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Presence of antibiotic resistant bacteria and antibiotic resistance genes in raw source water and treated drinking water

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ABSTRACT

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 drinking water treatment system is designed specifically to eliminate bacteria and pathogens in drinking water. The presence of ARB and ARGs in source water and drinking water may affect public health and it is an emerging issue in drinking water industry. Therefore, this study was conducted to study the presence of ARB and ARGs in a source water, treated drinking water (finished water), and in the distribution line (tap water) in a rural water treatment plant in Louisiana. The results showed the presence of several ARB in the source water including, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas*, *Enterococcus*, *Staphylococcus* and *Bacillus* spp. However, the water treatment plant effectively removed these bacteria in the treated water as none of these bacteria were found in the tap water as well as in the finished water at the water treatment plant. Bacterial DNA including 16s rRNA and ARGs of sulfonamides and tetracycline antibiotics were observed in raw water. The presence of 16s rRNA was found consistently in every month of sampling in raw water, finished water, and tap water. This suggests that the filtration system at the treatment plant was ineffective in filtering out small fragments of bacterial DNA. Also, the possibility of the presence of biofilms in the water pipeline exists, which may develop antibiotic resistance due to the selective pressure of chlorination in drinking water.

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Introduction

Increase in antibiotic resistance is reported almost daily in both popular and scientific literatures. 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. 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, 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 used antibiotics do not get always 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 varieties 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;

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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, microbial quality of 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, 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; Schwartz et al., 2003). Although many reports were available on the presence of ARB and ARGs in many parts of USA (Armstrong et al., 1981; Xi et al., 2009) there was no report available on the presence of ARB and ARGs in the drinking water in Louisiana. Therefore this study was conducted to look at the presence of ARB and ARGs in the drinking water in southeast Louisiana. This study focused on the presence of several antibiotic resistance genes, namely, *erm(B)*, *sul1*, *tet(A)*, *tet(W)*, *tet(X)*, and *mecA* for resistant to erythromycin, sulfonamides, tetracycline, and methicillin antibiotics respectively in the source, finished, and tap water. The results showed the presence of many ARB and few ARGs in the source water.

Materials and methods

Collection of sample

Monthly water samples were collected from raw source water, finished water from a water treatment plant, and tap water serviced by the same water treatment plant in southeast Louisiana for six months. Triplicate samples were collected each time from the above-mentioned sites. Samples were transported back to the lab on ice, and stored at 4 °C until analysis is completed.

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 pH meter (Denver Instruments, Denver, CO). The organic carbon in terms of chemical oxygen demand (COD), total nitrogen, and phosphate in the sample was analyzed by the methods described in APHA (1998).

Bacterial analysis

Total aerobic heterotrophic bacteria and fecal coliform were analyzed every month according to the method described by Everage et al. (2014). Various Pure cultures were isolated and identified using BIOLOG method and by various specific biochemical tests as described by Everage et al. (2014).

Antibiotic resistant test

Antibiotic resistance was determined using the Kirby–Bauer method (Brown, 2005). Different pure cultures isolated each month from the raw source water were subjected to antibiotic resistant assay. A bacterial lawn of the sample was grown on tryptic soy agar (TSA) media plate, using sterile cotton swabs as described by Everage et al. (2014). After the sample was streaked onto the TSA plates, the antibiotic discs of erythromycin, tetracycline, neomycin, chloramphenicol, kanamycin, streptomycin, oxacillin, clindamycin, and vancomycin were placed using an automatic, hand-held disk dispenser. The plates were then incubated at 37 °C for 24 h. The zone of inhibition for each antibiotic was measured in millimeters with a standard laboratory caliper at the end of the 24-h incubation period. The antibiotic resistance was consulted with the Kirby–Bauer chart as described by Brown (2005).

DNA extraction

Water samples were centrifuged at 13,000 RPM for 15 min. The pellet was transferred to a 1.5 ml microcentrifuge vial. A Fast ID DNA Extraction Kit was used according to manufacturer's instruction to extract the DNA. After the DNA was extracted, polymerase chain reaction (PCR) was used to amplify the DNA as described by Naquin et al. (2014, 2015). The presence of various antibiotic resistance genes was analyzed using the well known primers for methicillin (*mecA* gene), erythromycin (*ermB* gene), sulfonamides (*sul1*gene), tetracycline (*tetA*, *tetW*, and *tetX* genes for efflux pump, ribosomal protection, and enzymatic modification respectively) as shown in Table 1 based on Burch et al., 2013. The presence of *mecA* gene in the water samples was analyzed using the *mecA* primer (Table 1) as demonstrated by Suzuki et al. (1992). All primers were obtained from Sigma Aldrich Co. (St. Louis, MO). A 2% agarose gel with ethidium bromide was prepared and used to visualize the PCR samples. 10 μl PCR sample was mixed with 2 μl 6× loading dye and injected into each well. The gel was run at 100 V for an hour. The gel was visualized using FluorChem FC2 imaging system. Antibiotic resistant strains and primers served as a positive control and the DNA free water served as the negative control. A universal 16s rRNA gene was used as the housekeeping genes for the presence of bacteria in the samples.

Statistical analysis

All chemical data were subjected to an analysis of variance (ANOVA) test ($p \leq 0.05$) followed by a Tukey "*post hoc*" analysis when needed (SAS).

Results and discussion

Performance of water treatment plant

The water treatment plant is operating according to its designed purpose. It is very effective in removing aerobic heterotrophic bacteria and fecal coliform bacteria. Fecal coliform bacteria were observed in raw source water sample every month (Fig. 1). The bacterial counts varied from month to month, but it was always below 1000 coliform/100 ml of sample. There was no fecal coliform found in treated finished water at the plant and in the tap water at the distribution line. This result indicates that the filtration systems as well as chlorination process employed at the water treatment plant is working effectively and the plant meets Environmental Protection Agency's (EPA) water treatment standard. Similarly, the aerobic heterotrophic bacterial count showed the absence of bacteria in treated finished water and in the tap water. However, the raw source water contained significant amount of bacteria (Fig. 2) and the bacterial numbers varied from month to month. The organic carbon in the form of COD, total nitrogen, and phosphate were always present in the raw water and the concentration was always less than 25 mg/l (data not shown). In the treated water also organic carbon, nitrogen, and phosphate were found in every month of the sampling period. These results showed that the raw source water is contaminated with fecal coliform, heterotrophic bacteria, organic carbon, nitrogen, and phosphate. The source water is from Mississippi River and this water carries heavy pollution load as many farm lands and municipalities drain the water into Mississippi River all the way from its origin in the state of Minnesota, which results in one of the longest stretches of pollution in an aquatic ecosystem (Boopathy et al., 2012).

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