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The Prevalence of Antibiotic Resistance in *Escherichia coli* in Relation to Wastewater Treatment Plants

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ABSTRACT
E. coli is part of human's natural flora found within the intestinal system, but it can also grow within contaminated food. When there is an excess amount of *E. coli* that is when people can become really sick. As more and more antibiotics are being developed more research will need to be conducted with bacterial resistance and how it affects the daily lives of everyone else. The objective of the research was to compare the amount of antibiotic resistant *E. coli* within dry turkey creek located in McPherson, KS. The predictions were that there will be a greater amount of resistance downstream of the wastewater treatment plant vs. upstream of the wastewater treatment plant. 24 undiluted one milliliter samples were taken both from upstream and downstream of the treatment plant and grown on agar plates for *E. coli.* Those colonies were then transferred onto agar with ampicillin and tested for resistance. The results were not similar to that of other literature. Overall the stream had *E. coli* resistance of 72%, but there was no significant difference in resistance between upstream and downstream. There was a significant difference in the amount of *E. coli* colonies, with downstream having more than upstream which correlated with other literature.

Keywords: Wastewater treatment, *E. coli*

INTRODUCTION

Antibiotics are among the most successful group of pharmaceuticals used for human and veterinary therapy. However, because of widespread use, incomplete metabolism in humans, and disposal of unused antibiotics, large amounts of antibiotics are released into municipal wastewater (Danae and Dlamadopoulos, 2013). This is important for everyone to know because *E. coli* is found in all mammal feces at concentrations of 10^9cells/g but it does not multiply in the environment. In the 1890s it was chosen as the biological indicator of water treatment safety. Because of method deficiencies, *E. coli* surrogates such as the 'fecal coliform and total coliforms tests were developed and became part of drinking water regulations.

Public health protection requires an indicator of fecal pollution. It is not necessary to analyze drinking water for all pathogens with the advent of the Defined Substrate Technology in the late 1980s, it became possible to analyze drinking water directly for *E. coli* (and, simultaneously, total coliforms) inexpensively and simply.

During the wastewater treatment process the wastewater treatment plants add antibiotics and UV to the water to try and kill of any bacteria that is in the water. The bacteria that aren't killed off then become resistant to the antibiotics. Bacteria will continue to develop resistance during wastewater treatment by either new mutations or the exchange of genetic information. (Danae, 2013) The antibiotics that humans take and are not always metabolized and therefore defecated and make it into water systems or are dumped by medical facilities such as hospitals all eventually find their ways into different natural environmental compartments such as streams and rivers. They find their way into wastewater treatment plants and not all bacteria are eliminated such as *E. coli* and develop resistance.

Wastewater treatment reduces the numbers of *E.coli* bacteria, however antimicrobial-resistant studies have shown that *E. coli* was not eliminated, and *E. coli* resistant to cefotaxime, ciproflaxin, and cefoxitin was present in treated effluent samples. (Galvin, 2010) The bacteria are becoming resistant due to consistent small exposure of antibiotics in the wastewater treatment process as well. Indicator bacteria with resistance patterns were positively selected by the wastewater treatment processes based on activated sludge. Their number in treated wastewater periodically reached even 90% of the total fecal bacteria. (Aneta, 2010)

The objective of my research was to compare the amount of *E. coli* upstream vs. downstream of the wastewater treatment plant of McPherson, KS, and to compare the amount of *E. coli* that is resistant to ampicilin upstream and downstream.

MATERIALS AND METHODS

I gathered one ml of undiluted and diluted samples from both locations. When I diluted the samples I put one ml of sample into a test tube with nine ml of autoclaved deionized water. I placed the gathered samples on the Chem-dry agar plates (Hardy Diagnostics Santa Maria, CA) that specifically grow *E. coli* and incubated the plates in a 37 degree Celsius chamber for approximately 24 hours. The undiluted samples proved to provide the best amount

Figure 1. Map showing sampling locations. The northern and southern most circles are the sample locations and the middle location is where the wastewater treatment plant is located.

of *E. coli* colonies that I was able to work with. I made nutrient agar plates with ampicilin and when I had the *E. coli* I gridded each agar plate that contained the ampicillin and placed an *E. coli* colony within each grid square and then incubated the plates at 37C for 24 hours. If the colonies grew it meant that they are antibiotic resistant. I can also compare to the original plates without the antibiotics and compare how many bacteria forming colonies actually grew. My hypothesis is that not only will there be more *E. coli* forming colonies downstream of the McPherson, KS wastewater treatment plant but there will also be more antibiotic resistant colonies downstream of the wastewater treatment plant. The few bacteria that do survive wastewater treatment processes build resistance to any antibiotics that are in the water. A t test was performed to determine any significance within the *E. coli* colonies and a binomial distribution was used to determine any significance in the amount of resistance overall upstream vs. downstream within the *E. coli* colonies as well as resistance in the stream overall.

RESULTS

There was a significant difference in the amount of *E. coli* colonies upstream versus downstream of the wastewater treatment plant (*df=36, P=.01; Figure 2)*

There was no significant difference in antibiotic resistance upstream vs. downstream.

Overall the *E. coli* had 72% resistance to the ampicllin within the stream. Upstream *E. coli* colonies had an overall resistance of 60% and downstream had an overall resistance of 73%.

DISCUSSION

I found that there was no significant difference in antibiotic resistance in *E. coli* downstream vs. upstream of the McPherson wastewater treatment plant. But there was a significant difference in the amount of *E. coli* colonies downstream of the treatment plant. I expected for there to be a significant difference downstream vs. upstream in antibiotic resistance as well as a significant difference in the amount of *E. coli* colonies. I believe the reason behind there not being a greater significance in antibiotic resistance downstream of the wastewater treatment plant is due to the fact that I wasn't able get that many colonies of *E. coli* upstream. With not having a lot of colonies to work with it is unknown if a greater amount of colonies would have yielded a

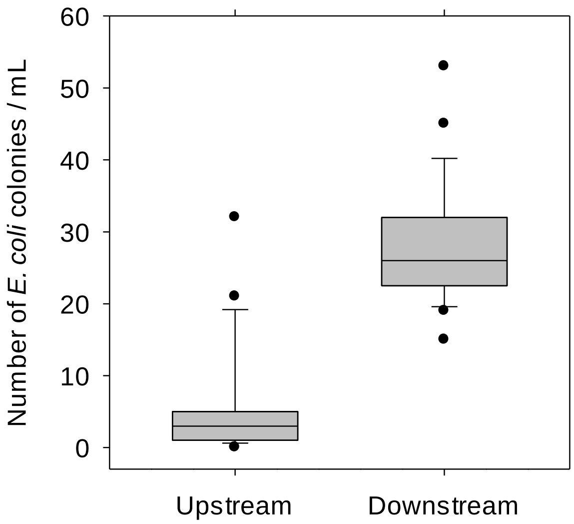

Figure 2. Amount of *E. coli* colonies within the stream.

significance in antibiotic resistance. Performing the experiment again and checking to see if the same results are yielded would be the most accurate way to truly determine if there is a significant difference in antibiotic resistance downstream of the wastewater treatment plant as indicated by literature.

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