Presence of antibiotic-resistant bacteria and antibiotic resistance genes in coastal recreational waters of southeast Louisiana, USA

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ABSTRACT

In the past few decades, the medical community has faced a rising problem in the spread of antibiotic-resistant bacteria (ARB) and the difficulty of treating related infections. The presence of these bacteria in high-traffic bodies of water, as well as the presence of the antibiotic resistance genes (ARG) floating freely in the water, pose the threat of antibiotic-resistant related infections in individuals living and recreating in these areas of southeast Louisiana. Water samples from coastal waters of southeast Louisiana were analyzed using chemical, microbial, and molecular methods to determine the presence of ARB and ARG. The main species analyzed include *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp. They were tested for resistance to carbapenem, monobactam, penicillin, tetracycline, sulfonamide, and cephalosporin antibiotics. The results indicated significant numbers of ARBs were consistently found in the coastal waters, and ARGs were found throughout testing. These results show that these high-traffic recreational bodies of water may be putting wildlife and humans at risk for antibiotic-resistant-related illnesses.

Key words | antibiotics, antibiotic resistance genes, antibiotic-resistant bacteria, coastal waters, fecal coliform, total coliform

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INTRODUCTION

In the modern healthcare industry, antibiotic-resistant bacteria (ARB) infections have become one of the most insidious epidemics seen throughout the world. The need for solutions to the growing antibiotic resistance problem has been the basis of a large number of recent healthcare research projects. The CDC (2017) estimates that each year, at least 2,000,000 people in the United States contract ARB infections, and at least 23,000 of those cases become directly fatal. This statistic also does not take into account the patients who were indirectly killed by the effects of these infections or never had their infection diagnosed. The methods necessary to treat these infections can be very costly and even harmful to the patient, according to the CDC (2017).

The spread of resistance is known to come about by the simple use of antibiotics, a practice that has dominated the medical field for the past 70 years since the development of penicillin. Up to 50% of antibiotic prescriptions are misused, involving misdiagnosis or overdosing, according to the CDC (2017). The CDC also states that antibiotics given either to humans or to livestock kill normal flora indiscriminately, allowing for the remaining resistant bacteria to prosper in a wealth of resources. Both human contact and poorly handled food allow for the spread of these ARB to new hosts that previously did not have the resistant bacteria. Antibiotic resistance genes (ARG) are a part of the genetic information of ARB, and they can be released into the environment and allow susceptible bacteria to integrate

the ARG into their genome and express antibiotic resistance.

In southern Louisiana, the bayous, waterways, and marshlands provide the location for many popular recreational activities. Hunting, fishing, boating, swimming, and other water-based activities play a huge part in the culture of the area and have done so for centuries before. According to the Louisiana Department of Wildlife and Fisheries (LDWF), there are at least 316,593 active motorboat registrations in Louisiana, which roughly equates to one motorboat per every six Louisiana homes. This statistic still does not include boats that do not have motors or may be registered exclusively by the United States Coast Guard. It also goes without saying that Louisiana's industry relies heavily on seafood and other water-based activities. The LDWF (2013) also states that there were 71,631 total active commercial fishing licenses as of 2012.

It should also be noted that in homes further down the bayous toward the Gulf of Mexico, there tends to be no state-run sewage operation; instead, homes typically have outdated septic tanks that are rarely maintained and discharge wastewater directly into the bayou water (Bergeron et al. 2016). As shown by Zhang et al. (2009), many antibiotics when taken are not fully metabolized in the body and can be excreted into the environment. These antibiotics in wastewater can lead to either a proliferation of ARB in wastewater treatment plants in the case of state-run sewage – as shown by Naquin et al. (2017) and Everage et al. (2014) – or in the bayou water itself in the case of outdated septic tanks (Bergeron et al. 2016). To date, there are no studies reported on the presence of ARB and ARG in the coastal waters of Louisiana. Therefore, this study was conducted in the coastal waters of Louisiana that are actively used throughout the year for various recreational activities.

The sites in this study were chosen specifically for their location downstream from insufficient waste disposal and their high human activity. Site 1, the Louisiana Universities Marine Consortium site in Chauvin, LA, is down Bayou Terrebonne and close to an outlet that drains into marshlands. It is surrounded by docks with plenty of daily watercraft activities. Site 2 in Port Fourchon, LA, is near the mouth of Bayou Lafourche at the Gulf of Mexico. The area is known for water-based commerce, fishing charters, and

recreational activities. Monthly water samples were analyzed for the presence of ARB and ARG for six months in 2017 and the results are reported. The main objectives of this study are to analyze water quality in terms of total coliform and fecal coliform bacteria in the coastal water samples, to monitor water chemistry of the coastal waters, and to study the presence of ARB and ARGs in the coastal waters.

MATERIALS AND METHODS

Collection of samples

Water samples were collected from two coastal sites: the Louisiana Universities Marine Consortium (LUMCON) site in Chauvin, Louisiana (Site 1) and from the side of Old Highway 1 in Port Fourchon, Louisiana (Site 2). Satellite images of the sampling sites are shown in Figure 1. The GPS coordinates of Site 1 in Chauvin, LA are 29°15′13.69″N, 90°39′40.82″W and the GPS coordinates of Site 2 in Port Fourchon, LA are 29°9′41.25″N, 90°10′58.35″W. Monthly samples were collected for six months from April to September of 2017. Samples were collected in sterile collection bottles in triplicates from both sites and transported to the laboratory on ice. Samples were labeled as being from either Site 1 or Site 2 (S1, S2).

Chemical analysis

Water temperature, dissolved oxygen, and salinity were measured using a YSI 63 (YSI Inc., Columbus, OH, USA) pH, salinity, dissolved oxygen, conductivity, and temperature probe at the site when samples were collected. Samples were returned to the laboratory and first analyzed for biological and chemical composition. Phosphate, nitrate, and ammonia concentrations were analyzed using the standard methods described by APHA (1998).

Bacterial quantification, isolation, and identification

An estimated quantification of both fecal and total coliforms in each sample was measured using the fecal coliform and total coliform most probable number (MPN) method
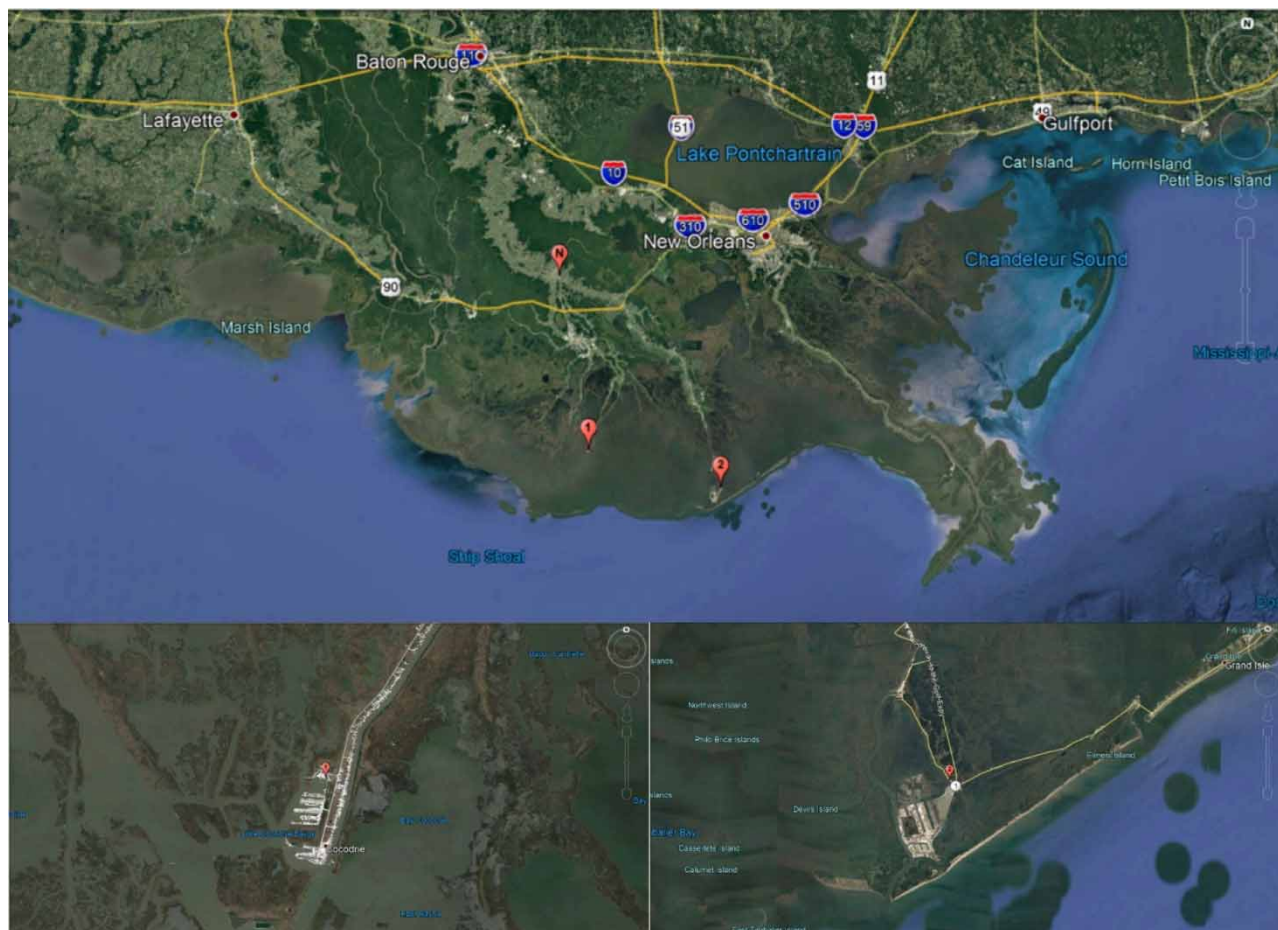
Figure 1 | Satellite imaging of sampling sites. Site 1 in Chauvin, LA (29°15′13.69″N, 90°39′40.82″W). Site 2 in Port Fourchon, LA (29°9′41.25″N, 90°10′58.35″W). (N) Nicholls State University in Thibodaux, LA (29°47′22.26″N, 90°48′.45″W).

described by Everage *et al.* (2014), using A1 broth. The total coliform test included three double-strength A-1 media tubes and six single-strength A-1 media tubes per sample. Each test tube contained one Durham tube. 10 mL of sample was inoculated into the three double-strength tubes, 1 mL of sample was inoculated into three single-strength tubes, and 0.1 mL of sample was inoculated into the remaining single-strength tubes. The total of nine tubes used to estimate total coliforms were incubated at 37 °C for 48 hours. Durham tubes containing gas accounting for at least 10% of the tube length were noted as positive.

Fecal coliforms' estimates were gathered using the same methods with the exception of incubation time and temperature. Fecal coliform tests were subjected to a 3-hour 37 °C adjustment period and then transferred to 44 °C for 21 hours. Durham tubes containing gas

accounting for at least 10% of the tube length were noted as positive. Total coliform tubes were incubated at 35 °C for 48 hours.

Coliform tubes were always inoculated on the same day of sample collection. The MPN index was used to estimate colony forming units/100 mL of sample water based on the number of tubes indicating positive gas production (APHA 1998). MPN estimates were averaged out of the triplicate samples per site. At least one pure culture isolate was collected from each fecal and total coliform set. Isolates were identified via biochemical assays as described by Everage *et al.* (2014), including eosin methylene blue (EMB) agar plates, triple sugar iron agar (TSIA) slants, and sulfide-indole-motility medium (SIM). Specific methods used to identify *Enterobacter* spp. are described by Delost (2014). *Enterobacter* spp., *Escherichia coli*, and

Klebsiella pneumoniae were isolated by quadrant streaking positive A-1 tubes onto EMB medium. At least two EMB quadrant streaks were performed per site. The EMB plates were then incubated at 37 °C for 24 hours. The EMB plates were then used to create a second EMB quadrant streak to assure the culture was pure. The second quadrant streak was then used to inoculate a sterile Tryptic Soy Broth (TSB) with 5–10 colonies of the same phenotype. TSB were then incubated at 37 °C for 24 hours. The pure TSB cultures were then used to inoculate triple sugar iron agar slants (TSIA), SIM medium, and Simmons citrate slants. *Enterobacter* spp. appeared as acid/acid with gas production in TSIA, motile and indole negative in SIM, and citrate positive. *Escherichia coli* appeared as acid/acid with gas production in TSIA, motile and indole positive in SIM, and citrate negative. *Klebsiella pneumoniae* appeared as acid/acid with gas production in TSIA, non-motile and indole negative in SIM, and citrate positive. Positively identified specimens were saved for antibiotic resistance testing and unidentifiable specimens were discarded.

For the isolation of *Enterococcus* spp., 1 mL of sample water from each site was used to inoculate sterile TSB tubes and incubated for 24 hours at 37 °C. The TSB was then used to quadrant streak Enterococcal medium. Positive plates were noted by a presence of blackened medium. Five to 10 colonies from positive enterococcel medium plates were then inoculated into a Purple Heart Infusion broth, which contained a salinity of 6.5%. The tubes were incubated at 37 °C for 24 hours and were deemed positive if the tube appeared yellow.

Antibiotic resistance assay

Antibiotic resistance was measured via the Kirby-Bauer method described by Delost (2014). After identification, each pure culture isolate was cultured in TSB and diluted to standard turbidity between 0.08 and 0.10 absorbance. Using sterile cotton swabs, a bacterial lawn of the diluted culture was streaked onto Mueller-Hinton (MH) agar plates. BD Sensi-Disc antibiotic discs (Becton Dickinson, Franklin Lakes, NJ, USA) containing aztreonam 30 μcg (ATM-30), ceftazidime/clavulanic acid 30/10 μg (CAZ-CLA), imipenem 10 μg (IPM-10), meropenem 10 μg

(MEM-10), piperacillin 100 μg (PIP-100), and sulfamethizole (sulfonamide) 0.25 mg (TH-0.25) were aseptically placed on the lawn using sterile forceps. Plates were incubated at 37 °C for 24 hours, and zones of inhibition were then measured using standard laboratory calipers. The BD Sensi-Disc zone size chart was used to determine whether the size of each zone of inhibition indicated that the species was sensitive (S), intermediate (I), or resistant (R) to each specific antibiotic as described by Delost (2014).

DNA extraction and qualitative PCR

Two mL of TSB containing confirmed resistant isolates were transferred to 2 mL microcentrifuge tubes. The tubes were centrifuged with a speed of 845 g for 15 minutes. The supernatant was discarded and the pellet was used for DNA extraction. DNA was extracted by the methods of Fast ID DNA Extraction Kit (Qiagen, San Diego, CA, USA). After extraction, DNA was subjected to polymerase chain reaction (PCR). The thermocycle (Fisher Scientifc, St Louis, MO, USA) used consisted of 30 cycles of initial denature 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 minutes. All DNA primers were obtained from Sigma Aldrich Co (St Louis, MO, USA) and can be seen in Table 1. A 2% agarose gel was created with ethidium bromide to identify DNA after PCR. The gel was then placed into a tris-acetate-EDTA (TAE) buffer. 7 μL of amplified DNA was combined with 2 μL of 6× loading dye and transferred into the wells. A 100 bp DNA ladder was used to determine the size of each DNA band and a Bio-Rad PowerPac Basic machine was used for electrophoresis. The gel was then examined using Alpha Innotech FluorChem FC2 transluminater using UV light. Fluorchem FC2 Imaging software was used to capture the image.

Statistical analysis

Water quality data from each site within a season were subjected to a one-way analysis of variance (ANOVA) ($p \leq 0.05$). All the data represent triplicate analysis with standard deviation.

Table 1 | Targeted antibiotic resistance genes and their resistance mechanism, primer sequence, size and annealing temperature for PCR, adapted from Burch et al. (2013)

Gene target	Resistance mechanism	Primer sequence (5′ → 3′)	Size (bp)	Annealing temperature (°C)
16S rRNA gene	NA	F: CCT ACG GGA GGC AGC AG; R: ATT ACC GCG GCT GCT GG	202	60
erm(B)	Ribosomal protection	F: GAT ACC GTT TAC GAA ATT GG; R: GAA TCG AGA CTT GAG TGT GC	364	58
sul1	Enzymatic modification	F: CCG TTG GCC TTC CTG TAA AG; R: TTG CCG ATC GCG TGA AGT	67	60
tet(A)	Efflux	F: GCT ACA TCC TGC TTG CCT TC; R: CAT AGA TCG CCG TGA AGA GG	210	60
tet(W)	Ribosomal protection	F: GAG AGC CTG CTA TAT GCC AGC; R: GGG CGT ATC CAC AAT GTT AAC	168	60
tet(X)	Enzymatic modification	F: AGC TTA CCA AT GGG TGT AAA; R: TTC TTA CCT TGG ACA TCC CG	278	60
mec(A)	β-lactum binding protein	F: ATG CGC TAT AGA TTG AAA GGA T; R: TAC GCG ATA TCT AAC TTT CCT A	163	60

RESULTS AND DISCUSSION

Chemical analysis

The salinity of Site 1 was found to average approximately 5 parts per thousand (ppt) with little variation and the salinity of Site 2 was found to average approximately 23 ppt, ranging anywhere from 20.3 to 25.8 ppt, (Table 2). Water temperature was very consistent between the two sites, and there is a clear increase in temperature in the summer months. Dissolved oxygen varied monthly, but there seemed to be

Table 2 | Water chemistry at Site 1 (S1) and at Site 2 (S2)

	April	May	June	July	August	September
S1						
Salinity (ppt)	5.1	5.3	4.7	4.6	4.4	4.7
Temperature (°C)	23.1	23.4	28.7	33.4	32.6	30.6
Dissolved oxygen (mg/L)	3.67	2.78	2.61	2.38	3.18	3.73
Phosphate (mg/L)	1.30	1.33	0.95	1.10	1.97	1.00
Nitrate (mg/L)	0.50	0.36	0.40	0.73	0.20	0.30
Ammonia (mg/L)	0.29	0.53	0.44	0.35	0.23	0.44
S2						
Salinity (ppt)	25.8	24.7	23.3	21.9	20.3	21.4
Temperature (°C)	23.4	23.6	28.2	32.0	30.4	31.1
Dissolved oxygen (mg/L)	2.62	2.62	2.44	2.26	3.13	2.65
Phosphate (mg/L)	1.33	0.66	0.86	0.77	0.51	0.83
Nitrate (mg/L)	0.66	0.46	0.56	0.76	0.33	0.56
Ammonia (mg/L)	4.52	4.50	2.47	4.29	4.30	5.27

The data represent the average of triplicate analysis. The standard deviation is less than 5%.

little to no correlation between the two sites. Phosphate, nitrate, and ammonia concentrations in both sites were below 5 mg/L throughout the study period (Table 2). There is very little variation in these parameters within these two sites other than salinity and ammonia.

Bacterial quantification, isolation, and identification

As shown in Figures 2(a) and 2(b), fecal and total coliform data showed rather unpredictable patterns, although Site 1 was generally found to have more microbial life overall. Site 1's fecal coliforms varied anywhere from single digits to thousands of CFU/100 mL, while Site 2's fecal coliforms generally stayed around 10 CFU/100 mL. Similarly, total coliforms in Site 1 greatly varied from month to month, while total coliforms in Site 2 generally stayed in the 10 to 100 CFU/100 mL range. There was no clear correlation between coliforms in one site and coliforms in the other; however, the fecal coliforms in Site 1 did seem to follow a very similar trend to the total coliforms in Site 1. These data illustrate that there are certainly enough bacteria present to facilitate the spread of antibiotic resistance in the recreational water. The less fecal and coliform numbers in Site 2 may be attributed to high salinity, which was 23 ppt in Site 2 compared to 5 ppt in Site 1. The fecal and total coliforms are susceptible to high salinity (Everage et al. 2014; Bergeron et al. 2016). Bergeron et al. (2016) reported the presence of ARB and ARGs in three different salinities of 0, 6, and 12 ppt salinity water in salt marshes. As the salinity increases there is a decrease in the survival of fecal and
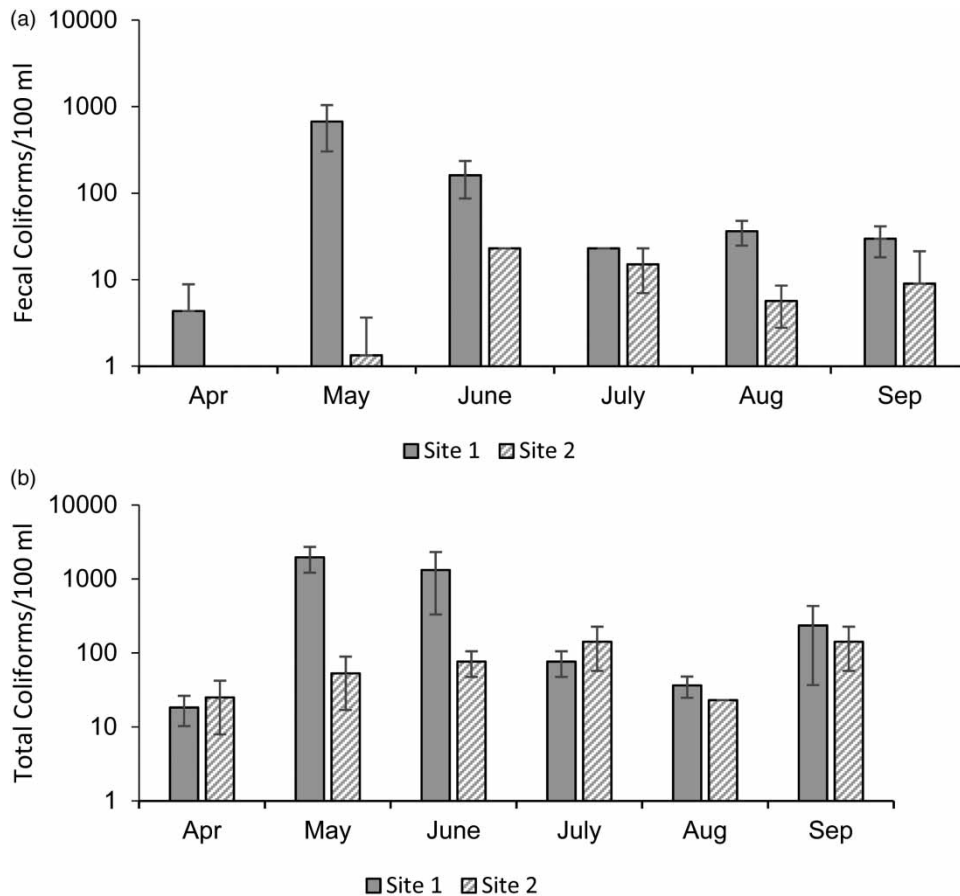
Figure 2 | Fecal coliform (a) and total coliform (b) in Sites 1 and 2 over a six-month period in 2017.

total coliform bacteria as these bacteria are not known for salt tolerance.

Antibiotic resistance assay

Several pure cultures were isolated and identified and the bacteria that were present consistently every month in the water sample were *Escherichia coli*, *Enterobacter cloacae/aerogenes*, and *Enterococcus* spp. Isolates were identified by species using biochemical and assays, and grouped into categories based on speciation. The number of each isolates that read as susceptible, intermediate, or resistant according to the Kirby-Bauer assay is shown in Table 3. The data indicate very few instances of resistance to the carbapenems, ceftazidime/clavulanic acid, and aztreonam; however, each of these drugs had at least one isolate with intermediate resistance. Many more cases of both intermediate

resistance and complete resistance to piperacillin and sulfa-methizole were present. Resistance seemed to be rather evenly spread among the species of bacteria present. All these isolates were equally present in both sites regardless of salinity variations. A number of previous studies have reported that ARB are common in water including raw sewage, treated sewage, and drinking water (Everage *et al.* 2014; Bergeron *et al.* 2015). Interestingly, in this study, ARB were found in the coastal waters with two different salinities. The presence of ARG were analyzed in the ARB isolated from these two sites. The isolates include *E. coli* and *Enterobacter cloacae*. As indicated in Figure 3, ARG were consistently present for erythromycin, with *erm*(B) gene, sulfonamide and tetracycline with the specific genes of *Sul1* and *tet*(A) and *tet*(W). This may be due to higher use of these antibiotics in this region as demonstrated in the past research (Everage *et al.* 2014; Bergeron *et al.* 2017).

Table 3 │ Number of antibiotic-resistant isolates from both sites showing resistance to various antibiotics

Bacteria and the number of isolates	Antibiotic sensitivity	ATM	CAZ	IPM	MEM	PIP	TH
E. coli (n = 28)	S	23	28	28	28	24	20
	I	3	0	0	0	2	5
	R	0	0	0	0	2	3
Klebsiella spp. (n = 30)	S	28	30	29	30	21	20
	I	2	0	1	0	5	6
	R	0	0	0	0	4	4
Enterobacter spp. (n = 10)	S	8	9	9	9	8	6
	I	2	2	1	1	1	2
	R	0	0	0	0	1	2
Other (n = 6)	S	5	6	5	6	4	1
	I	1	0	1	0	1	0
	R	0	0	0	0	1	5

Isolates were identified and grouped for ease of representation. 'S' indicates that isolates were susceptible to the drug. 'I' indicates that isolates had an intermediate level of resistance to the drug. 'R' indicates that isolates were completely resistant to the drug.
Antibiotics tested include aztreonam (ATM) ceftazidime (CAZ), imipenem (IPM), meropenem (MEM), piperacillin (PIP), and sulfamethizole (TH). The concentrations of the antibiotics are given in the Materials and methods section.

The bacteria studied in this investigation are human enteric bacteria, which do not tolerate higher salinity of more than 12 ppt because these organisms are not adapted to high salt conditions as they lack adaptation to osmoregulation for high salinity conditions (Everage *et al.* 2014; Bergeron *et al.* 2016) and thus we anticipated the presence of lower numbers of these bacteria in Site 2, which contained the salinity of more than 20 ppt. Hgher salinities did have some effect on the presence of ARB, which showed lower numbers of total and fecal coliform bacteria in Site 2 coastal water samples. Both sites were commonly used for recreational purposes by a significant number of people. The presence of ARB in these water samples is of concern to public health. The *Sul1* gene for sulfonamide resistance was found in both sites (Figure 3). Sulfonamides act as competitive inhibitors of the enzyme dihydropteroate synthase in the folic acid pathway. The gene *sul1* encodes alternative sulfonamide-resistant dihydropteroate synthases in Gram-negative clinical bacteria (Huovinen *et al.* 1995). Tetracycline resistance genes were most commonly found in both sites. The three tetracycline resistance genes represent each of the three known mechanisms of tetracycline resistance, namely, efflux pumps modification, ribosomal protection, and enzymatic modification (Levy *et al.* 1999; Burch *et al.* 2013). Molecular analysis was done to study the presence of these genes that encode resistance to
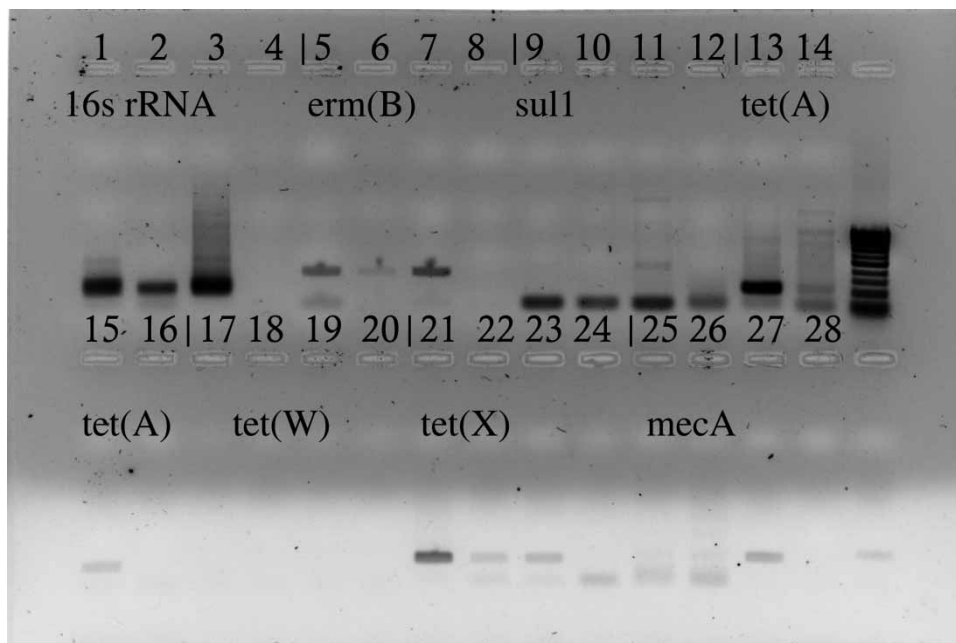

Figure 3 │ Presence of ARGs in bacterial isolates from Site 1 and Site 2. Lanes are organized by genes, with the lanes being in order of Site 1, Site 2, positive control, and negative control. In Site 1, the bacterial isolate was *E. coli* and in Site 2, the bacterial isolate was *Enterobacter cloacae*.

tetracycline, namely, *tetA, tetW*, and *tetX* and the results are presented in Figure 3. The *tetA* gene, which codes for efflux pump modification, was consistently present in both sites. The *tetW* gene that codes resistance to tetracycline via ribosomal protection proteins was also found in the water samples. The gene *tetX* responsible for enzyme modification to confer tetracycline resistance was not observed in this study. We also found *mecA* gene responsbible for vancomycin resistance in isolates from both sites.

With the threat of resistance always on the rise, certain drugs are reserved as treatments of last resort. Carbapenems are a class of beta-lactam antibiotics that are used primarily to treat infections caused by multi-drug-resistant (MDR) bacteria and they are considered to be drugs of last resort. Carbapenems are broad-spectrum antibiotics, which means they are able to treat both Gram-positive and Gram-negative bacteria, although they are more commonly used to treat Gram-negative bacteria (Papp-Wallace *et al.* 2011). Carbapenems function by entering the periplasmic space, which is found on Gram-negative bacteria, through porins. These porins are proteins that allow for the diffusion of molecules across the bacterial membrane. Once inside the periplasmic space, carbapenems inhibit penicillin-binding proteins, which are enzymes that aid in bacterial cell wall synthesis by catalyzing the formation of peptidoglycan. The binding of carbapenem to penicillin-binding proteins causes the irreversible loss of catalytic activity, which causes weakening of the peptidoglycan. Ultimately, the bacterial cell ends up lysing due to osmotic pressure. Carbapenems include several clinically used drugs, such as imipenem, meropenem, eratapenem, and doripenem (Jacob *et al.* 2013). Compared to other beta-lactams, carbapenems are more likely able to resist extended-spectrum beta-lactamases, making them an effective treatment option for these types of infections (Nicolau 2007); however, carbapenems are not perfect drugs. Recently, carbapenem-resistant Enterobacteriaceae (CRE) has become a concern in the healthcare industry. CRE are a class of rod-shaped, Gram-negative bacteria that are resistant to carbapenems. Unlike methicillin-resistant *Staphylococcus aureus*, which has one mechanism for resistance in only one species of bacteria, CRE possess several different mechanisms of resistance in several different species (Jacob *et al.* 2013). This makes CRE even more difficult to manage. The main reason for the spread of CRE is because

of their ability to produce the enzyme, carbapenemase. Carbapenemase is a specialized beta-lactamase that uses Zn^{2+} to hydrolyze carbapenems, rendering them ineffective. It cleaves the beta-lactam ring, which is the portion of carbapenems that binds to and inactivates penicillin-binding proteins in Enterobacteriaceae (Papp-Wallace *et al.* 2011). CRE can also be resistant by producing extended-spectrum beta-lactamases in addition to having a mutation in the bacteria's cell membrane that prevents diffusion of carbapenem into the periplasmic space. Extended-spectrum beta-lactamases are still susceptible to carbapenems; however, in light of the emergence of carbapenemases, treatment of extended-spectrum beta-lactamases with carbapenems is in question. In this study, we did not find any carbapenem-resistant bacteria in the coastal waters of southeast Louisiana.

Although antibiotics have saved the lives of many since their discovery, they have also been used excessively and indiscriminately, leading to new problems. The application of antibiotics may be increasing frequency of antibiotic resistance in the environment. For instance, soil samples in the Netherlands were shown to contain up to 15 times more genes encoding resistance in 2008 when compared to soil samples from 1970 (Knapp *et al.* 2009). Although many isolates did not appear positive for the genes tested, there are many other genes that could be responsible for resistance to both tetracyclines and sulfonamides, which were not tested in this study.

Practical application and future research prospects

ARG and bacteria are found in wastewater from hospitals and animal production, sewage treatment effluent, drinking water, and natural waters and sediments (Zhang *et al.* 2009). Environmental bacteria maintain the ability to infect human hosts either directly, such as through the consumption of contaminated food and water, or indirectly, as it is speculated that environmental resistance and nosocomial resistance are closely associated (Iversen *et al.* 2004; Rodríguez *et al.* 2006; Prabhu *et al.* 2007; Zhang *et al.* 2009).

While environmental ARB and ARG are known in many places around the world, they have also been noted in the region of this study. ARB and ARG have been found in Thibodaux sewage treatment plant, wetlands, marshes, and the bayous of southeast Louisiana (Everage

et al. 2014; Naquin et al. 2015, 2017; Bergeron et al. 2016, 2017). In this study, we report the presence of ARB and ARGs in the coastal recreational waters of Louisiana. The presence of antibiotic-resistant *E.coli* was reported in UK coastal water by Leonard et al. (2015) and they concluded that surfers are at risk of exposure to and colonization by clinically important antibiotic-resistant *E. coli* in coastal waters. Maloo et al. (2014) reported the occurrence and distribution of multiple ARB of the Enterobacteriaceae family in surface and bottom waters along the Veraval coast of India. This study revealed that imprudent use of antibiotic in humans, aquaculture, poultry, and livestock may pose high ecological risk to coastal water users and there is a need to control anthropogenic activities in coastal water bodies to avoid the occurrence of multiple ARB. The occurrence and patterns of antibiotic resistance in bacteria isolated from vertebrates of coastal waters in the northeastern United States was studied by Rose et al. (2009). Out of 472 isolates, 58% were resistant to at least one antibiotic and 43% were resistant to multiple antibiotics. Antibiotic resistance was more widespread in bacteria isolated from seabirds than marine mammals. The study showed that antibiotic resistance is widespread in marine vertebrates, and they may be important reservoirs of ARB in the marine environment. Multiple antibiotic resistance of *Vibrio* isolates were reported on the Kerala coast of India by Manjusha et al. (2005). *Vibrio* spp. were resistant to 22 different antibiotics and the resistance was attributed to nearby aquaculture farms. Asian countries like Vietnam, Indonesia, China, and Malaysia have a significant presence of ARB in the coastal waters due to intense aquaculture industries and their heavy use of antibiotics, especially in shrimp production (Kern & Boopathy 2012; Boopathy et al. 2015). Apart from improper disposal of sewage, aquaculture industry is the major cause for the presence of ARB and ARG in the coastal recreational waters in Asian countries. The practical implication of the present study is to educate the public and create awareness about the presence of multi-drug resistant bacteria and their genes in recreational coastal waters. The only way forward is by reduction at source by reducing the presence of antibiotics in the environment and so future generations can enjoy coastal waters not only for recreational activities but also for their livelihoods of commercial fishing and tourism in many developing countries in Asia, Africa,

and South America. Further research is needed to expand the antibiotic-resisant survey with more antibiotics and also source tracking the origin of ARB and ARGs in coastal waters.

CONCLUSIONS

The results from this study showed the environmental conditions of two sites in southeast Louisiana that seemed to allow for prosperous bacterial growth. The two sites had significantly different salinity levels, but this only allowed for more variety of species between sites. Chemical data showed that nutrient concentrations were found to be more than sufficient for abundant bacterial growth. Fecal and total coliform data confirmed that microbial life was plentiful at both sites. Kirby-Bauer assay data showed a considerable number of drug-resistant isolates, with up to one-third of some species being resistant to the same drug. Some isolates were resistant to two or even three of the antibiotics used. Resistance was distributed rather evenly among species, and all species tended to show more resistance to sulfonamide and piperacillin. The presence of ARG in these two sites indicates a possible public health concern for recreational users of these water bodies.

ACKNOWLEDGEMENTS

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