected positive, appears as a large pink mucoid colony. Moreover, purple colonies indicated *Enterobacter spp.* An isolated colony was inoculated into Tryptic Soy Broth (TSB) tube and incubated for 24 hours at 37°C. *Enterococcus spp.* was identified by the method described by Everage et al. (2014). The sample from positive coliform test was inoculated into an enterococcal media plate, and the presence of black colonies indicated a positive result. *Staphylococcus spp.* were identified by inoculating the water sample into the Mannitol Salt Agar (MSA) plate and were then incubated at 37°C for 24 hours. The presence of golden yellow color colonies indicates the presence of *Staphylococcus aureus*.

Bacterial colonies that were not able to be identified by the three-sugar fermentation test were identified by the BIOLOG method. Unknown samples from TSB were inoculated onto BIOLOG Universal Growth (BUG) agar plate. The BUG plate was incubated for 24 hours at 37°C and then transferred to BIOLOG MicroPlates. The Microplates were incubated for 24 hours at 37°C. BIOLOG MicroStation was used to read the plate after 24 and 48 hours.

Antibiotic Susceptibility Test and Analysis of Antibiotic Resistance Genes

Isolated pure culture bacteria were tested for antibiotic resistance using the Kirby Bauer method. After streaking on Muller-Hinton agar from Tryptic Soy Agar (TSA) plate, an antibiotic disk of Meropenem, Amoxicillin, Tetracycline, Trimethoprim, Penicillin, Bacitracin, and Erythromycin were stamped on the plate and incubated for 24 hours at 37°C. A standard laboratory caliper was used to measure the zone of inhibition. The antibiotic resistance was categorized by a Kirby-Bauer index of zone diameter interpretive standards (Delost, 2020).

Extraction of resistance gene were done using polymerase chain reaction (PCR) followed by the technique used by A. Naquin et al. (2015). After isolation of desired bacteria into TSB, 2mL of each isolate were centrifuged in microcentrifuge tubes at 4000 RPM for 10 minutes. After discarding the supernatant, the pellet was used for DNA extraction. The DNA were extracted using a Fast ID DNA Extraction Kit. PCR was done using the primers from Sigma Aldrich Co. (Table 1) to identify resistance genes. Each PCR tube contained 50µL of mixture containing 25µL Promega GoTaq Green, 2µL forward primer, 2µL reverse primer, 16µL sterile DI water and 5µL of extracted DNA. The tube then underwent 35 cycles in thermal cycler for 94°C for 5 minutes, denaturing at 94°C for 30 seconds, annealing at 53°C for 1 minute, extension at 72°C for 30 seconds, and final annealing at 72°C for 7 seconds. A 2% agarose gel in 1x TAE were made with 2-3µL ethidium bromide to perform the gel electrophoresis. The 1x TAE were poured over the gel until submerged. 10µL of DNA was pipetted into the wells of the gel and a 100bp DNA ladder was used to determine the band length. A Bio-Rad Powerpack machine were used for this procedure at 100V setting for 1 hour. The gel was visualized under a FluroChem UV light and imaging software to obtain the result. Various antibiotic resistant gene primers used in the study are given in Table 1.

Table 1. Genes for antibiotic resistance, forward and reverse primer, size of base pair sequence, and resistance mechanism. (Bird et al. 2019).

Gene Name	Resistance Mechanism	Primer Sequence 5' → 3'	Size of predicted product (bp)
ermB	Ribosomal protection	F: GAT ACC GTT TAC GAA ATT GG R: GAA TCG AGA CTT GAG TGT GC	364
sul1	Enzymatic modification	F: CGC ACC GGA AAC ATC GCT GCA C R: TGA AGR RCC GCC GCA AGG CTC G	163
sul2	Enzymatic modification	F: TCC GGT GGA GGC CGG TAT CTG G R: CGG GAA TGC CAT CTG CCT TGA G	191
sulA	Inhibition of cell division	F: TCT TGA GCA AGC ACT CAA GCA G	299

Table 1. Genes for antibiotic resistance, forward and reverse primer, size of base pair sequence, and resistance mechanism. (Bird et al. 2019).

Gene Name	Resistance Mechanism	Primer Sequence 5' → 3′	Size of predicted product (bp)
		R: TCC AGC CTT AGC AAC CAC ATG G	
tetA	Efflux	F: GCT ACA TCC TGC TTG CCT TC	210
		R: CAT AGA TCG CCG TGA AGA GG	
mecA	β-lactam binding protein	F: GAC AAA TCG CAA ACG TGG CGA AGA A	541
		R: CTT CTG TTC TGT CTG TTG GAT AAG CAA ATT CAA GC	
bacA	Cell wall synthesis	F: GTG AAA GCA AAG GTC GTT TAA CGC TGA TCC	349
		R: CGC CAG CAG GAA CGA AAA CTC GGA AGC AGC	
ampA	β-lactam binding protein	F: CCG CTA AAC AGT GGA ATG GGA TCA C	497
		R: CGG TTT GCC AGT AGC GAG ATT GTG C	

Statistical Analysis

All analysis were done in triplicates. Water Quality data which includes water chemistry and coliform number from each site within each season was subjected to one-way ANOVA ($p \leq 0.05$) as described by Valiela (2009).

RESULTS AND DISCUSSION

Water Chemistry

Water Chemistry data, including dissolved oxygen, temperature, pH, chemical oxygen demand, nitrate, ammonia, sulfate, and phosphate, were monitored from October 2021 to September 2022. Data are represented in seasons' average for statistical comparison.

Temperature for Fall (August, September, October) (Table 2) ranged from 24.1-25.2°C. Dissolved oxygen was in the range of 3.97-5.19 mg/L. The low dissolved oxygen may result in poor water quality and frequent fish kill events (Bird et al. 2019: Bernhardhorton et al 2023; Andrzejak et al. 2023). The bayou did not experience any major fish kills during the study period. Salinity was in the range of 0.16-5.47 ppt. pH for Fall ranged from 8.22-8.59. The average pH for Site 4 (8.37 ± 0.36, p= 0.0028) was significantly higher than Site 3 (7.89 ± 0.3). Chemical oxygen demand ranged from 15.89-23.889 mg/L. The Chemical oxygen demand for Site 4 (23.89± 41.77, p= <0.0001) was significantly lower in Fall than in Winter (111 ± 26.09). Nitrate ranged from 0.4-0.71 mg/L. No statistical difference in average nitrate was observed between sites for the fall season. Ammonia ranged from 0.39-0.77 mg/L. The average ammonia concertation for Site 3 (0.3 ± 0.16, p= 0.0077) was significantly lower than for Site 4 (0.74 ± 0.3). Sulfate ranged from 8.67-750 mg/L. The average sulfate concentration for Site 1 (805.56 ± 408.65, p= 0.0001) was significantly higher than the concentration of Site 1 (8.11 ± 7.62) and Site 2 (34.44 ± 9.08) for the fall season. Also, sulfate concentration for Site 4 (805.56 ± 408.65, p=0.0003) was significantly low compared to site 4 (1152.78± 271.12) in the spring season. The phosphate ranged from 0.3-1.37 mg/L. The average phosphate concentration for Site 1 (1.25 ± 0.37, p= 0.0002) was significantly higher than Site 2 (0.45 ± 0.13) and Site 4 (0.37± 0.22, p= <0.0001) for the fall season. Site 3(0.97 ± 0.63, p= 0.0205) was also significantly higher than Site 4 (0.37± 0.22).

Table 2. Mean ± (SE) water quality variable in all four sites during the Fall sampling season.

Water Quality Variable	Site 1	Site 2	Site 3	Site 4
Temperature (°C)	24.1	25.2	24.7	25.5
DO (mg/L)	3.97	5.04	3.58	5.19
Salinity (ppt)	0.16	0.33	0.87	5.47

Table 2. Mean ± (SE) water quality variable in all four sites during the Fall sampling season.

Water Quality Variable	Site 1	Site 2	Site 3	Site 4
pH	8.04±0.35	7.99±0.35	7.89±0.3	8.37±0.36
COD (mg/L)	19.44±16.81	17.22±11.68	34.1±31.93	23.89±41.77
Nitrate (mg/L)	0.74±0.69	0.77±0.2	0.69±0.66	0.67±0.38
Ammonia(mg/L)	0.69±0.24	0.35±0.06	0.3±0.16	0.74±0.3
Phosphate(mg/L)	1.25±0.37	0.45±0.13	0.97±0.63	0.37±0.22
Sulfate (mg/L)	8.11±7.62	34.44±9.08	34.67±21.12	805.56±408.65

Temperature for Winter (November, December, and January) (Table 3) ranged from 19.1-22°C. The dissolved oxygen ranged from 8.06-11.94 mg/L—salinity ranged from 0.05-8.5ppt. pH for winter ranged from 8.22-8.6. pH for Site 1 (8.46± 0.51, p=0.0002)) was significantly higher than Site 1 (7.6± 0.48) during the spring season. Also, the pH for Site 3 (8.59 ± 0.11, p= 0.0007) was significantly higher than Site 3 in the fall season. Chemical oxygen demand ranged from 15.33-91.67 mg/L. The COD concentration for Site 4 (111± 26.09, p= <0.0001) was significantly higher than Site 1 (16.5 ± 10.6) for the winter season. Nitrate ranged from 0.12-0.88 mg/L. Site 2 (0.88 ± 0.83, p=0.0182) was significantly higher than site 3 (0.22 ± 0.24) for the winter season. Ammonia ranged from 0.24-1 mg/L. The ammonia concentration was significantly lower in site 3 (0.23 ± 0.04 p= <0.0001) compared to site 4 (1 ± 0.63) during the winter season. Sulfate ranged from 13.16- 725 mg/L. The phosphate ranged from 0.19-0.71 mg/L. No statistical difference in average phosphate was observed between sites for the winter season. The sulfate concentration in site 4 (725 ± 210.95, p= <0.0001) was significantly higher than all the other sites.

Table 3. Mean ± (SE) water quality variable in all four sites during the Winter sampling season.

Water Quality Variable	Site 1	Site 2	Site 3	Site 4
Temperature (°C)	19.1	21.05	21.35	22.0
DO (mg/L)	10.13	8.06	8.65	11.94
Salinity (ppt)	0.05	0.25	0.3	8.5
pH	8.46±0.51	8.22±0.1	8.59±0.11	8.5±0.18
COD (mg/L)	16.5±10.6	16.83±11.81	21.67±10.37	111±26.09
Nitrate (mg/L)	0.48±0.37	0.88±0.83	0.22±0.24	0.17±0.2
Ammonia(mg/L)	0.39±0.1	0.34±0.04	0.23±0.04	1±0.63
Phosphate(mg/L)	0.71±0.11	0.58±0.17	0.44±.34	0.19±0.14
Sulfate (mg/L)	14.67±6.22	27.33±17.92	66.33±13.94	725±210.95

Spring temperatures (February, March, and April) ranged from 19.1-22°C (Table 4). The dissolved oxygen was in the range of 2.31-5.71mg/L. Salinity ranged from 0.06-1.23 ppt. pH for spring ranged from 7.59-7.92. The pH of Site 3 (7.86± 0.37, p= 0.0023) during the spring season was significantly lower than Site 3 (8.59 ± 0.11) in the winter season. Chemical oxygen demand ranged from 13.67- 68.78 mg/L. The COD concentration of Site 3(21.44± 20.31, p=0.0154) was significantly lower than Site 4 (69.89± 44.53) in the spring season. Nitrate ranged from 0.37-0.79 mg/L. No statistical difference in average nitrate was observed between sites for the spring season. The ammonia ranged from 0.34-1.09 mg/L. The ammonia concentration of Site 3 (0.37± 0.22, p=0.0074) was significantly lower than Site 4 (0.82 ± 0.45) in the spring season. Sulfate ranged from 4.33-1152.83 mg/L. The sulfate concentration of Site 4 (1152.78 ± 271.12, p=<0.0001) was significantly higher than all the other three sites. The phosphate ranged from 0.18- 1.24 mg/L. The concentration of Site 1 (1.24 ± 0.3, p=<0.0001) was significantly lower than Site 4(0.19± 0.09).

Table 4. Mean ± (SE) water quality variable in all four sites during the Spring sampling season.

Water Quality Variable	Site 1	Site 2	Site 3	Site 4
Temperature (°C)	19.1	21.05	21.35	22.0
DO (mg/L)	2.31	5.71	5.07	5.06
Salinity (ppt)	0.06	0.1	1.23	3.3
pH	7.6±0.48	7.92±0.34	7.86±0.37	7.93±0.26
COD (mg/L)	20.78±16.66	17.67±10.16	21.44±20.31	69.89±44.53
Nitrate (mg/L)	0.47±0.35	0.79±0.27	0.37±0.22	0.41±0.21
Ammonia(mg/L)	0.67±0.2	0.47±0.15	0.34±0.08	0.82±0.45
Phosphate(mg/L)	1.24±0.3	0.49±0.24	0.5±0.16	0.19±0.09
Sulfate (mg/L)	5.33±2.87	24.67±3.46	41.89±13.74	1152.78±271.12

Temperature for Summer (May, June, July) (Table 5) ranged from 19.1-22°C. The dissolved oxygen ranges from 0.63-2.15mg/L. Salinity ranged from 0.1-7.73 ppt. pH for summer ranged from 7.25-7.5. There was a significant difference between site 1 (7.34± 0.16, p=<0.0001) of the summer season and site 1 (8.46± 0.51) of the winter season. Chemical oxygen demand ranged from 11.55-76.78 mg/L. No statistical difference in average COD was observed between sites for the summer season. Ammonia ranged from 0.39-0.61 mg/L. The concentration of Site 1 (0.83 ± 0.39, p=0.0023) was significantly higher than that of Site 2 (0.34 ± 0.14) in the summer season. Sulfate ranged from 1.57- 487.22 mg/L. The sulfate concertation of Site 1 (3.33 ± 3.28, p=<0.0001) was significantly lower compared to Site 4 (813.89 ± 240.8) during the summer season. The phosphate ranged from 0.34- 1.42 mg/L. The phosphate concertation for site 1 (1.65 ± 0.44, p=<0.0001) was significantly higher than all other sites.

Table 5. Mean ± (SE) water quality variable in all four sites during the Summer sampling season.

Water Quality Variable	Site 1	Site 2	Site 3	Site 4
Temperature (°C)	19.1	21.05	21.35	22.0
DO (mg/L)	0.63	2.15	1.86	2.01
Salinity (ppt)	0.2	0.1	0.2	7.73
pH	7.34±0.16	7.33±0.18	7.38±0.16	7.6±0.28
COD (mg/L)	33±44.28	12.22±11.69	20.89±22.77	66.78±41.72
Nitrate (mg/L)	0.54±0.24	0.5±0.07	0.36±0.22	0.38±0.12
Ammonia(mg/L)	0.83±0.39	0.34±0.14	0.36±0.29	0.41±0.12
Phosphate(mg/L)	1.65±0.44	0.7±0.31	0.77±0.55	0.28±0.12
Sulfate (mg/L)	3.33±3.28	13.22±9.4	34.78±11.52	813.89±240.8

Antimicrobial resistance in the aquatic environment positively correlates with eutrophication (Li et al., 2020). Nutrients are estimated to contribute 19.6% of antibiotic gene transfer in the aquatic environment (Zhou et al., 2017). Moreover, adding nutrients like nitrogen and phosphorus affected the antimicrobial resistance of E.coli in artificial ecological water (McPhearson et al., 1991). However, significant differences in water quality between sites were observed only for sulfate (Tables 2-5). The pH of all four sites was within the limit specified by the Environmental Protection Agency (EPA) – the criteria being 6-8.5 (EPA, 2012). The EPA water quality regulation also states that pH should not vary more than one pH unit, and no sites have shown a difference of more than one pH unit.

The EPA estimates that 250 mg/L or greater sulfate levels can be observed in about 3% of the public drinking water systems (EPA, 2012). Site 4 had a significantly higher level of sulfate than all the other sites for fall (p= 0.0001), spring (p=<0.0001), and summer season(p= <0.0001) (Tables 2-5). No significant differences were observed during the winter season. Site 4 has also surpassed the Louisiana Department of Environments Quality criteria (<50mg/L). The increase could be because of the sediments in Site 4 as it runs off into the Gulf of Mexico. Agricultural runoff denotes another source of sulfate in the water (Andrzejak et al., 2023).

Nitrate and ammonia are a form of nitrogen. Excess nitrate can cause hypoxia and be toxic to warm-blooded animals at a higher concentration of 10 mg/L. The natural ammonia and nitrate level in water is as low as one mg/L (EPA, 2012). Phosphorus is a nutrient in short supply in fresh waters. A concentration deficient as 0.01 mg/L can dramatically impact the streams (EPA, 2012). However, nitrate and phosphate were within the limit set by the Environmental Protection Agency standard. Chemical Oxygen demand (COD) measures the water's biologically active and inactive organic matter. High COD levels lead to more oxidizable material in the sample, which reduces the dissolved oxygen available for the aquatic life forms. Site 4 had the highest COD for Winter ($p = <0.0001$) and Spring ($p=0.0154$). The reason can be attributed to the high level of decaying plants, human waste, or effluent from sewage treatment plants.

The antibiotic content in the water samples was tested for antibiotics and the results are given in Table 6 The antibiotics found in the water include Amoxicillin, Erythromycin, Sulfamethoxazole, Trimethoprim, Bacitracin, Penicillin, and Tetracycline in nanogram per liter. It was observed that Sulfamethoxazole was high in Site 1 compared to other sites. Similarly, Tetracycline was consistently high in all sites of the collected water samples. Similar levels of antibiotics were reported previously in the waters of southeast Louisiana (Bird et al., 2019; Andrzejak et al., 2023; Bernharthorton et al., 2023). Significant reductions were noted in sulfonamide, β-lactams, tetracycline, and aminoglycoside antibiotic classes concentrations in the nearby wetlands (Bernharthorton et al., 2023). Amoxicillin and the macrolide erythromycin had lesser, but apparent reductions, concurrent with literature that describes these antibiotics as more persistent than many -lactams also undergo hydrolysis. While these reductions could reflect historic and current environmental antibiotic concentrations, it could also indicate that while enzymatic hydrolysis may be effective between sites, there are likely other mechanisms affecting the system like soil assimilation, biodegradation, or photodegradation that enable the degradation of these other antibiotic classes throughout the entire system. Several antibiotics and sulfonamide medications contain sulfate, including sulfamethoxazole-trimethoprim and erythromycin-sulfisoxazole (Bernharthorton et al., 2023). This could indicate successful degradation of antibiotics in the bayous, especially with increased phytic acid/plant presence and acidity at some part of the waterways that may encourage biodegradation. Dilution plays a role in the reduction of antibiotic concentration during the rainy days. Because biodegradative mechanisms act synergistically within bayou system it can be assumed that many are influencing nutrient and antibiotic degradation given the presence of multiple mechanistic indicators.

Table 6. Mean ± (SE) water quality variable in all four sites during the Summer sampling season.

Antibiotic	Site 1	Site 2	Site 3	Site 4
Amoxicillin (ng/L)	0.28	0.15	0.1	ND
Erythromycin (ng/L)	0.44	0.22	0.16	ND
Sulfamethoxazole (ng/L)	4.2	1.83	0.57	0.23
Trimethoprim (ng/L)	2.9	1.93	0.68	1.2
Bacitracin (ng/L)	0.27	0.1	ND	ND
Penicillin (ng/L)	3.27	1.35	2.7	ND
Tetracycline (ng/L)	4.63	3.53	3.4	1.9

ND - Not determined

Total and Fecal Coliform Bacteria in the Water

Over the one-year sampling period, the average total coliform for all sites ranged from 54-1100 MPN/ 100mL (Figure 2). The highest average total coliform was detected during the Fall season. Also, Site 1 had consistently high total coliforms for all seasons. Site 1 and Site 3 had the highest average total coliform values in Winter. Furthermore, Site 1 and Site 2 were the highest in the Spring. Summer had relatively low average total coliform values compared to other seasons. The

reason for high total coliforms in Site 1 may be due to the presence of higher number of households in this site with septic tank system compared to other sites and sometimes these septic tank systems are not functioning properly.

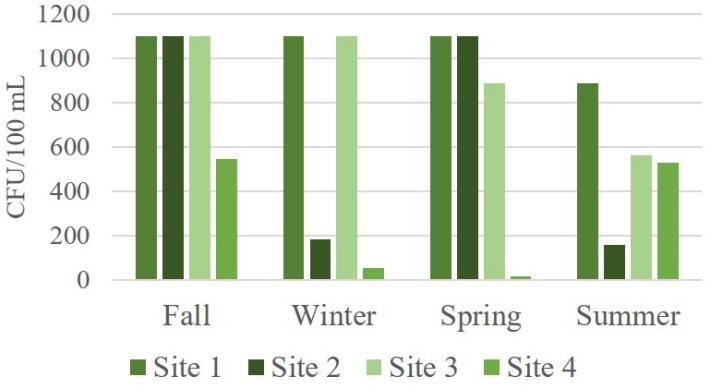

Figure 2. Mean total coliform values of water samples

The average fecal coliforms ranged between 44-1100 MPN/ 100mL (Figure 3). The highest fecal coliform was detected in site 1, exceeding the Environmental Protection Agency's criteria for primary water usage (400 MPN FC/100mL) during the Fall season. Similarly, Site 1 surpasses the guidelines for primary water usage in the Winter and Spring season. The lowest fecal coliform estimate was detected during the summer season.

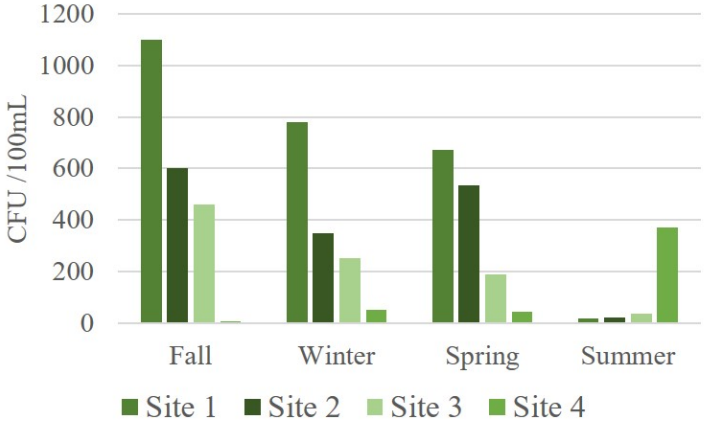

Figure 3. Mean fecal coliform values of water samples

Coliforms are used as an indicator of sewage contamination as they are found in human and animal feces. Although harmless, they indicate the presence of pathogenic bacteria and viruses that live in human and digestive systems. Therefore, high fecal and total coliform values indicate the presence of pathogenic microorganisms that pose health risks. As Andrzejak et al. (2023), mentioned high fecal coliforms are observed during summer because of the increased bacterial growth. However, in this study fall had the highest fecal coliform estimates. Site 1 and Site 2 have passed the criteria set by EPA for primary contact (maximum of 400 MPN FC/ 100mL) . This makes the waterbody unfit for swimming. Previous research on the Bayou Lafourche and adjacent wetlands have confirmed the

fecal contamination and presence of antibiotic resistance genes (Bergeron et al., 2016; Belding and Boopathy, 2018; Bird et al., 2019; Martinez et al., 2019; E. Naquin et al., 2021).

The water bodies are within the limit set for the secondary contact (2000 MPN FC/ 100mL), making them safe for fishing. Andrzejak et al. (2023), also observed similar findings in the boat launch site of Terrebonne Parish. Also, less fecal contamination at some sites correlates to high salinity (Belding and Boopathy, 2018). The study by Everage et al. (2014) and Bergeron et al. (2016), proved that since the coliform bacteria are less salt tolerant, the total and fecal coliform bacteria decrease as the salinity increases. However, salinity did not affect the presence of antibiotic-resistance genes in the water sample (Bergeron et al., 2016).

Antibiotic Resistance

Samples were selected randomly each month to check the sensitivity with the seven antibiotics (meropenem, amoxicillin, erythromycin, tetracycline, sulfamethoxazole/ trimethoprim, bacitracin, and penicillin). These antibiotics were selected for this study based on previous reports of their presence in the waterbodies of southeast Louisiana by many researchers (Everage et al., 2014; Bergeron et al., 2016; Andrzejak et al., 2023; Bird et al., 2019; Bernharthorton et al., 2023; Belding and Boopathy, 2018). The results were compared based on Delost's Interpretive Guide for Aerobic Bacteria: Zone Diameter Interpretive Standards (Delost, 2020). A total of 60 isolates were subjected to testing. Penicillin, Erythromycin, and Bacitracin showed the highest resistance percentage (Figure 4). There were even 12% of meropenem-resistant bacteria found in the isolates tested, which is the most potent last resort antibiotics available for the treatment of multi drug resistant infections. Multi drug-resistant bacteria were found in the samples. A bacterium, Pseudomonas aeruginosa, isolated from Site 3, was resistant to all six antibiotics, excluding meropenem (Figure 5). Isolates that are multi-drug resistant were subjected to molecular analysis. Many bacterial isolates were tested for the presence of antibiotic resistance genes. A representative of bacA and ampA genes presence in few isolates are shown in Figure 6 and 7. These two genes are responsible for resistance to bacitracin and ampicillin.

Figure 4. Antibiotic Resistant Disc Assay for a Bacterial Isolate, Pseudomonas aeruginosa

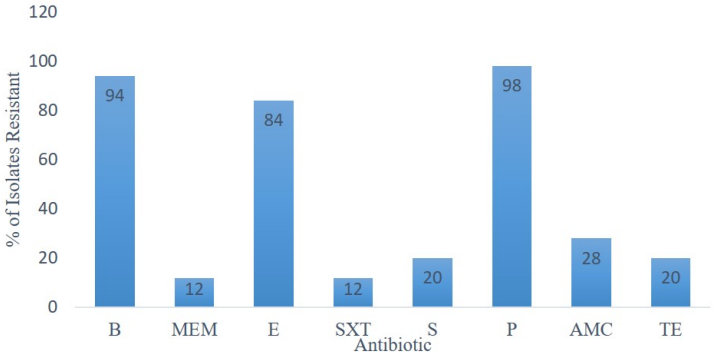

Figure 5. Percent antibiotic resistance during the study period (n=60). B-Bacitracin, MEM-Meropenem, E-Erythromycin, SXT-Sulfamethoxazole/Trimethoprim, P-Penicillin, AMC-Ampicillin, and TE-Tetracycline

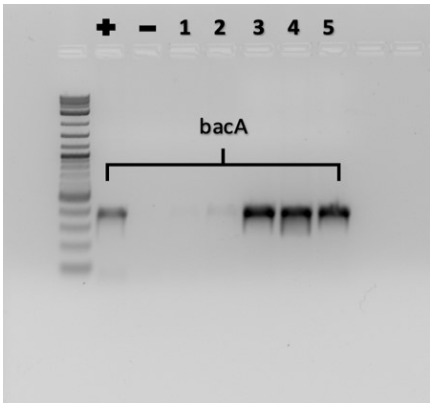

Figure 6. Antibiotic resistance genes in bacterial isolates from the water samples Bacitracin resistant gene, bacA. + Positive control, - Negative control, Lanes, 3, 4, and 5 E.coli isolates from January, March, and October samples. and Lanes 1,2, and 3 E.coli isolates from January, July, and October samples

The isolates were mainly resistant to penicillin, bacitracin, and erythromycin. Carbapenem resistance gene was confirmed in Bayou Lafourche by T. Naquin et al. (2017). Meropenem was found in the lowest percentage. However, results still showed 12% resistance. Meropenem compounds, a type of carbapenem, were first discovered in the 1980s. As bacteria produce -lactamase enzymes, it hydrolyzes the antibiotics or bacterial cell wall's porin channel, reducing the permeability into the organism.

The ARB and ARGs are mainly due to the runoff from agriculture, hospital, and wastewater treatment plant (Andrzejak et al., 2023). The ARB and ARGs results were similar to previous studies of Bird et al. (2019); A. Naquin et al. (2015); T. Naquin et al. (2017); Belding and Boopathy (2018); Bergeron et al. (2016); and Bernharthorton et al. (2023), all these studies showed the presence of ARB and ARGs in wastewater treatment plant, wetlands, and the Bayous of southeast Louisiana.

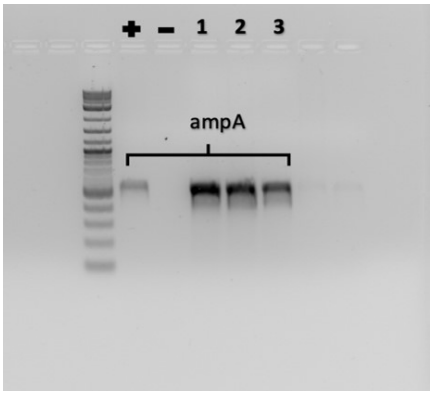

Figure 7. Antibiotic resistance genes in bacterial isolates from the water samples Ampicillin resistant gene, ampA. + Positive control, - Negative control, Lanes, 3, 4, and 5 E.coli isolates from January, March, and October samples. and Lanes 1,2, and 3 E.coli isolates from January, July, and October samples

CONCLUSIONS

The water chemistry of the bayous falls within the range set by LDEQ except for sulfate. Fecal and total coliforms were higher than the criteria set by the LDEQ and has passed the limit for the primary contact for some sites. Bayou Terrebonne and Bayou Petit Caillou is heavily contaminated with fecal matter. The factor of localized runoff or septic system cannot be ignored as a reason. Many septic systems in Terrebonne parish were installed 30 years ago. According to LDEQ, 50% of the septic systems are failing. Resistance to penicillin, bacitracin and erythromycin was higher in the aquatic environment. Many multidrug resistant bacteria were found in the water sample. The water is highly impaired and must be studied for public safety. Further research should be done to find effective ways to reduce ARB and ARGs in the environment.

Author contributions

Bincy Biju, Emily Arceneaux, Zachary Ledet: Conceptualization, analysis, draft writing, and editing; Raj Boopathy: Conceptualization and Data analysis.

Conflict of Interest

There is no conflict of interest in this study.

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Data availability

Data related to the findings of this study are available at the corresponding author.

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