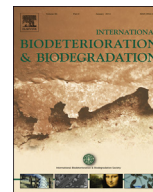




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Presence of antibiotic resistance genes in different salinity gradients of freshwater to saltwater marshes in southeast Louisiana, USA



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ABSTRACT

One of the major public health problems facing the world today is the occurrence and spread of antibiotic resistant bacteria (ARB) in the environment. The main reservoir for ARB is the aquatic ecosystems. Culture based methods and qualitative molecular techniques were used to screen and determine the presence of antibiotic resistance genes (ARG) and ARB in three different salinity gradients of wetland marsh in the southeast Louisiana of USA. The bacteria of interest include *Enterobacter cloacae/aerogenes*, *Enterococci spp.* and *Escherichia coli*. The antibiotic resistance genes of interest include *ermB*, *sul1*, *tetA*, *tetX*, *tetW*, and *mecA* that are responsible for resistance to erythromycin, sulfonamide, tetracycline, and methicillin antibiotics. The water salinity ranged from 0 to 12 parts per thousand (ppt). Monthly samples were taken for a six-month period and analyzed for the presence of ARB and ARGs along with carbon, nitrogen, and phosphorous levels in the water samples. The results indicated salinity did not have significant difference in the presence of ARB and ARGs in the wetlands. Significant numbers of ARB were found in all three salinity levels (0, 6, and 12 ppt) in the marshes of Southeast Louisiana. ARGs were more prevalent in site 2 with the salinity of 6 ppt followed by site 1 with the salinity of 12 ppt and site 3 with 0 ppt salinity. Bacterial load and the pollution load varied from month to month and among the three salinities. This study indicates the presence of ARB and ARGs in the wetland habitat is a cause for concern as the potential threat of the spread of ARGs into native bacteria and into fish and wildlife exists due to human activities even under high salinity habitat.

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1. Introduction

Antibiotic resistance is becoming a very large problem throughout the world. The spread of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in the environment is a major public health issue. Aquatic ecosystem is a significant source for ARB and ARGs. The rise of antibiotic resistance has led to much discussion on the spread of antibiotic resistance genes and the future of antibiotic resistance on public health. Since the production of antibiotics there has been a noted impact with resistant bacteria. Each year there are over 23,000 deaths with at least 2 million people becoming infected with antibiotic resistant bacteria in the United States (CDC, 2015). Antibiotics are among the most commonly used and successful group of pharmaceuticals used for human medicine (Bouki et al., 2013). Rapid spread in resistance to these antibiotics has caused medical concerns to both public and health professionals.

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Resistance is a result of both the appropriate use of antibiotics, such as normal exposure due to usage, and inappropriate use, such as not finishing a prescription or over-use of the drugs. Other reasons include the selective pressure of antibiotic use in the human body and in the environment, as well as change in genome that enhance the transmission of resistant organisms. The goal of the medical professional is to slow down the rise in antibiotic resistance genes (ARGs) by implementing better hygiene, preventing infections, controlling the nosocomial transmission of organisms, treating the source of the causative agent, and changing and developing new treatment methods (Dzidic and Bedekovic, 2003). The general public also plays a key role in control and spread of antibiotic resistant bacteria in the environment through their prudent use of antibiotics and proper disposal of unused antibiotics and also ensuring their waste disposal system is functioning properly.

Some used antibiotics do not always get fully metabolized by the body and are mostly excreted in its original form into the environment (Zhang et al., 2009). There is a growing problem of discharge of antibiotic residues into the environment due to the common use of antibiotics (Zhang et al., 2009). Presence and

spread of antibiotics into the environment have arisen antibiotic resistance in bacteria (Auerbach et al., 2007) especially in wastewater treatment plant, where there is high variety of antibiotics and bacterial densities, bacteria can easily acquire resistance against those antibiotics and release their antibiotic resistance genes (ARGs) into the environment during their release from the treatment plant (Everage et al., 2014; Naquin et al., 2015). These released ARGs through genetic transformation can get easily be transferred to the environmental bacteria and pathogens, increasing risks and dangers to environment and human (Liu et al., 2012). Recent studies show that incomplete metabolism in humans and improper disposal of antibiotics to sewage treatment plants has been a main source of antibiotic release into the environment (Rizzo et al., 2013; Everage et al., 2014). This gives bacteria enough time and sufficient contact to shield themselves selecting for strains that have genes and cellular mechanisms, favoring their growth and reproduction (Galvin et al., 2010). These bacteria have the potential to infect the wildlife in nature, where the treated water is released.

Louisiana is known as “Sportsman’s Paradise”, and has over 300,000 registered boats, with approx. 41,500 of these belonging to southeast Louisiana (Louisiana Department of Wildlife and Fisheries (2013)). Recreational activities such as hunting, fishing, and boating are economically important to Louisiana and with so much physical interaction with the waterways and bayous, water quality becomes a major concern. There are various ways in which water can become polluted and fecal content in aquatic environments can increase, such as agricultural and storm runoff, the waste of animals, and human sewage. In southeast Louisiana, USA, most of the rural household is responsible for their own septic system to treat the wastewater. These systems are effective but require

maintenance, are costly, and if not taken care of properly, can lead to water pollution. When waste is improperly disposed, the risk of antibiotic resistance increases. In this study, a site was chosen, where people reside near the wetlands, which include freshwater, brackish, and saltwater marshes and the waste disposal in these households is mainly individual septic tank. The effectiveness of these septic tanks is not always reliable leading to fecal contamination of wetlands. The purpose of this research is to test antibiotic resistance in three salinity gradients in southeast Louisiana in order to observe whether salinity affects fecal coliforms and their contribution to antibiotic resistant bacteria and antibiotic resistance genes to the environment.

2. Materials and methods

2.1. Collection of sample

Monthly water samples were collected from wetlands that are interconnected with a salinity gradient of 0 (site 3), 6 (site 2), and 12 (site 1) parts per thousand (ppt) in Bayou Petit Caillou in southeast Louisiana, USA. The sampling sites are shown in Fig. 1 with GPS coordinance. The water samples were collected for six months from April to September in 2015. Duplicate samples were collected from the above-mentioned sites using sterile containers. Samples were transported back to the lab on ice, and stored at 4 °C until analysis was completed.

2.2. Analysis of sample

Once the samples were received in the lab, they were manually mixed by shaking the sample bottles. The pH was measured using a

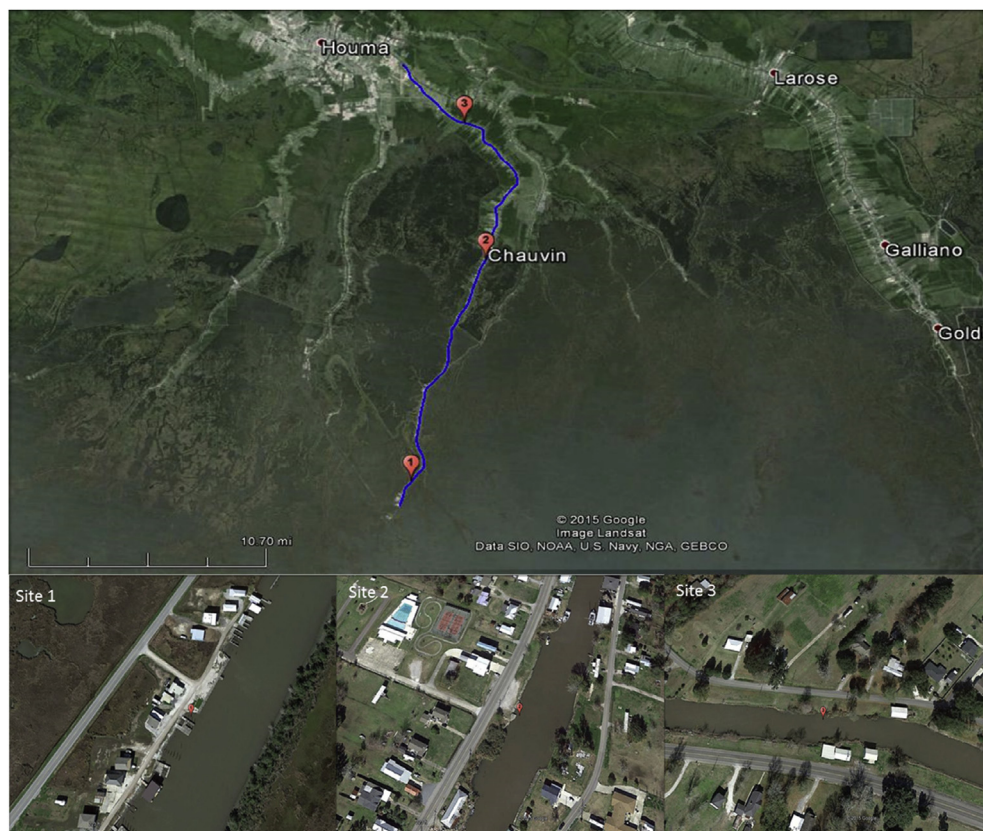


Fig. 1. The sampling sites on Bayou Petit Caillou. Site 1 (12 ppt salinity) in Cocodrie, Louisiana (29°15'49.44"N, 90°39'9.81"W), site 2 in Chauvin (6 ppt salinity), Louisiana (29°25'53.24"N, 90°35'49.43"W), and site 3 in Houma (0 ppt salinity), Louisiana (29°32'5.75"N, 90°36'46.85"W).

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