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Presence of antibiotic resistance genes in raw source water of a drinking water treatment plant in a rural community of USA

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ABSTRACT

Very few studies have been reported on the presence of antibiotic resistance genes in the raw source water of rural communities in the USA. Therefore this study was conducted to study the presence of few specific antibiotic resistance genes, which imparts resistance to commonly used antibiotics in a rural drinking water plant in Louisiana, USA. Samples were taken from raw intake water, the treated water in holding tank at the water treatment facility, and an offsite consumer source serviced by the distribution line. Water quality analysis uncovered increase in organic carbon in distributed water. No live bacteria were found in the treated or distributed water, but bacterial DNA in the form of 16s rRNA was consistently found. Isolates of antibiotic resistant *E. coli, Enterobacter cloacae, Pseudomonas aerugenosa, and Klebsiella pneumonia* were found in the raw intake water. Antibiotic resistance genes, *tetA* and *sul1* were observed in the raw intake showing the presence of antibiotic resistance genes (ARG) and antibiotic resistant bacteria (ARB) in the raw intake water. However, no ARGs or ARB were found in the treated and distributed water.

1. Introduction

Antibiotic resistance is a result of both the appropriate use of antibiotics, such as normal exposure due to usage, and inappropriate use, such as not completing a prescription or over-use of the drugs. The selective pressure of antibiotic use, as well as change in genome that enhance the transmission of resistant organisms also play a key role in antibiotic resistance. Antibiotics are among the most commonly prescribed and successful group of pharmaceuticals used for human medicine (Bouki et al., 2013). The goal of the public health official 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).

When antibiotics are prescribed, they do not get always fully metabolized by the body and can be 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. Presence and spread of antibiotics into the environment have given rise to antibiotic resistance in bacteria especially in wastewater treatment plant, where there are high quantities 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 sewage 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 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 the 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 by altering their genes and cellular mechanisms, favoring their growth, and reproduction (Galvin et al., 2010). These genes can go on to infect the wildlife in nature, where the treated water is released.

Historically, eliminating microbial load in drinking water in the distribution systems was the major focus of the water treatment plants (Xi et al., 2009). The presence of trace levels of antibiotics, antibiotic resistant bacteria (ARB), and ARGs in source and finished water may also greatly affect public health and this is an emerging problem for the drinking water industry (Armstrong et al., 1981;

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Schwartz et al., 1998, 2003). Although many reports were available on the presence of ARB and ARGs in many parts of USA (Auerbach et al., 2007; Xi et al., 2009) there are very few reports available on the presence of ARB and ARGs in the drinking water in Louisiana (Bergeron et al., 2015). Therefore this study was conducted to test for the presence of ARB and ARGs in the drinking water in southeast Louisiana. This study focused on several antibiotic resistance genes, namely, *ermB, sul1, tetA, tetW, tetX,* and *mecA* for resistance to erythromycin, sulfonamides, tetracycline, and methicillin antibiotics respectively in the source, finished, and tap water.

2. Methods

2.1. Sample collection

Water samples were collected from September 2014 to September 2015 in 250 mL sterile plastic bottles for each site, as shown in Fig. 1. Site one was collected from the raw intake pipeline for the Schriever water plant (GPS 29.745118, −90.768077). Site two was collected from the service pipe that water exits the holding tanks at Schriever water plant after water treatment but before it is released into the distribution line (GPS 29.731739, −90.785479), 1.4 miles away from site 1. Site three was taken at a local residence that gets serviced by the Schriever water plant (GPS 290537301, -90.609428), 17.09 miles away from site 2. In each site samples were collected in triplicates, and they were kept on ice while transported to the lab. After collection of the sample, the sample bottles were stored at 4° until analysis.

2.2. Water analysis

The pH was measured using a pH meter (Denver Instruments, Denver, CO). The organic carbon was measured in terms of chemical oxygen demand (COD). Nitrate, nitrite, ammonia, and phosphate were analyzed by the methods described in APHA (1998). The fecal coliforms were determined using the most probable number method (MPN) (APHA, 1998). Pure cultures of bacteria including *E. coli, Klebsiella pneumonia, Enterobacter cloacae, Pseudomonas aerugenosa, Staphylococcus aureus*, and *Enterococcus* spp., were identified by the methods described by Everage et al. (2014) and Bergeron et al. (2015).

2.3. Antibiotic resistance test

Antibiotic resistance in the identified bacteria was measured using the Kirby-Bauer disk assay. The identified bacteria were incubated in TSB at 37 °C for ≈24 h, and diluted to a 0.5McFarland standard. The identified bacteria were used to cover the entire Mueller-Hinton agar plate and various antibiotic discs were placed on the agar. The antibiotics tested include bacitracin, clindamycin, erythromycin, kanamycin, neomycin, oxacillin, penicillin, streptomycin, tetracycline, vancomycin, ampicillin, and chloramphenicol. The plates were incubated at 37 °C for ≈24 h. The zones of inhibition were measured with a caliper in mm for each antibiotic disk. The zones of inhibition were compared using a chart in Leboffe and Pierce (2010) to determine if each isolate is susceptible, intermediately resistant, and totally resistant.

2.4. Detection of antibiotic resistance genes

The methods used for the detection of antibiotic resistance genes were done according to Naquin et al. (2015). One mL of sample from each site was inoculated into a 50 mL centrifuge tube containing 10 mL of TSB and incubated at 37 °C for ≈24hrs. After

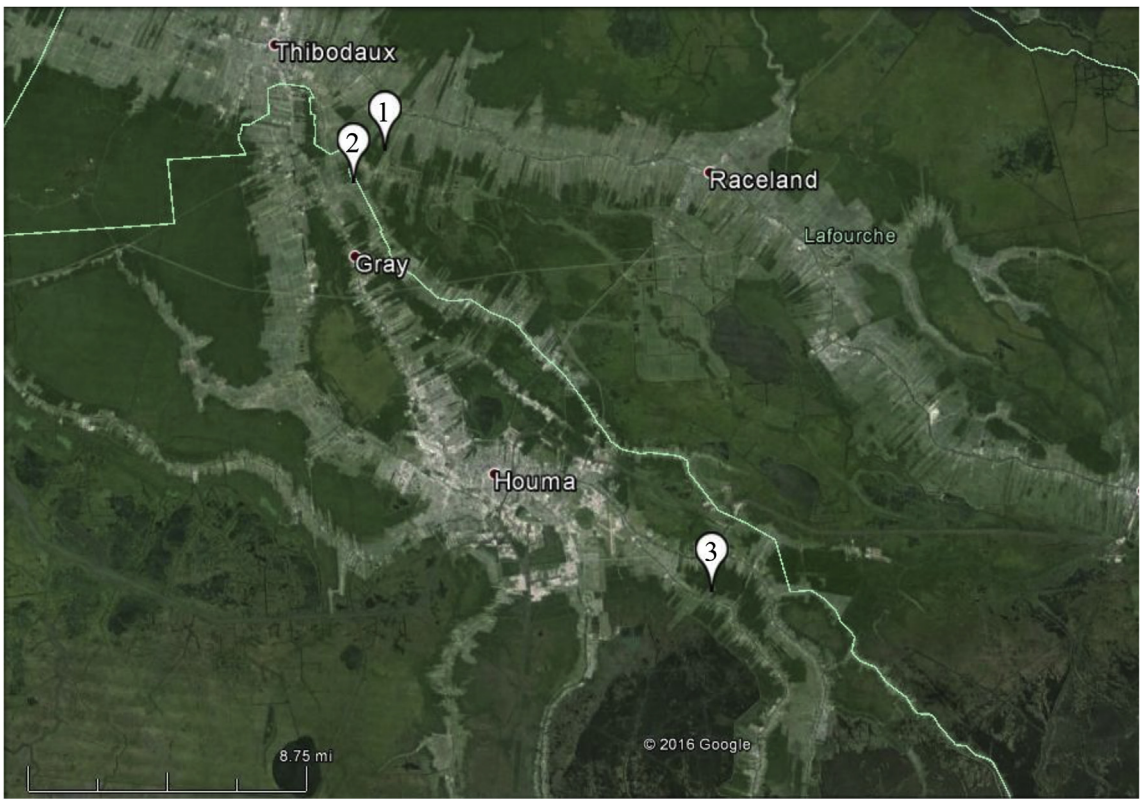

Fig. 1. The three sample sites studied from September 2014 through September 2015. From the raw intake pipeline at Bayou Lefort (Site 1), from the holding tanks at the Shriever Water Plant after treatment (Site 2), a residence that is serviced by this water plant (Site 3).

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