

Bard

Bard College
Bard Digital Commons

Senior Projects Spring 2020

Bard Undergraduate Senior Projects

Spring 2020

Antibiotics in the Saw Kill and Rhinebeck Kill Creeks: Tracking Anthropogenic pollution with Bacteria and Integrons

Timothy William Matsakis
Bard College, tm5109@bard.edu

Follow this and additional works at: https://digitalcommons.bard.edu/senproj_s2020

 Part of the [Environmental Studies Commons](#)



This work is licensed under a [Creative Commons Attribution-NonCommercial-No Derivative Works 4.0 License](#).

Recommended Citation

Matsakis, Timothy William, "Antibiotics in the Saw Kill and Rhinebeck Kill Creeks: Tracking Anthropogenic pollution with Bacteria and Integrons" (2020). *Senior Projects Spring 2020*. 117.
https://digitalcommons.bard.edu/senproj_s2020/117

This Open Access work is protected by copyright and/or related rights. It has been provided to you by Bard College's Stevenson Library with permission from the rights-holder(s). You are free to use this work in any way that is permitted by the copyright and related rights. For other uses you need to obtain permission from the rights-holder(s) directly, unless additional rights are indicated by a Creative Commons license in the record and/or on the work itself. For more information, please contact digitalcommons@bard.edu.

Bard

Antibiotics in the Saw Kill and Rhinebeck Kill Creeks:
Tracking Anthropogenic pollution with Bacteria and Integrons

Senior Project Submitted to
The Division of Social Studies
of Bard College

by
Timothy Matsakis

Annandale-on-Hudson, New York
May 2020

Acknowledgements

I would like to thank my friends and family for their continued support throughout my time working on this project. I would also like to thank my advisor Eli Dueker for his support and guidance. Finally, I would like to thank Lindsey Drew for her assistance in the water lab and with providing supplies.

Table of contents

Abstract	3
Introduction	3
Fecal Indicator Bacteria	5
ARG's and Integrons	6
Integrons in the Environment	8
How Integrons Spread	8
Antibiotics and ARG's in WWTP	9
Hospitals and Antibiotic Resistance in WWTP's	11
Combined Sewer Overflows	13
Antibiotics and Agriculture	14
Sewage Sludge	15
The Case for Tracking Antibiotics	17
Current Practices for Limiting ARB in WWTPS	17
Materials and Methods	18
The Rhinebeck and Saw Kill Creeks	18
The Bard and Rhinebeck WWTP's	19
Sample Collection and Pretreatment	20
Results and Discussion	22
Next Steps	23
Conclusion	23
Figures and Tables	25
Bibliography	27

Abstract

Antibiotic resistance is an increasingly researched topic along with concerns regarding emerging organic contaminants. Antibiotic resistance is a risk to public health and therefore a better method for tracking antibiotics and antibiotic resistance is needed to develop regulations and protect the public. Several studies have shown that the use of class 1 Integrons and fecal indicator bacteria (FIB) prove effective for conducting these tests. This study aimed to assess the extent to which FIB and Integrons were present in the Saw Kill and Rhinebeck Kill creeks and serve as effective indicators of antibiotic resistance. The results show that there were no statistical differences in environmental variables and FIB concentrations between sites. Because this study was cut short, the presence of Integrons and antibiotic resistance were not measured; however, samples were stored for DNA extraction at a later date.

Introduction

Emerging organic contaminants are becoming an increasingly concerning threat to people's health and well-being as personal care products, antibiotics, and other harmful organic compounds are released into sewage. To make matters worse, existing wastewater treatment plant (WWTP) infrastructure struggle to process these contaminants resulting in their

introduction into the environment. Once they are present in the environment they pose a threat, not only to humans, but also to the overall ecosystem (Sánchez-Avila et al, 2012).

Among these contaminants antibiotics are particularly concerning as they don't break down in the human digestive tract and are often flushed down toilets instead of being properly disposed. As a result, antibiotics and antibiotic residuals end up in the environment and WWTP's increasing the potential for bacteria to become antibiotic resistant (Müller et al, 2018).

WWTP's have also been shown to increase the chance of antibiotic resistance occurring as well as promoting antibiotic resistance genes (ARG) (Voigt et al, 2020). ARG's have the potential to spread within the environment and cause bacteria to become antibiotic-resistant in the future. This is a major concern for public health as antibiotic resistance accounts for around 23,000 deaths a year (CDC) and yet is only recently being discussed as a contaminant. As a result, very little consideration is given to the presence of antibiotics and the associated residuals when treating sewage.

Treatment plants have been found to be a hot spot for the introduction of antibiotic-resistant bacteria and residuals into the environment, as they essentially act as a breeding ground where a mixture of high concentrations of antibiotics and bacteria combine, increasing the chances of Horizontal Gene Transfer (HGT) occurring which in turn increases the rate at which antibiotic resistance occurs (Müller et al, 2018). There is currently a lack of research on monitoring the presence of ARG's antibiotic-resistant bacteria (ARB), antibiotics, and antibiotic residuals (AR) in WWT processes from the sewers to the effluent and receiving water bodies. The ability to track these contaminants is not only important for understanding the

current effectiveness of WWTPS and how to improve them but also providing the public with better information on water quality and safety.

This study aims to determine whether or not fecal indicator bacteria and or class 1 Integrons are an effective means for monitoring antibiotic pollutants. More specifically to assess whether or not WWTPS increases the concentrations of antibiotic resistance. The impact that environmental factors such as conductivity, turbidity, and temperature have on this will also be assessed. By generating data around this issue, a better understanding of how to prevent these contaminants from entering water bodies can be generated.

Fecal Indicator Bacteria

Because having the ability to effectively track antibiotic resistance is so vital to public health, it is important to have effective testing methods. Fecal indicator bacteria can be used to perform this role as they serve as an indicator of anthropogenic pollutants (Gillings, 2015). and because of their associations with antibiotic-resistant bacteria. While they do not have the ability to identify specific antibiotic-resistant strains, studies have shown that it is more effective to look at a broad range of antibiotic resistance instead of wasting resources isolating specific antibiotics (Urriza et al, 2000). The relative simplicity of analyzing fecal indicator bacteria makes them particularly useful for sites that are regularly assessed, and are ideal for citizen science purposes and non clinical environmental testing. Additionally fecal indicator bacteria concentrations are often collected as part of regular water quality assessments, meaning that existing practices can be left relatively unchanged.

ARG's and Integrons

One of the major ways genetic information is spread between bacteria is through class 1 and class 2 Integrons. Integrons contain gene cassettes, that are so named for their ability to store genetic information (Gillings, 2015). When a cassette containing an ARG is picked up by a non-antibiotic-resistant bacteria, the genetic information is capable of being transferred without disrupting existing genes,(Gillings, 2014) foregoing the process of natural selection. Class 1 Integrons are more effective at doing this than the other classes as they also contain their own promoters which gives them the ability to express genes that can potentially be immediately beneficial far faster (Gillings, 2014). Because of the class 1 Integrons' ability to readily develop resistance that can be transferred between different taxa, they are extremely versatile Having the ability to easily move between genomic locations (Gillings, 2017) and can be found in a wide range of environments around the world, (gillings, 2014) meaning that they have a high potential to spread ARG's outside the immediate vicinity of a WWTP. Integrons have also been shown to thrive in the human gut, and as such serve as have a strong correlation to human presents and can be used as indicators for anthropogenic pollutants other than ARB's such as heavy metals and personal care products (Gillings, 2017). Although other methods of tracking ARB's in water bodies exist, the versatility of Integrons makes them highly promising for environmental monitoring where conditions are subject to frequent change.

Because there are a wide range of antibiotic classes in use with the potential for hundreds of types to enter the influent of a single WWTP (Yang et al, 2014), it is important that

monitoring methods can reveal resistance to antibiotic families instead of specific antibiotics themselves. This will provide a big picture perspective of the study area which is useful for monitoring purposes, being able to assess general trends will also make it easier for monitoring the effectiveness of wastewater treatment plants. If specific classes of antibiotics are found to be present in a system treatment methods designed to specifically deal with them can be introduced. This is also a more realistic approach in an environment setting with constantly changing factors. Generality is better than precision in these situations because assays are more likely to detect pollutants that would have otherwise gone unnoticed. Integrons are highly effective at this type of monitoring as they are capable of representing a variety of antibiotic-resistant types present in water. In one study, Integrons as well as aminoglycoside and beta-lactam resistant genes were present in both clinical and environmental isolates of gram-negative bacteria (Zheng et al, 2020). In this situation the presence of Integrons are correlated to the presence of antibiotic resistant factors such as the amount of antibiotics present in the WWTP as well as the occurrence of two types of resistance among gram-negative bacteria.

It can therefore be concluded that if Integrons are found to contain the genetic material associated with these factors, antibiotic resistance can occur. This is further supported by findings that showed that not only were Integrons present in WWTP's, but they also may be responsible for introducing antibiotic resistant genes in the activated sludge (AS) stage of treatment processes, thereby assisting in the propagation of antibiotic resistance in sewage sludge (Zheng et al, 2020). IntI1 and clinical intI1 were both shown to have positive associations with ARG's and the AS. Despite class two Integrons being present in a large number of samples the other classes of Integrons did not have a significant association. As a result, class 1 Integrons are

the only classifications that can serve as indicators for the overall ARG abundance in activated sludge. Class two integrons are further limited by the fact that they cannot capture new gene cassettes (Zheng et al, 2020).

Integrons in the Environment

Integrons have been present in the environment for millions of years and can be found in a wide range of substances including soil and water (Gillings, 2014). Four classes of integron exist however only the clinical ones classes 1-3 are of interest and of those only classes 1 & 2 are effective at moving antibiotic resistance, with class 1 Integrons being the primary focus for research into tracking ARBs (Deng et al, 2015). Their potential as a means to track antibiotic resistance was not recognized until fairly recently and so more research is needed on their uses.

How Integrons Spread

Integrons have been found on every continent and most countries in the world, which is indicative of their ability to survive in a wide range of environments as well as their tendency to be transported to new locations, potentially bringing genetic information with them. (Gillings, 2014) Because of this, it is important to understand the ways in which Integrons move around in the environment and some of the major points of transmission.

Humans most likely help to mobilize class 1 Integrons and their associated ARBs because they are commonly found to be present in our microbiome, enabling their spread to our pets and agricultural animals when we travel (Muller et al, 2018). In fact concentrations of

Integrans were found to rise and lower in correlation with travel seasons (Müller et al, 2018). Water is also a major method of transportation for Integrans giving them the ability to reach a wide range of environments. As a result special attention must be paid to the introduction of pollutants upstream and in tributaries.

As was mentioned above, not all Integrans have the potential to be used for tracking ARBs. There are four different classes of Integrans, however only class 1 and 2 are capable of actively spreading antibiotic resistance due to their ability to store genetic information. While Integrans contain coding sequences, they do not have promoters meaning they are not independently mobile. Cassettes can include resistance to up to 130 distinct antibiotic resistant genes (Deng et al, 2015). Class1 are also the only classes that have been shown to have positive associations with ARGs and activated sludge (AS) (Zheng et al, 2020). The other classes of Integrans do not have this association, meaning only class 1 Integrans can be effectively used as indicators for the overall ARG abundance in WWTP.

Antibiotics and ARG's in WWTP

When attempting to limit antibiotic resistance in WWTP it is important to consider the potential for horizontal Gene transfer (HGT). One key concern is limiting the amount of antibiotics and antibiotic residuals(AR) that make it through the treatment process and into the effluent. Furthermore, the transfer rate between plasmids and bacteria in certain WWTP processes such as membrane bioreactors (MBRs) and activated sludge has been shown to be

greater than that of waste water and conventional bacterial sludge (Yang et al, 2013). As a result consideration must be given to the specific methods used by a given WWTP.

Antibiotic residuals can commonly be found in wastewater effluent and often coincide with some level of ARBs presence as well. The rate at which they are present is dependent on whether or not they're urban or rural, if they are receiving sewage from a wide range of sources such as hospitals, farms, industry, or simply domestic sewage. The time of year is also an important factor. Clinical urban wastewater was found to have less antibiotic resistance (86%) than that of rural WWTP's (96%) showing that although Intergrons have been linked to the human gut, they are clearly not the only factor that affects the potential for antibiotic resistance (Voigt et al, 2020). This also shows that although hospitals may be using greater amounts of antibiotics than domestic WW they may not actually have a large impact on the total amount of antibiotic-resistant present in the receiving WWTP. However, most of the strains found in the clinical WW were more susceptible to more than two strains of antibiotics indicating that while overall concentrations of antibiotic resistance attributed to hospitals is low the potential for multidrug-resistant bacteria is high. Significance has also been found between the relationships of antibiotic bacteria and residuals, particularly when looking at clinically used antibiotics, indicating the hospital effluent contains antibiotics that are not properly removed in WWTPs.

Clustering has been shown to occur only in clinical Wastewater. This type of environment increases the chance of bacteria becoming multi-drug resistant, as bacteria become resistant to a family of antibiotics that are commonly seen in hospital waste (Voigt et al, 2020). This does not mean that resistance cannot occur in other ways, as the same study found ARs in all their sample sites along with a large number of sites showing some level of resistant bacteria.

Another factor in WWTP's ability to effectively process sewage is the time of year. This is particularly true for certain aspects of the treatment process, particularly those that rely on biological activity. Treatment plans that use bacteria for secondary treatment have been shown to be less effective in winter months when the cold weather inhibits them from effectively treating sewage (Koivunen et al, 2003).

Because there are such a wide range of environmental factors that impact antibiotic resistance in water, it is important to measure not just antibiotic resistance genes, bacterial loads and antibiotics present but also factors such as conductivity, turbidity, weather and more when testing (Young et al, 2013). Gaining a better insight into these factors could help not only predict when bacterial loads will be high, but would also aid in the process of limiting the presence of organic pollutants in affluent receiving water bodies.

Precipitation in particular is a highly influential variable as antibiotic-resistant bacteria have been shown to increase dramatically after rain events suggesting they have a greater association with the occurrence of antibiotic-resistant bacteria than resistance developed under normal circumstances (Young et al, 2013). Weather events therefore have the potential to dramatically change the effectiveness of sewage treatment plants ability to function. Combined with the fact that abundant genres of ARB have been associated with antibiotic-resistant infections, effluent receiving water bodies have a high potential to be dangerous to the General public, particularly with immunocompromised individuals (Young et al, 2013).

Hospitals and Antibiotic Resistance in WWTP's

Because of the high use of antibiotics in hospitals they would seem to be a major source of antibiotics and antibiotic resistant bacteria into WWTPs. As is the case with other point source contaminants into WWTPs, the effectiveness at which hospital effluent can be treated depends on a variety of factors. A study by Buelow et al assessed this by looking at the influence that hospital effluent has on the microbiome and resistome of wastewater systems in different scenarios, comparing two WWTP.

The first serviced an urban community of 290,000 people and three hospitals, while the second serviced a smaller community of 14,000 people and did not contain a hospital. Both of the treatment plants used similar processes with the highlighted features being an activated sludge process to perform nitrification and denitrification. The only difference was in the way they dealt with phosphorus with the urban plant using chemicals while the other plant used biological systems. The microbiome was found to be diverse at all sample sites with diversity being the greatest in the effluent influenced river samples and lowest in sewage samples coming directly from the hospitals. The hospital sewage had relatively high levels of anaerobic bacteria that was theorized to have originated from the human gut. Both the urban and Suburban wastewater treatment plants influents were clustered together on a principal coordinates analysis and had the same three most abundant bacterial taxa and no significant difference was found in the microbiota of the two influences.

Although the urban wastewater treatment plant received a far greater variety of sources, this did not seem to have an effect. The only major differences that were found were those of the hospital sewage samples, however these differences became nullified after being processed by a WWTP. Despite hospitals having no effect on the end result of the microbiome, antibiotic resistant genes were found only in samples taken from hospital sewage, indicating that hospitals may still increase the risk of ARG's and turn the environment.

Combined Sewer Overflows

While the impact of source contaminants has been shown to affect the presence of antibiotic-related contaminants in WWTPs, another potential for contamination is when treatment plants are not able to effectively treat sewage before it is released. In fact, one major way that ARGs, ARBs, and pharmaceuticals end up in water bodies is through combined sewer overflows.(CSO) These events have been shown to overload WWTPs significantly, increasing the amount of antibiotics and other pollutants in the water during brief shocks to a system. A CSO occurs when there is a significant rain event that the normal sewer system cannot handle and WWTPs let large amounts of water go by untreated. Treatment systems that require time to work such as the settling process cannot handle the additional influx of rain and therefore large amounts of effluent are released untreated. Additionally, FIBs and antibiotics that were lining sewer pipes and had become dislodged were re-suspended and mixed in with the effluent, increasing the concentrations in the influent to above average levels (Passerat et al, 2013). Preventing combined sewer overflows would mean a complete overhaul of the sewer system,

separating domestic sewage and storm drains into their own pipes and facilities. As a result, other methods must be used in order to prevent combined sewer overflows from affecting local water bodies each time it rains. Improving and or including tertiary methods of treatment such as using UV light or chlorine can dramatically decrease the amount of genetic pollution entering the environment after one of these events. Tertiary methods are not perfect however, and combined sewer overflows do not only have an effect on bacterial loads (Passerat et al, 2103). Up to 86% of suspended material in effluent receiving waters has been found to originate from a location other than a WWTP after a CSO. CSOs have also been shown to increase environmental conditions that have been correlated with a higher percentage of fecal indicator bacteria in the water, such as suspended matter (sm) and conductivity. Because these events are unpredictable and lead to a dramatic rise in fecal indicator bacteria, monitoring around water bodies that have CSO systems is vitally important.

Antibiotics and Agriculture

Antibiotics are also commonly released into the environment through their use in agriculture (Gillings, 2015). Unlike in human use, where antibiotics are only given when someone is showing signs of a bacterial infection, many farming practices use antibiotics as growth promoters and to prevent bacterial infections from occurring in the first place. The antibiotics used on farms also have the potential to be spread through runoff, entering water bodies without ever being processed.

Just as antibiotics are not processed down to a state that prevents resistance in the human gut, the same holds true for animal's and as a result, agricultural waste contains high amounts of antibiotics. Unfortunately, because agricultural waste is not viewed as hazardous like hospital waste is, it often undergoes no special treatment. Park et al analyzed bacterial and antibiotic resistant loads in WWTPs that processed swine effluent. They found that like those that treat hospital sewage, they were overall effective at removing the bacteria they analyzed (*E coli*), with the greatest decreases occurring during the anaerobic digestion phase. Ampicillin (AMP) resistance was the most frequently observed throughout the study, and it was proposed that this was because the farm had a history of using an antibiotic with a similar structure. The WWTPs were not capable of removing AMP resistant bacteria despite being able to remove those resistant to β -lactam antibiotics. Strains carrying multi-drug resistance and Integrons were also not affected during the treatment process, and because a large percentage of the non multidrug-resistant strains carried the *Int1* gene they have the potential for becoming an MDR strain. In order to limit these effects, tertiary treatment should be included specifically in wastewater treatment plants that process agricultural sewage with high amounts of antibiotic use (Park et al, 2018). The lack of proper treatment and the potential for runoff is further evidence that constant monitoring, in a wide area around known contamination areas, is essential for public health and preventing (as much as possible) the spread of antibiotic resistance. The findings of this study are similar to those above concerning hospital sewage, in that significant sources of similar antibiotics appear to increase the probability of finding multidrug-resistant bacteria.

Sewage Sludge

Activated sludge is used in the treatment process of many WWTPs to settle out nutrients and biological matter from sewage. The amount of bacterial loads present in the end result of sludge depends on the method used by a given treatment plant (Kim et al, 2010). Because sludge is high in nutrients it is often used as fertilizer, and therefore has the potential to be a significant source of antibiotic-resistant bacteria in the environment. The amount of antibiotics and AR (Antibiotic Resistance) in the sludge is dependent on how the sludge is treated. Sewage sludge was found to harbor and accumulate antibiotic-resistant bacteria and fecal indicator bacteria, unless treated with chemicals such as $\text{Ca}(\text{OH})_2$ (Reinthal et al, 2003).

Antibiotic-resistant concentrations in primary effluent and AS have been shown to overall have no statistical difference in antibiotic concentrations. Different stages after treatment did have an effect on this however, with the potential for AS to increase the abundance of ARG's and ARB'. AS that was treated with a secondary clarification resulted in lower concentrations downstream for bacteria resistant to specific antibiotics. UV treatment on the other hand was found to significantly increase resistance to specific antibiotics, however it was not significant on average. Additionally chlorine was shown to have no effect on the concentrations of antibiotic-resistant bacteria (Kim et al, 2010).

Despite studies showing that activated sludge can have an effect on the concentrations of antibiotic-resistant bacteria, Kim et al found no significant correlations in their study indicating that only antibiotic resistant genes are spread via HGT in activated sludge (Kim et al, 2010).

Given that activated sludge does nothing to limit antibiotic-resistant bacteria, WWTPs continue to be a significant point source contaminant regardless of this step in the treatment process. Because antibiotic-resistant concentrations downstream of AS have been shown to decline, further processing should be included after the AS process to account for its inability to remove antibiotics (Kim et al, 2010).

The Case for Tracking Antibiotics

The difficulty in predicting the bacterial loads from wastewater treatment plants relative to location, inputs, and season means that there is no simple way to predict the presence of antibiotic-resistant bacteria and genetic information from being emitted via a WWTP at any given time. Because monitoring these contaminants is vital for human health they must be monitored regularly along with other common water quality measurements. There is still limited research on the best approach to monitoring these contaminants in the environment however, and both fecal indicator bacteria and the use of Integrons have their benefits. Whatever method is used it is important that a broad range of antibiotics are assessed instead of only observing a few strains. Because class 1 Integrons have such a high potential to increase the rate of genetic transfer, it is important that they are monitored alongside fecal indicator bacteria in order to determine not only the presence of antibiotic resistance, but also the potential for HGT. Fecal indicator bacteria can effectively reveal existing antibiotic resistance, while Integrons are better at predicting the rate at which antibiotic resistance can occur.

Current Practices for Limiting ARB in WWTPS

Although there are a number of ways antibiotics can proliferate in the environment, WWTPs still represent a major point source of these contaminants. While WWTPs have been shown to effectively remove the concentrations of certain bacteria, they do not effectively remove fecal indicator bacteria and antibiotic resistance genes. As a result, antibiotic residuals (ARs) are commonly found in WWTPs and their effluents. ARs have been shown to affect the concentrations of antibiotic-resistant bacteria meaning that WWTPs are not effective in preventing the spread of antibiotic resistance (Voigt et al, 2020). The influence of WWTP can vary dramatically from hospital, agricultural, or simply urban sewage as has been shown above. Each of these has their own unique set of bacterial makeups but despite this, the bacterial concentrations seen in the treatment process and effluent remain generally the same. Resistance levels on the other hand vary depending on the type of treatment used at a given WWTP.

This study aimed to determine if fecal indicator bacteria and or class 1 Integrons were present and can be effective indicators of antibiotic resistance and associated genes in water bodies. The study looked at two creeks, the Saw Kill and Rhinebeck Kill. I hypothesized that there would be a greater quantity of antibiotic-resistant bacteria, FIBs, and Integrons at the Rhinebeck treatment plant.

Materials and Methods a

The Rhinebeck Kill and Saw Kill Creeks

The Rhinebeck Kill (Creek) is monitored by the Rhinebeck Conservation Advisory Council and has been given class HE designation by the Department of Environmental Conservation (DEC). This means that while the water is not safe to swim in or drink it must be high enough quality to ensure that fish are capable of surviving. The town has asked the DEC to improve the water quality in the Rhinebeck Kill and the surrounding tributaries. The Rhinebeck Kill is also located in a swampy hardwood area that is prone to flooding and as a result the potential for the land surrounding it to be developed is limited. Further downstream from the sampling location the Rhinebeck Kill joins with the Landsman Kill and proceeds to dump into the Hudson River via the Vanderburgh Cove.

The Saw Kill Creek and its tributaries are monitored by The Saw Kill Watershed Community who collect samples of overall water quality including FIBs monthly. At the moment the Saw Kill only receives effluent from the Bard WWTP and also provides water for Bard College. There are plans to introduce a treatment upstream in Red Hook in the future. The Saw Kill drains into South Tivoli Bays as can be seen in figure 3 which eventually leads out into the Hudson.

Samples were collected near the effluent pipes of two local WWTPs located on the Rhinebeck Kill and Saw Kill creeks. The area surrounding the Saw Kill and Rhinebeck Kill as well as their tributaries are both rural, with farmland livestock and apple production as the major sources of industry. Much of the infrastructure that was in the area is old and dilapidated at this

point and there are a number of dams dating back to the late 18th century and early 19th century, many of which are in need of repair. As a result it is likely that the major pollutants in these water bodies are related to fertilizers and antibiotics associated with agriculture, as opposed to heavy metals or plastics associated with industry.

The Bard and Rhinebeck WWTP's

Both creeks receive effluent from only one wastewater treatment facility. The Rhinebeck WWTP treats water from the village of Rhinebeck (population of 7,800) as well as from Northern Dutchess Hospital. The treatment process listed on the (SPDES) permit (NY0110281) consists of first an initial VFR screening before going through an influent pump station into a splitter box. After this point either in an aerator or oxidation ditch is used before it is sent into secondary clarification, at which point the sludge is recirculated before going into a sludge holding tank. The wastewater continues on to a dual media filtration tank, UV disinfection, and finally cascade step aeration, before releasing into the Rhinebeck Kill. The sludge is pressed using a sludge filter for commercial disposal.

The Bard Water Treatment Plant, on the other hand, only processes water from Bard's campus (population of 2,500) and has a rated design flow of 1 million gallons a day. As listed on the SPDES permit (NY0031925) It consists of three preliminary treatment processes which include bar screening, a flow equalization basin, and a primary treatment. The secondary treatment is conducted using rotating biological containers. Bard's WWTPS also has tertiary treatment which consists of nitrification as well as a chlorination and a dechlorination stage. Solids generated at this facility undergo aerobic digestion, and are shipped off for processing.

Sample Collection and Pretreatment

Three samples were taken at each location in order to get a full range of data (upstream, effluent, and downstream) and YSI measurements were taken (making sure to keep the sensor fully submerged) in order to get an overall sense of the quality of the water (dissolved oxygen, conductivity and temperature). The samples were collected by using a dipper to pour water into a sterilized 2 liter bottle (both rinsed with sample water three times before use). Once each sample was collected, it was placed into an insulated backpack with ice before being transported back for processing. General site observations were recorded for future reference.

Once back at the lab, the samples were placed into a conventional refrigerator before being used for assays and run through sterivex filters. IDEXX Enterolert and Colilert tests were used to assess the total colorforms of each sample location. For the Enterolert, this was conducted by first inverting the sample three times before pipetting 10 mL of the sample into a 125 mL vessel after which 90 mL of autoclaved deionized water was added to create a 1 in 10 dilution. Next a Entero Coliert alert media packet was emptied into each vessel and mixed until it dissolved. The solution was then added to Quanti Trays which were sealed and incubated at 35° Celsius for 24 hours. After the incubation period, the number of large and small cells that were more yellow than the comparator were recorded along with the number of cells that fluoresced. Finally the IDEXX Quanti Tray/2000 MPN Table was used to determine the total number of coliforms per sample.

The IDEXX Enterolert protocol was used to assess the amount of enterococcus per sample. Samples were again inverted three times before pipetting 100mL of the sample into each

125mL vessel. Enterolert media packets were then emptied into the vessels and stirred until they dissolved. The solution was then poured into Quanti Trays which were sealed and incubated at 41° C for 24 hours. After the incubation period the trays were placed under a UV light and the number of fluorescent cells was recorded.

Turbidity was assessed by first inverting the sample bottles three times before filling a clean sample cell (rinsed with tap water three times) to the 15mL line. After wiping the cell down with a microfiber cloth and the provided oil solution it was placed into, the turbidimeter and the NTUs were recorded. In order to collect the DNA for PCR and QPCR processing sample water was first run through Sterivex filters. This was done using a peristaltic pump which ran a minimum of 100mL through each filter before they were stored in a -80 degrees Celsius freezer for DNA extraction.

Results and Discussion

The variables assessed in order to determine the overall quality of water were temperature conductivity and turbidity (Table 1). FIBs were also assessed in the lab. After running a three way Anova, there were no significant correlations between any of the variables and there was also no significant difference between sample location and date. The lack of significance was expected as both creeks are in relatively close proximity to one another, and therefore are affected by the same weather events and have similar pollution inputs (with the exception of Northern Dutchess Hospital). Although the presence of class 1 Integrons was not assessed in this study, it is likely that the concentrations would be similar between plants given that they have been found to have a high presence in other WWTPs. As can be seen in table 1,

there was also no significant difference between the dissolved oxygen in sample sites above the effluent compared to those below, despite previous studies finding that wastewater treatment plants lower the concentration of dissolved oxygen. This could be due to the implementation of the stepping system that both WWTPs use as a final step in the treatment process. The lack of difference in fecal indicator bacteria between the sites in both creeks Fig1 and 2 was not expected, as it was hypothesised that the effluents would have an impact on total coliforms. Further analysis should be conducted on the presence of these environmental factors in a multi-seasonal study, given that previous papers have shown that there can be a connection between the time of year and the concentration of fecal indicator bacteria. The turbidity remained fairly low throughout the sampling, with the average NTU being 5 (Table 1). Given that FIBs were correlated with turbidity it makes sense that FIB concentrations were low.

Next Steps

Because this study was cut short, further testing should be conducted to determine whether or not class 1 Integrons or fecal indicator bacteria are more successful in determining concentrations of antibiotic-resistant bacteria, or if they should be used in combination. The data that was collected in this study showed that the effluent of both plants had little to no impact on environmental variables and FIB concentrations downstream. This data should be expanded on both by the collection of more data and further analysis. A metagenomic analysis should be run to see if there is a significant difference in the biome between treatment plants. Particularly because the wastewater of hospitals has been shown to result in the clustering of specific families of resistant bacteria. qPCR should also be performed to detect the presence of Integrons,

antibiotic resistant bacteria, and antibiotic resistant genes to look for correlations. This information is particularly important given that both creeks run into the Hudson River which serves as drinking water for many communities.

Conclusion

This study aimed to assess the extent to which fecal indicator bacteria and Integrons were present in the Saw Kill and Rhinebeck Kill creeks, and serve as effective indicators of antibiotic resistance. It was found that there were very few differences in environmental variables and FIB concentrations between sites. Because this study was cut short, no DNA was able to be analyzed. This information could help to provide better insight into how to properly monitor antibiotic resistance, and provide more accurate safety standards, particularly for people who are immunocompromised.

Figures and Tables

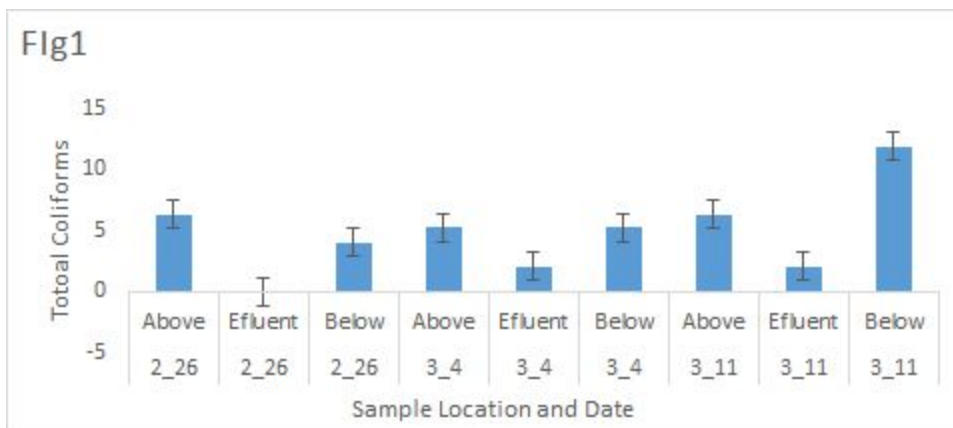


Figure 1. Total coliforms by site (Above, effluent and below) for the Rhinebeck Kill creek. Samples are organized by date (oldest to most recent).

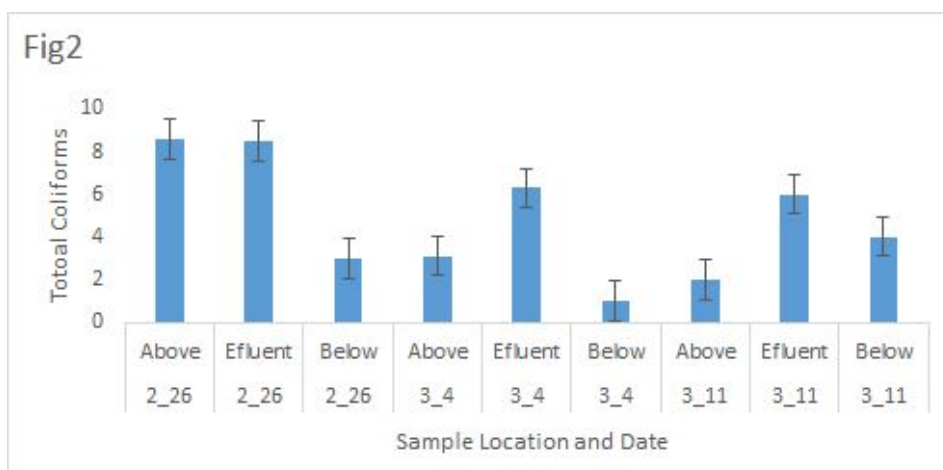


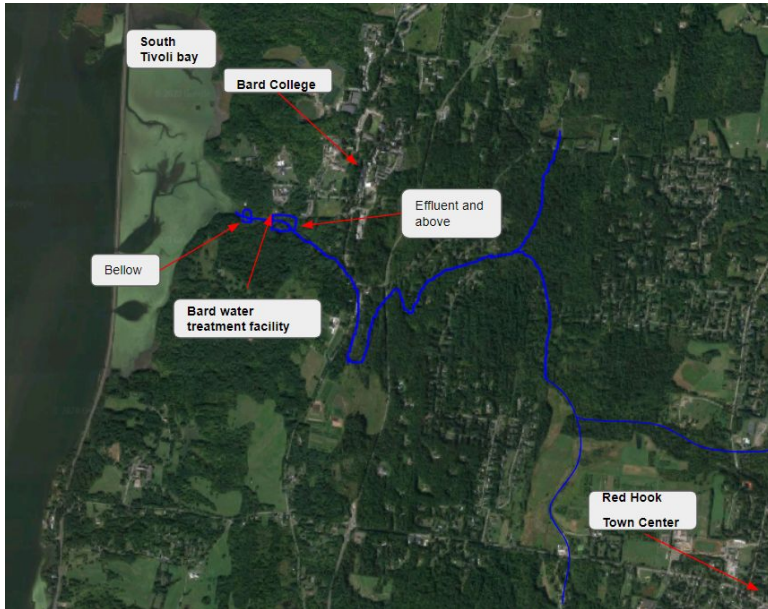
Figure 2. Total coliforms by site (Above, effluent and below) for the Sawkill Kill creek. Samples are organized by date (oldest to most recent).

Table 1

	Mean all	Stdev all	MeanRBK	MeanSK	StdevRBK	StdevSK
Temp	8.04	183.48	8.43	7.66	2.28	1.4
Conductivity	369.79	113.96	401.28	338.3	140.45	75.09
Turbidity	5.03	3.6	6.43	3.64	3.49	3.31

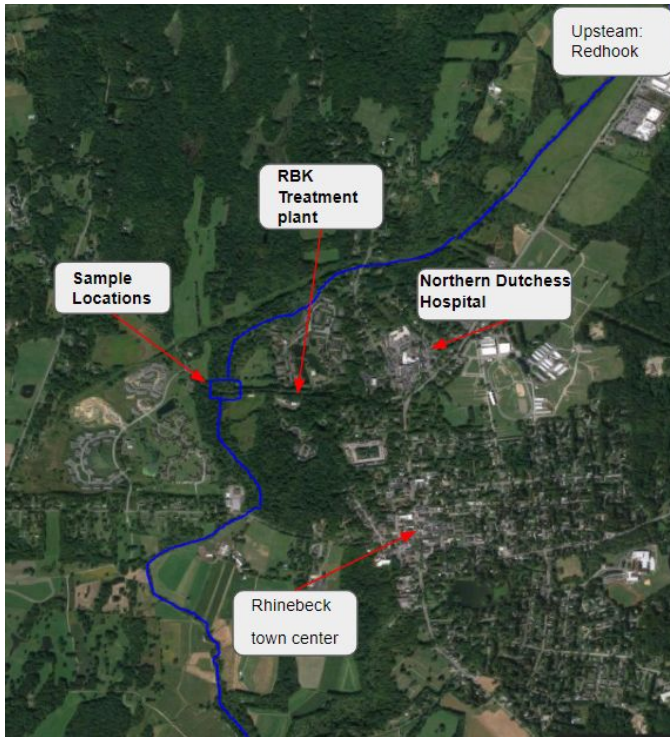
Table 1. The mean standard and deviation for temperature, conductivity and turbidity for both creeks and between creeks.

Fig 3



Map of the lower Saw Kill and local feachers of interest.

Fig 4



Map of the Rhinebeck kill around the sample site and local feachers.

Bibliography

- Voigt, A. M., Zacharias, N., Timm, C., Wasser, F., Sib, E., Skutlarek, D., ... Schreiber, C. (2020). Association between antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes in anthropogenic wastewater – An evaluation of clinical influences. *Chemosphere*, 241, 125032. <https://doi.org/10.1016/j.chemosphere.2019.125032>
- Müller, H., Sib, E., Gajdiss, M., Klanke, U., Lenz-Plet, F., Barabasch, V., ... Bierbaum, G. (2018). Dissemination of multi-resistant Gram-negative bacteria into German wastewater and surface waters. *FEMS Microbiology Ecology*, 94(5), 1–11. <https://doi.org/10.1093/femsec/fiy057>
- Buelow, E., Bayjanov, J. R., Majoor, E., Willems, R. J. L., Bonten, M. J. M., Schmitt, H., & van Schaik, W. (2018). Limited influence of hospital wastewater on the microbiome and resistome of wastewater in a community sewerage system. *FEMS Microbiology Ecology*, 94(7), 1–9. <https://doi.org/10.1093/femsec/fiy087>
- Deng, Y., Bao, X., Ji, L., Chen, L., Liu, J., Miao, J., ... Yu, G. (2015). Resistance integrons: Class 1, 2 and 3 integrons. *Annals of Clinical Microbiology and Antimicrobials*, 14(1), 1–11. <https://doi.org/10.1186/s12941-015-0100-6>
- Zheng, W., Huyan, J., Tian, Z., Zhang, Y., & Wen, X. (2020). Clinical class 1 integron-integrase gene – A promising indicator to monitor the abundance and elimination of antibiotic resistance genes in an urban wastewater treatment plant. *Environment International*, 135(August 2019), 105372. <https://doi.org/10.1016/j.envint.2019.105372>
- Park, J. H., Kim, Y. J., Binn-Kim, & Seo, K. H. (2018). Spread of multidrug-resistant *Escherichia coli* harboring integron via swine farm waste water treatment plant. *Ecotoxicology and Environmental Safety*, 149(October 2017), 36–42. <https://doi.org/10.1016/j.ecoenv.2017.10.071>
- Young, S., Juhl, A., & O'Mullan, G. D. (2013). Antibiotic-resistant bacteria in the Hudson River Estuary linked to wet weather sewage contamination. *Journal of Water and Health*, 11(2), 297–310. <https://doi.org/10.2166/wh.2013.131>

- Kim, S., Park, H., & Chandran, K. (2010). Propensity of activated sludge to amplify or attenuate tetracycline resistance genes and tetracycline resistant bacteria: A mathematical modeling approach. *Chemosphere*, 78(9), 1071–1077. <https://doi.org/10.1016/j.chemosphere.2009.12.068>
- Koivunen, J., Siitonen, A., & Heinonen-tanski, H. (2003). Elimination of enteric bacteria in biological–chemical wastewater treatment and tertiary filtration units, 37, 690–698.
- Reinthaler, F. F., Posch, J., Feierl, G., Wüst, G., Haas, D., Ruckebauer, G., ... Marth, E. (2003). Antibiotic resistance of *E. Coli* in sewage and sludge. *Water Research*, 37(8), 1685–1690. [https://doi.org/10.1016/S0043-1354\(02\)00569-9](https://doi.org/10.1016/S0043-1354(02)00569-9)
- Phillips, P. J., Chalmers, A. T., Gray, J. L., Kolpin, D. W., Foreman, W. T., & Wall, G. R. (2012). Combined sewer overflows: An environmental source of hormones and wastewater micropollutants. *Environmental Science and Technology*, 46(10), 5336–5343. <https://doi.org/10.1021/es3001294>
- Goñi-Urriza, M., Capdepuy, M., Arpin, C., Raymond, N., & Pierre Caumette, C. Q. (2000). Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and *Aeromonas* spp. *Applied and Environmental Microbiology*, 66(1), 125–132. <https://doi.org/10.1128/AEM.66.1.125-132.2000>
- Passerat, J., Ouattara, N. K., Mouchel, J. M., Vincent Rocher, & Servais, P. (2011). Impact of an intense combined sewer overflow event on the microbiological water quality of the Seine River. *Water Research*, 45(2), 893–903. <https://doi.org/10.1016/j.watres.2010.09.024>
- Koivunen, J., Siitonen, A., & Heinonen-tanski, H. (2003). Elimination of enteric bacteria in biological–chemical wastewater treatment and tertiary filtration units, 37, 690–698.
- Antunes, S., Dionísio, L., Silva, M. C., Valente, M. S., & Borrego, J. J. (2007). Coliforms as indicators of efficiency of wastewater treatment plants, (1), 26–29.
- Yang, Y., Li, B., Zou, S., Fang, H. H. P., & Zhang, T. (2014). Fate of antibiotic resistance genes in sewage treatment plant revealed by metagenomic approach. *Water Research*, 62, 97–106. <https://doi.org/10.1016/j.watres.2014.05.019>
- Stange, C., Sidhu, J. P. S., Tiehm, A., & Toze, S. (2016). Antibiotic resistance and virulence genes in coliform water isolates. *International Journal of Hygiene and Environmental Health*, 219(8), 823–831. <https://doi.org/10.1016/j.ijheh.2016.07.015>

Gillings, M. R. (2017). Class 1 integrons as invasive species. *Current Opinion in Microbiology*, 38, 10–15. <https://doi.org/10.1016/j.mib.2017.03.002>

Di Cesare, A., Eckert, E. M., D'Urso, S., Bertoni, R., Gillan, D. C., Wattiez, R., & Corno, G. (2016). Co-occurrence of integrase 1, antibiotic and heavy metal resistance genes in municipal wastewater treatment plants. *Water Research*, 94, 208–214. <https://doi.org/10.1016/j.watres.2016.02.049>

Sanderson, H., Fricker, C., Brown, R. S., Majury, A., & Liss, S. N. (2016). Antibiotic resistance genes as an emerging environmental contaminant. *Environmental Reviews*, 24(2), 205–218. <https://doi.org/10.1139/er-2015-0069>

Gillings, M. R., Gaze, W. H., Pruden, A., Smalla, K., Tiedje, J. M., & Zhu, Y. G. (2015). Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *ISME Journal*, 9(6), 1269–1279. <https://doi.org/10.1038/ismej.2014.226>

Adelowo, O. O., Helbig, T., Knecht, C., Reincke, F., Mäusezahl, I., & Müller, J. A. (2018). High abundances of class 1 integrase and sulfonamide resistance genes, and characterisation of class 1 integron gene cassettes in four urban wetlands in Nigeria. *PLoS ONE*, 13(11), 1–15. <https://doi.org/10.1371/journal.pone.0208269>

Sánchez-Avila, J., Tauler, R., & Lacorte, S. (2012). Organic micropollutants in coastal waters from NW Mediterranean Sea: Sources distribution and potential risk. *Environment International*, 46, 50–62. <https://doi.org/10.1016/j.envint.2012.04.013>

Yang, D., Wang, J., Qiu, Z., Jin, M., Shen, Z., Chen, Z., ... Li, J. W. (2013). Horizontal transfer of antibiotic resistance genes in a membrane bioreactor. *Journal of Biotechnology*, 167(4), 441–447. <https://doi.org/10.1016/j.jbiotec.2013.08.004>