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Presence of sulfonamide and carbapenem resistance genes in a sewage treatment plant in southeast Louisiana, USA

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ABSTRACT

To date only sporadic studies have reported the occurrence of carbapenem and sulfonamide resistance genes in rural sewage treatment plants of USA. Most of the reports on this subject are from hospitals and urban aquatic habitats. Therefore, this study was conducted to find the presence of these genes in a rural sewage treatment plant in southeast Louisiana, USA. Raw and treated sewage samples were analyzed for five months in 2016 for the presence of antibiotic resistance genes. The results showed the presence of carbapenem and sulfonamide resistance genes in both raw and treated sewage. Many sulfonamide and piperacillin resistant bacteria were consistently present in raw and treated samples. This is the first report on the presence of carbapenem resistance genes in a small town sewage treatment plant in USA. Carbapenem possesses potent antibiotic capabilities coupled with inhibition of bacterial enzymes and it has been the last resort of physician's medication to treat infection from many multidrug resistant bacteria. The presence of carbapenem resistance genes in treated sewage sample is a cause for concern and further research should be done to see how prevalent these genes are in many aquatic habitats including rural sewage treatment plants, which in many cases discharge the treated sewage into nearby streams and rivers and may act as a reservoir for these genes in the environment.

1. Introduction

Since the discovery of penicillin in 1928, antibiotics have become an effective means of treatment for bacterial infections (Martin and Kaye, 2004). In fact, they have increased the average expected lifespan by changing the outcome of bacterial infections (Ventola, 2015). Antibiotics can vary in bacterial spectrum, treating a broad or narrow range of pathogens, and can have different types of activity, being bactericidal or bacteriostatic. There are now over 20 classes of antibiotics; however, antibiotic resistance has become an increasing concern with each year (Coates et al., 2011). It has been reported that in the United States, two million people become infected with antibiotic resistant bacteria, and 23,000 people die from these infections each year (CDC, 2016). Antibiotic resistance refers to the ability of bacteria to resist antibiotics that were once used to treat them. The reason for increasing resistance has been attributed to both the rapid evolution of bacteria and the widespread abuse of antibiotics.

Antibiotic overuse, inappropriate prescription, extensive agricultural use, availability of few new antibiotics, and regulatory barriers preventing the use of new antibiotics have all attributed to increased antibiotic resistance (Ventola, 2015). In addition to the increased abuse of antibiotics, the rapid evolution of bacteria has also attributed to increased antibiotic resistance. Even before the use of antibiotics, antibiotic resistance genes were present; however, the widespread use of antibiotics has made bacteria more resistant through evolutionary pressure. By reducing reproductive success in non-resistant bacteria through the use of antibiotics, antibiotic resistant bacteria are naturally selected. A bacterium can develop antibiotic resistance through spontaneous mutation of its own genome or by acquiring antibiotic resistant genes via plasmids. Plasmids may code for antibiotic resistance genes and are capable of transferring their genetic information to another plasmid or to the genome of a bacterium (Hawkey, 1998). Bacteria may acquire plasmids through horizontal gene transfer, which is when a plasmid is transferred from one bacterium to another through transformation, conjugation, or transduction process. Once a bacterium has the genes that allow for antibiotic resistance, it can pass on resistance to other bacteria through vertical or horizontal transmission.

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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) have become a concern in the healthcare industry.

Carbapenem-resistant Enterobacteriaceae are a class of rod-shaped, gram-negative bacteria that are resistant to carbapenems. Unlike Methicillin-resistant *Staphylococcus aureaus*, 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 β-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.

CRE have become a major problem in healthcare because they are resistant to virtually all antibiotics, and infection can be deadly. CRE usually do not infect healthy people and mainly affect people in hospitals and places of long-term healthcare, such as nursing homes. Particularly, CRE have the greatest effect on patients who have extended hospital stays, experience greater severity of illness, have had exposure to higher levels of antibiotics, and who seek treatment in a facility with increased CRE colonization (Ray et al., 2016). One study found that CRE are able to persist in intensive care unit (ICU) sinks and drains, despite disinfection and sterilization efforts. Although the ICU sinks could not be identified as the official cause of CRE in patients, this suggests that hospitals are a reservoir and source of infection (Kotsanas et al., 2013). It should also be noted that once infected with CRE, treatment options are limited because of the bacteria's high levels of antibiotic resistance. It has been reported that there is almost a 50% mortality rate out of patients who become infected with CRE (Jacob et al., 2013).

Presence and spread of antibiotics in the environment have arisen antibiotic resistance in bacteria, especially, in wastewater treatment plant, where there is a possibility of the presence of many kinds of antibiotics and the occurrence of high bacteria densities can lead to bacteria easily acquire resistance against many antibiotics. (Naquin et al., 2015). These resistance genes can get into many aquatic habitats during their release from treatment plant. Sewage treatment plant is an ideal habitat for the development of antibiotic resistant bacteria as large numbers of bacteria come in close contact with many types of antibiotics discharged in the wastewater (Boopathy, 2017; Everage et al., 2014; Naquin et al., 2015; Bergeron et al., 2016). There are many reports on the presence of antibiotic resistance genes in urban environment (Liu et al., 2012; Auerbach et al., 2007; Zhang et al., 2009; Galvin et al., 2010). However, very limited studies have been reported on the presence of carbapenem resistance genes in sewage treatment facility from rural areas. Therefore, this study was conducted to study the presence of CRE and sulfonamide resistance genes in a small rural sewage treatment plant, which serves 15,000 people in Thibodaux, Louisiana, USA. The results showed the presence of carbapenem and sulfonamide resistance genes in the raw as well as treated sewage.

2. Materials and methods

2.1. Collection of sample

Monthly samples were collected in sterile collection bottles from an aerated lagoon where the raw sewage is held for initial treatment (site 1) and treated effluent that has undergone UV treatment (site2) (Fig. 1). The water samples were collected for five months from February 2016 to July 2016 in triplicate. Samples were transported to the lab on ice and analyses were performed the day of collection.

2.2. Water quality

All sample bottles were manually mixed by shaking before all analyses were performed. Total heterotrophic bacteria (Pour Plate Method) were quantified by performing logarithmic serial dilutions with phosphate buffer solution (PBS). One mL of each diluted sample was pippeted onto a sterile petri plate, and liquid tryptic soy agar (TSA) agar was poured over the sample. The plate was mixed, allowed to solidify, and incubated for 24 h at 37 °C. Colonies were counted from the lowest dilution that had countable colonies (30–300 colony forming units (CFU)). Duplicate plates were made for each dilution. Fecal coliforms (A1 Most Probable Number (MPN) Method) were quantified by inoculating three sets of 10 mL 2x A-1 media/durham assembly with 10 mL of sample, three sets of 10 mL of 1x A-1 media with 1 mL, and three sets of 10 mL of 1x A-1 media 0.1 mL of sample. The tubes were incubated for 3 h at 37 °C and 21 h at 44.5 °C. Tubes containing no less than 10% of the durham tube filled with gas were considered positive, and the MPN chart published by the American Public Health Association was consulted to determine MPN of fecal coliforms based on the number of positive tubes (APHA, 1998).

2.3. Bacterial identification

Escherichia coli and Klebsiella pneumonaiae were isolated by quadrant streak plate method with eosin methylene blue (EMB) medium with a positive A1-2X tube incubated at 44.5 °C for 21 h. After the EMB plate was incubated for 24 h at 37 °C, colonies displaying a green sheen were suspected as *Escherichia coli*, and mucoid red or purple colonies were suspected as *Klebsiella pneumonaiae.* Positive *Escherichia coli* and *Klebsiella pneumonaiae* colonies were inoculated into triple sugar iron agar (TSIA) medium and SIM's tubes and incubated for 24 h at 37 °C. Positive *E. coli*

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