CHARACTERIZING THE PREVALENCE AND MITIGATING POSSIBLE RISKS OF WASTEWATER BORNE ANTIBIOTIC RESISTANCE

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Introduction

Antibiotic resistant bacteria (ARB) are a growing public health concern (CDC, 2019a). In the U.S. alone, 2 million people contract antibiotic resistant infections annually with approximately 23,000 fatal infections (CDC, 2019a). According to Ventola (2015), a medical researcher, antibiotic resistance is often “attributed to the overuse and misuse of antibiotics" (p. 1) and can be found in several bacteria, such as Carbapenem-Resistant *Enterobacteriaceae* (CRE), which will be used as a model organism in this study to investigate the general movement of all ARBs. These bacteria are resistant to almost all antibiotics, including Carbapenem, listed by the CDC as “antibiotic of last resort” (CDC, 2019b, para. 5). Antibiotic resistant bacteria have been found by Mathers et al. (2011) in the University of Virginia (UVA) hospital system. ARB from infected patients in the UVA hospital have been shown to colonize hospital sinks and toilets leading to an increased risk of hospital-borne infections (Mathers et al. 2019). The wet and humid environment of the sink plumbing promotes microbial growth. The P trap of the sink provides a water barrier to prevent sewer smell from reaching the sink user which inadvertently creates a favorable environment for microbial and biofilm growth (Kotay et. al. 2017). Microbial communities are exposed to any materials going down the drain such as antimicrobial soap, discarded beverages, cleaning products, and bacteria from users’ hands (Kotay et al., 2017). Figure 1 outlines the parts of a typical hospital sink.

The presence of ARB in the plumbing of the hospital also raises concerns about the transfer of ARB to the local wastewater treatment plant (WWTP) and eventually into the environment. The Moores Creek Wastewater Treatment Plant in Charlottesville, VA accepts and treats the UVA hospital’s wastewater. It is unknown to what extent ARBs are transmitted to the WWTP via hospital sewage and, from there, into the environment. Depending on their persistence in the environment, ARB may contaminate water downstream leading to ill-defined public health effects through contaminated drinking water or recreational contact. More research is also needed to understand the potential for vertical and horizontal gene transfer in the WWTP setting (Rizzo et. al, 2013). ARBs in the environment pose a potential risk to public health, however, their transport has not been fully investigated.

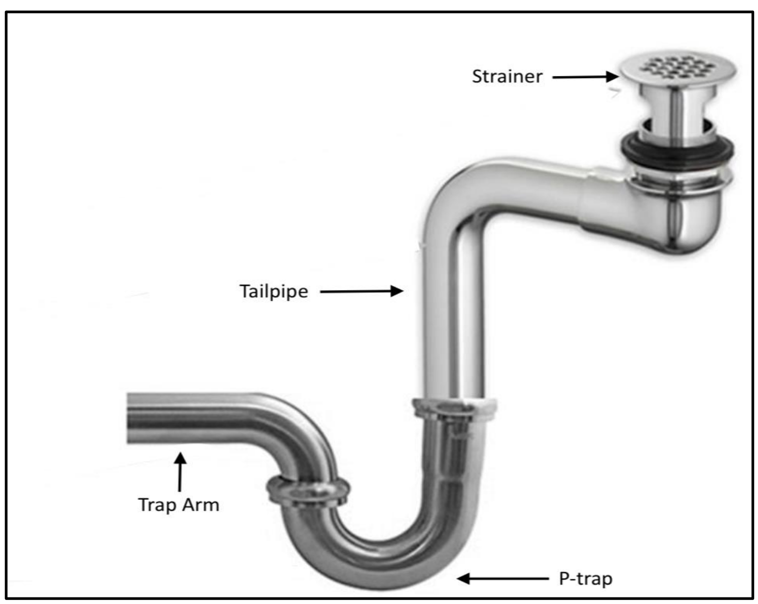

Figure 1: Diagram of a typical hospital sink (Kotay et al., 2017)

In order to develop a comprehensive strategy for mitigating the possible public health risk posed by hospital-borne antibiotic resistance, both within the hospital and at downstream locations including the municipal wastewater treatment facility and its receiving water, for hospital-borne ARB, it is necessary to first characterize possible transport through the system of interest. Once the prevalence of ARB and associated wastewater constituents has been fully characterized, it will then be possible to evaluate and design possible interventions.

With an ultimate goal of protecting human health at both a hospital level and at a community-level, this work has two specific objectives: 1) characterize the movement of antibiotic resistant bacteria within the UVA Hospital wastewater system and various downstream compartments, including municipal wastewater treatment, and 2) evaluate the possible usefulness of conventional water/wastewater treatments in mitigating the spread of hospital wastewater-borne antibiotic resistance, both within the hospital and at utility scale.

PART I.
Characterizing Antibiotic Resistant Bacteria in Wastewater Treatment System

1.0 Overview

In order to meet the first objective, characterizing the movement of hospital-derived antibiotic resistance flowing into and out of a municipal WWTP, the movement of a model ARB in the UVA and Charlottesville wastewater system was investigated through measurement of *Klebsiella pneumoniae* cabapenmase (KPC) positive bacteria at several key locations along this system. This CRE was chosen as a model group for ARB's and KPC as a model ARG for several reasons. First, as a drug of "last resort", Carbapenems are important tools against complicated bacterial infections, especially those presenting a broad spectrum of antibacterial activity (Meletis, 2016). Tracking resistance to a specific antibiotic drug of very high clinical relevance is important because the rapid spread of resistance through local and global health care institutions pose a massive healthcare risk. Second, the emergence of carbapenem resistance has occurred relatively recently, and it is currently thought that all carbapenem resistance conferred by the KPC plasmid can be traced to a single point of origin in the southeastern US; as opposed to multiple organisms becoming individually resistant (Mathers et al., 2011). This combination of features makes KPC-conferred carbapenem resistance uniquely easy to track within environmental systems, because it is fairly new and relatively centralized with respect to spatial distribution. Once the transport processes for ARB and ARG are better understood, traditional water/wastewater treatment processes will be investigated for which might be most appropriate for mitigating the spread of ARB and ARG originating in a hospital setting.

1.A Methods for Characterizing the Movement of ARB and ARG

Sampling

The UVA Hospital system was previously sampled by Mathers et al. (2011), in order to understand the source of an outbreak of CRE in the hospital which was linked to the KPC plasmid. This study was furthered by Mathers et al. (2018), in which investigators evaluated the possible role of hospital wastewater plumbing in the spread of KPC amongst patients. The current study expands on the previously performed sampling, adding environmentally relevant sample locations. Samples of wastewater, sludge, sediments, and water from upstream and downstream of the discharge point were collected on September 10, 2019 from the WWTP and Moores Creek. There was no rain in the preceding week. Sampling locations are listed in Table 1 and illustrated in Figure 2. Employees of the Rivanna Water and Sewer Authority collected 1-L samples of water and sludge from within the Moores Creek Wastewater Treatment Plant (sites a-d). 250 mL samples from Moores Creek were collected by student researchers (sites e-f). Bottles used for collection were previously sanitized using an autoclave. The total suspended solids (TSS) and volatile suspended solids (VSS) of each of the samples was measured and recorded. Based on the levels of TSS and VSS in Moores Creek Wastewater Treatment Plant and Moores Creek conditions would be favorable for bacterial growth. Complete results of the TSS and VSS tests are included in Appendix A. Samples were collected again from the Moores Creek WWTP in December but not from Moores Creek.

Table 1: Locations of sampling corresponding with the locations denoted on Figure 2.

Location	Sample(s)
A	Secondary Sludge
B	Post-digester
C	Influent
D	Final Effluent
E	Upstream Samples
F	Downstream Samples

Figure 2: Map of Sampling Locations at the Moores Creek Wastewater Treatment Plant: Possible sampling locations include multiple areas within the wastewater treatment process (a-d) as well as along Moores Creek on both sides of the final effluent outflow (e-f) (Google map adapted by Sutton, 2019).

Culture Analysis

Quantitative and enrichment analyses were performed for each sampling point. Samples were plated on to ChromAgar plates and incubated for 24 hours at 34°C. The mean number of colony forming units (CFUs) per mL was recorded for each plate and then pigmented colonies were subcultured for species identification and PCR screening. For enrichment analysis, sample swabs were vacuum-filtered (0.22 µm filters), and the filters were placed extraction tubes with 4.5 mL of tryptic soy broth (TSB) along with a 10-µg ertapenem disk. After incubating for 24 hours at 34°C, 10 µL was swabbed onto ChromAgar plate and incubated for another 24 hours at 34°C. Individual colonies were subcultured for species identification and PCR screening.

PCR screening was completed using Hot Start Taq and primers and analyzed using a Thermal Cycler (Instrument info here) added to a boil prep of the isolated colonies at cycling parameters of 94°C for 10 min, then 35 cycles of 94°C for 40 sec, 55°C for 40 sec, 72°C for 1 min, and then a final temperature of 72°C for 7 min. [EL1] Samples were injected into wells in a 2% agarose gel and run for 30 minutes at 100V and viewed under ultraviolet light and confirmed using a KPC-positive control and negative control.

1.B Results and Discussion on ARB Movement in the Wastewater System

Culture Analysis

In this study, selective culture methods were used to analyze whether carbapenam resistant bacteria were present in the samples collected from the UVA hospital, Moores Creek WWTP, and the receiving water at locations upstream and downstream from the effluent discharge. Culture methods refer to experimental protocols wherein samples are plated on selective augers in order to determine the concentration of bacteria with a specific characteristic. Organisms that were able to grow in the presence of the carbapenam selective pressure were then further analyzed using polymerase chain reaction (PCR) techniques, to determine their species and assess whether they were positive or negative for the KPC plasmid. Results from the culture methods and subsequent PCR analyses are summarized in Figure 3 and Figure 4.

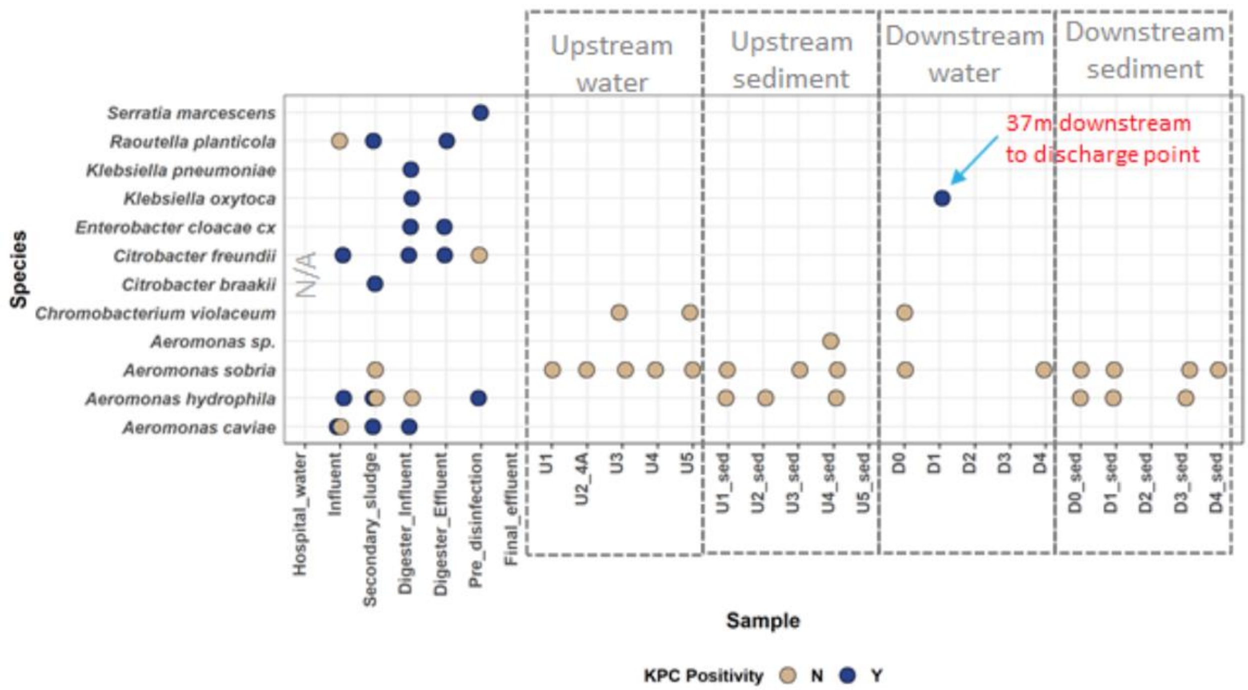

Figure 3: Diversity of Carbapenem Resistant *Enterobacteriaceae* (CRE) with and without KPC plasmid from water and sediment samples collected upstream, downstream, and within the Moores Creek WWTP in different species at sampling locations (Kotay, 2019)

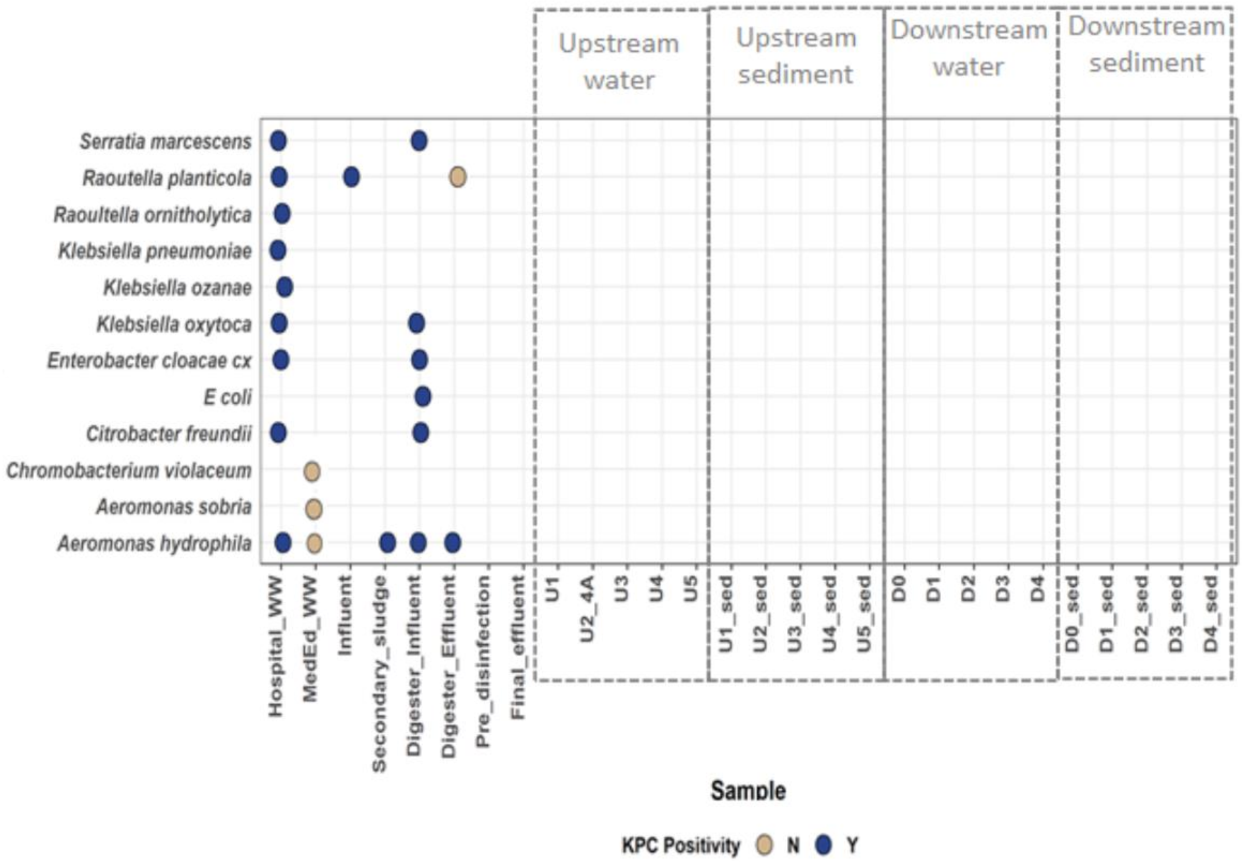

Figure 4: Diversity of Carbapenem Resistant *Enterobacteriaceae* (CRE) with and without KPC plasmid from water samples collected within the Moores Creek WWTP in different species at sampling locations (Kotay, 2019)

We observed interesting diversity and spatial distribution of carbapenam resistant organisms with and without the KPC plasmid in various water and sediment samples collected upstream, downstream, and within the Moores Creek facility. In the stream samples, two instances of KPC were detected: one in the upstream and one in the downstream. The first in upstream water in the species *Chromobacterium violaceum* and the second in downstream water in the species *Klebsiella oxytoca*. Neither the upstream or downstream sediment samples showed instances of KPC. In relation to samples collected from stages in the wastewater process, KPC positive *K. oxytoca* was detected in the digester influent but *C. violaceum* was not detected in any of the wastewater samples. Out of all of the wastewater samples, the widest range of species that tested KPC positive were found in the digester influent, followed by the secondary sludge. No instances of KPC positive bacteria were found in the final effluent.

Environmental Flow

KPC positive bacteria were found in seven of the eleven areas sampled as can be seen below in Figure 5. Hospital Plumbing at the University of Virginia Hospital has shown instances of bacteria containing KPC since 2011 when it was recorded by Mathers et al. The wastewater was then sampled at several intervals in the wastewater treatment process. Despite being largely diluted after leaving the hospital wastewater system and entering the municipal system, wastewater influent at the treatment plant still tested positive for KPC bacteria. As the treatment process progressed KPC positive bacteria were found at all stages, as was expected due to the optimal microbial growth conditions that are facilitated to promote microbes used in the wastewater treatment process. After the disinfection process there are no KPC positive samples, however some are found in the downstream water samples, which proposes questions about their source in the surrounding environment. As KPC positive bacteria are being used as an indicator for other ARB, we would expect that other ARB would also be found in these same locations and would move in a similar manner.

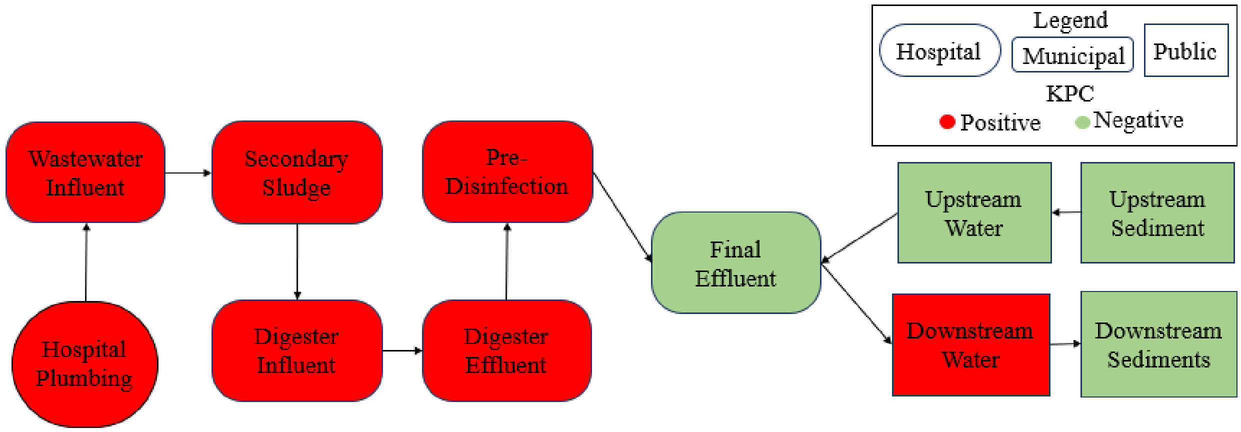

Figure 5: Flow of Wastewater and Instance of KPC positive Bacteria.

Implications

Despite a lack of KPC detected in the final WWTP effluent, KPC was still present in downstream waters in one of the same species found in the digester influent. This raises questions as to how this antibiotic resistant gene (ARG) was spread outside of the wastewater treatment plant. The complete elimination of KPC positive bacteria in the final effluent indicates that a treatment including UV radiation, the method of final disinfection at the Moores Creek plant, may be a viable method of treatment for ARB. This is consistent with emerging literature. For example, Zhang et al. (2019) observed a decrease in bacteria resistant to certain antibiotics after UV treatment and Silva et al. (2018) noted that the use of UV disinfection decreased the occurrence of Enterobacteriaceae that were resistant to cefotaxime (CTF) antibiotics. More importantly, because the results of this study suggest that UVA Hospital is indeed discharging KPC-positive CRE, it is of interest to investigate what kinds of interventions may be appropriate for mitigating health risk associated with CRE release into the natural environment.

PART II.
Design of Possible Interventions to Reduce Public Health Risks Associated with Hospital-Borne Antibiotic Resistance

KPC was found at points in the hospital pipes, wastewater treatment system, and the environment. Therefore, it is of interest to evaluate possible interventions that could be used to protect human and ecosystem health from possible health risks associated with exposure to KPC-positive CRE. These interventions could be deployed at different points in the system including at the point source, on-site, and municipal level. Criteria were developed to evaluate and compare the efficacy of different interventions; however, KPC is a relatively new biological pollutant, so more stakeholder input may be needed for more holistic decisions.

2. A Literature review methodology

To understand the state of practice of ARB treatment and removal a literature review was conducted. Relevant papers and information came from government agencies, such as the Environmental Protection Agency (EPA) as well as peer-reviewed journals. Although the literature review included scientific papers outside the United States, the search was limited to countries with similar wastewater treatment infrastructure. The literature review provided the necessary background to design the criteria and scoring for interventions.

2.B Methods of Scoring Interventions

Intervention Criteria Scoring Guideline

Based on findings from the literature review, criteria were formed to evaluate possible interventions at the point source, on-site, and municipal levels. Criteria were selected with the knowledge that a single point of intervention may not be sufficient to eliminate ARBs in the wastewater system and protect patient health, therefore the criteria are applicable to interventions at all three of these scales. Ratings of (+), 0, and (-) will be assigned for each criteria and will be explained in each section. An intervention with more (+)'s will be considered as a more favorable option for implementation. The rating system, however, should not be used as an absolute answer but instead as a way of narrowing down solutions. Although this project focuses specifically on the UVA hospital and Moores Creek wastewater treatment plant, the assessment is based on wastewater treatment plants and hospitals in general.

Table 2: Intervention Criteria: A description of criteria used to evaluate treatment interventions

Criteria	**Description**
Cost to achieve acceptable ARB removal	Cost of technology that meets current regulations in the Clean Water Drinking Act National Discharge Elimination System (NPDES) Compliance Monitoring
User Safety	Use of hazardous materials or mechanisms.
Ease of Implementation	Potential for application to existing infrastructure. Ease of installation in the current wastewater treatment procedure.
Sustainability	Energy efficiency.
Adaptability	Efficacy in treating different levels of contamination in the wastewater.
Maintenance	Active vs. Passive systems: how often does it need to be monitored and managed
General Removal Efficiency	Removal of other contaminants of concern such as suspended solids, other pathogens, etc.
Residuals & Byproducts	Ex: chloramines from chlorination.
Potential to Prevent Reactivation	Potential to prevent reactivation of ARB after disinfection.

Primary Concerns

Cost to Achieve Acceptable ARB removal

To date, regulatory agencies in the US and elsewhere have not provided definitive guidance or requirements for ARB management or removal. Until such time that they may decide to do so, WWTP operators will likely not be able to justify expensive treatments to remove unregulated constituents that may pose as yet undocumented health risk. Accordingly, the intervention must be as inexpensive as possible, ideally leveraging a treatment that would have been already applied for some other reason (e.g., to achieve some other current regulatory requirement). This would make the marginal cost for removal or deactivation of ARB and ARG essentially zero ("free").

User Safety

This category was put in place to examine the possibility of exposure to dangerous processes or materials. The higher the level of safety, the more points an intervention is awarded.

Additionally, due to added risk, access to the intervention by untrained individuals is treated as a higher safety risk. A (-) is awarded to interventions that have the potential for individuals without training to come into contact or be harmed by the intervention. A zero will be awarded to interventions that have the potential for trained professionals to be harmed and a (+) for interventions that pose little to no potential harm to trained or untrained individuals.

Ease of Implementation

This criteria discusses the ease at which the intervention can be integrated into existing infrastructure and includes both cost and technical feasibility. Additionally, whether the treatment procedure can be easily integrated into the existing water and wastewater regulatory framework will also be considered. For this criteria, a (-) will be awarded if there are significant barriers to implementation, such as high upfront and maintenance costs or the technology requires significant changes to pre-existing water infrastructure. A 0 will be awarded if there are moderate barriers to diffusion or if the intervention can be easily implemented in certain situations but not others. For example, copper piping may be easy to implement when building a hospital but difficult to install in retrofit. Finally, a (+) will be awarded if there are few or no barriers to implementation. This could include disinfection methods that are already implemented or would take few inexpensive changes within the system to additionally disinfect for ARBs.

Secondary Concerns

Adaptability

Adaptability is the ability for the intervention method to be installed without extra infrastructure, such a pretreatment or residual removal downstream. A (-) will be awarded if additional infrastructure is needed, a (+) will be awarded if the intervention method does not require additional infrastructure.

Maintenance

This criteria looks at the technological sophistication required to operate and maintain the potential intervention method. Operations and maintenance take up a large portion of long-term costs for systems and are highly considered. Sophisticated and complex systems that require specialized workers to operate have a higher cost than simple solutions that may not even require workers to operate. Having simpler solutions is ideal because repairs and operation can be done by a wider range of workers. If the potential solution is complex with constant maintenance and/or requires multiple operators, a (-) will be awarded. A 0 will be awarded if there is a need for occasional maintenance and one operator, and a (+) if the solution requires minimal maintenance and no operators.

Sustainability

The energy consumption, and corresponding greenhouse gas emissions per volume of treated water is important to contextualize as it allows for general assessments of efficiency and costs. Water treatment consumes a lot of material that are needed in society, however, it is desired to minimize consumption and greenhouse gas emissions. We understand that tertiary treatment consumes a lot of matter and electricity to run because of the large volume of relatively dilute wastewater (relative to ARB and ARG). On site and point source treatments that specifically aim to eliminate certain unique pollutants tend to be more efficient due to the lower volume needed to treat. We will be prioritizing cheaper and more sustainable options given that effectiveness is similar or the same. A (+) will be given to treatments that are highly efficient in its energy use, a 0 will be given for moderate efficiency, and a (-) will be given for low efficiency.

Tertiary Concerns

General Removal Efficiency

This criteria examines the removal of other contaminants of concern such as suspended solids and other pathogens. Infrastructure is costly, and devoting resources to address a relatively minor issue is not feasible. Good intervention methods offer secondary benefits or can remove other contaminants as well. A (+) will be given if it removes other contaminants, a score of (-) will be given to those who cannot.

Reactivation Potential

After treatment, some organisms may have the potential to repair their DNA and continue to spread. Many treatments may denature and unravel proteins to deactivate them; however, bacteria can repair certain damage and reactivate certain genes. A (-) will be awarded if the possibility is there, and a (+) will be awarded if the possibility is not.

Residuals and Byproducts

This criteria examines if the specific intervention method produces any harmful byproducts that need to be removed downstream. The infrastructure needed increases costs and risks needs to be addressed. A (-) will be awarded if residuals are produced; a (+) will be awarded if they are not.

2.C Candidates for Intervention

The following paragraphs provide relevant technical and contextual information about candidate treatments that appear to potentially useful for reducing the public health risk associated with hospital-derived antibiotic resistance. The candidate interventions are divided into three broad categories: point source (Section 2.C.i), on site (Section 2.C.ii), and municipal utility (Section 2.C.iii). All candidate interventions are evaluated with respect to the criteria laid out in Table 2. The final portion of this document (Sections 2.D and 2.E) will present a comparison of scores for all evaluated interventions and provide some design recommendations based on these outcomes.

2.C.i Point Source

Point source disinfection methods commonly treat water from single faucets, drains, and other outlets. Wet areas, such as sinks and toilets, and commonly touched surfaces, such as door handles and counters, are common breeding grounds and vectors for microbes and pathogens (WHO, n.d.). In hospitals, there is an increased proportion of immunocompromised and at-risk individuals who contract more severe infections at higher rates than the average person. Specifically minimizing the spread of hospital acquired infections (HAI) is a major design focus for point source intervention. Containment and deterrents are more cost effective and practical than complete removal at the point source due to safety and cost concerns. For the purposes of this system design, we envision that it might be appropriate to use point source treatments underneath sinks and other drains in hospital rooms because of the high risk of HAI they pose to patients.

Thermal Disinfection

With thermal disinfection, heat is applied to denature and kill bacteria and other pathogens. Using an autoclave, boiling water, and cooking food are examples of thermal disinfection. Most harmful bacteria, such as *E. coli* are mesophilic: they thrive in temperatures between 20C-45 ℃ (Robinson, 2000). Outside of the ranges, bacteria either grow slowly or not at all (at lower temperatures) or start to denature and die (at higher temperatures). Disinfection through the application of thermal energy is not applicable due to high energy costs; however, using heat as a deterrent was studied by a previous UVA Biomedical Engineering capstone team as a point source intervention. A prototype tailpiece heater was tested to create an inhospitable hot and dry environment for bacteria to dissuade colonization and movement past the specific pipe section. By pushing biofilm further into the pipes, the risk of bacteria spreading out of the drain is minimized. However, results were inconclusive due to inconsistencies with the control and experimental setup (Hughes et al., 2016).

Antimicrobial Surfaces

Surfaces that prevent the growth and attachment of microorganisms are extremely useful in biomedical applications. Copper is a common material used for antimicrobial surfaces as bacterial and other microorganisms rapidly die on copper and copper alloyed surfaces (Grass, 2010). In a Department of Defense (DoD) sponsored hospital trial, rooms with copper-based surfaces had a 58% decrease in contamination and infection rates than their non copper counterparts (Copper Development Association, n.d.). Copper and other microbial surfaces have shown promise in decreasing general HAI; however, research on the efficiency of antimicrobial surfaces on this specific vector is not available. One possible intervention method using antimicrobial surfaces is to use copper, or copper based, piping underneath the sinks to limit growth. Biofilms will not, or will rarely, grow on the surfaces and due to their passive nature, seem to offer promising results if tested. However, usage seems to be more suited for new construction as opposed to retrofit due to the difficulty in replacing piping in active hospital rooms.

Physical Covering

An additional method of preventing spread of wastewater-born antibiotic resistant bacteria includes simple preventative measures such as a physical covering of drains. Within the hospital setting, physical covering prevents the spread of bacteria through the dispersal of water droplets formed from activities such as flushing the toilet and using hoppers (Matherset al., 2018; Johnson et al., 2013). Hoppers are found in hospital rooms and are commonly used to wash bedpans and dispose of waste. They can easily splash waste products into the air and contaminate surfaces. The interventions, such as hopper covers, present a low-cost intervention that require little installation. A study conducted by Mathers et al. (2018) showed that the installation of hopper covers showed a decrease in patient acquisition of KPC producing organisms.

Table 3: Scoring of Intervention Criteria for Physical Coverings

Criteria	Intervention		
	Thermal Disinfection	Antimicrobial Surface	Physical Covering
Cost to achieve acceptable ARB removal	-	0	+
User Safety	0	+	+
Ease of Implementation	-	0	+
Sustainability	+	+	+
Adaptability	+	0	+
Maintenance	+	+	+
General Removal Efficiency	-	0	-
Residuals & Byproducts	+	+	+
Potential to Prevent Reactivation	-	-	-

2.C.ii On-site Hospital Water or Wastewater Treatment

On-site treatment would include sanitation of water before use within the hospital or the collection and treatment of wastewater before transport to the WWTP. Currently, this intervention is uncommon but scientists and engineers are studying the potential benefits. On-site wastewater treatment is considered since hospital discharge contains considerable amounts of pollutants including X-ray contrast agents, pharmaceuticals, and ARBs (Pauwels et al., 2006). On-site *wastewater* treatment will not protect patients within the hospital but instead is aimed at preventing the dissemination of ARBs. *Water treatment* at the hospital may have the potential to prevent bacterial growth in pipes and thus protect patient health but further research is necessary to support this hypothesis. Scoring of each of the on-site interventions is available in Table 4.

Chlorine application before water use

This method would include on-site treatment of the hospital water using chlorine before the water is used by physicians, hospital staff, patients, and visitors to the hospital. This additional application of chlorine would increase chlorine residuals in hospital water with the intention of preventing antibiotic resistant bacteria from growing within pipes. A similar process has been implemented in an Italian hospital with the use of a chlorine dioxide for treatment of *Legionella pneumophila* (Casini et al., 2008). Additional research is required to understand if this disinfection method would be equally effective on multiple types of ARB. The need for additional scientific evidence to verify the efficacy of this treatment is a significant drawback of this sanitation method. Additionally, to appropriately implement this method, water must be monitored for compliance under the US Safe Drinking Water Act. Meeting federal health-based standards is required for any system that serves at least 25 persons (EPA, 2013). The requirement to meet the rigorous standards is a significant barrier to the implementation of this intervention. For chlorination, the general removal efficiency rating is typically a (+). Since the effectiveness of this intervention for a broad range of ARBs is unknown, the general removal efficiency in this case is rated lower.

Significant maintenance and monitoring is required to operate this new system, which would require the hiring of additional personnel. Chlorine can be dangerous to handle so trained professionals are necessary for operation (EPA, 1999a, p.2). Pre-treatment of the influent will not likely be required since water entering the hospital will have already undergone sanitation at a municipal scale. Since this method is not fully tested, however, the adaptability rating is lowered as the potential need for pre-treatment is necessary. Chlorination does result in disinfection byproducts such as chloramines which need to be removed before distribution (EPA, 1999a, p.2). Chlorination has been shown to increase antibiotic resistance in some cases (Liu et al., 2018).

Treatment after water use

If the hospital wastewater is collected before transport to the WWTP there is an opportunity to disinfect the water before it enters the municipal treatment system. Hospital wastewater has been shown to contain higher ARB and ARB gene concentrations than municipal wastewater (Paulus, 2019). Treating before transport to the WWTP would help contain ARBs to the hospital but would not improve patient health or safety as the disinfection is applied after the point-of-use stage. Initial studies have concluded that on-site treatment is "highly advantageous in regard to antibiotic and ARG reduction" (Paulus, 2019). The evidence of success is a significant advantage this intervention has over on-site water treatment.

The UVA Hospital has 612 beds as of 2019 and hospitals consume 165GPD/bed (UVA Health, 2019; Georgia DPH, 2002, pg 2). The UVA Hospital, according to our calculations, produces 100,908 gpd (0.1 MGD). Moores Creek Wastewater Treatment Plant has a capacity of 15 MGD; however, daily flow rates average about 9.33 MGD (Albemarle County, n.d). Therefore, we estimate that the Hospital produces about 1% of the total daily load of the Moores Creek Wastewater Treatment Plant. Since the hospital wastewater comprises a small percentage of the total wastewater at the WWTP, the ARBs from the hospital are diluted in the larger system. Implementing on-site wastewater sanitation, which treats a smaller but more concentrated amount of wastewater would be a more efficient method since a significantly smaller portion of wastewater would need to be treated with the goal of ARB removal (Pauwels et. al, 2006).

The implementation of this intervention may be difficult as most hospitals do not have water treatment facilities. Primary and secondary treatment of the wastewater would likely be needed in order for chlorination to be effective. The construction and operation of a wastewater treatment facility this size would cost roughly $42,325 to build and $2,117/year to operate. assuming 9.33 MGD (EPA, 1999a, p. 2). This cost includes the dechlorination cost which is necessary to remove harmful by-products. In comparison, a 15 MGD WWTP would cost $4,242,445 to build and $211,689/year to operate. Both these costs are extrapolated from the expected cost construction and operation of a 12 MGD plant in 1999 (EPA, 1999a, p. 2). The creation of this system would ultimately be expensive, complex, and require strict monitoring and maintenance from trained professionals (Pauwels et. al, 2006) making the acceptance of this intervention less likely. Although the cost could be lower for the city, since a lower volume of water will need to be treated, the hospital may not support the intervention if it bears the cost of operation.

Table 4: Scoring of Intervention Criteria for On-Site Water and Wastewater Treatment

Criteria	Intervention	
	Treatment before water use	Wastewater treatment after use
Cost to achieve acceptable ARB removal	-	-
User Safety	0	0
Ease of Implementation	-	-
Sustainability	0	0
Adaptability	-	-
Maintenance	-	-
General Removal Efficiency	0	+
Residuals & Byproducts	-	-
Potential to Prevent Reactivation	-	-

2.C.iii Municipal Utility Scale

Preliminary studies have addressed the efficacy of different traditional treatment methods for ARB inactivation at the WWTP scale, but further research is required to fully understand their efficacy and cost-effectiveness (Bouki, 2013). Within the United States, the most common wastewater treatment plant disinfection methods include chlorination, UV disinfection, and ozone disinfection. Within these three, chlorination is the most commonly used disinfectant according to the EPA (1999a), however this information is outdated and the concentration of facilities that may use one of the other two disinfection methods may now be higher (p. 1). More recent statistics on the distribution of disinfection methods implemented by WWTP's in the United States is not recorded in the EPA Facilities Registry Service Database which primarily covers the compliance of WWTP in the U.S. (EPA, 2020). Figure 6, below, shows the current rates of compliance of all WWTPs in the United States as of January, 2020. Within these wastewater treatment plants approximately 39% are not in compliance with EPA standards and another 9% have significant violations.

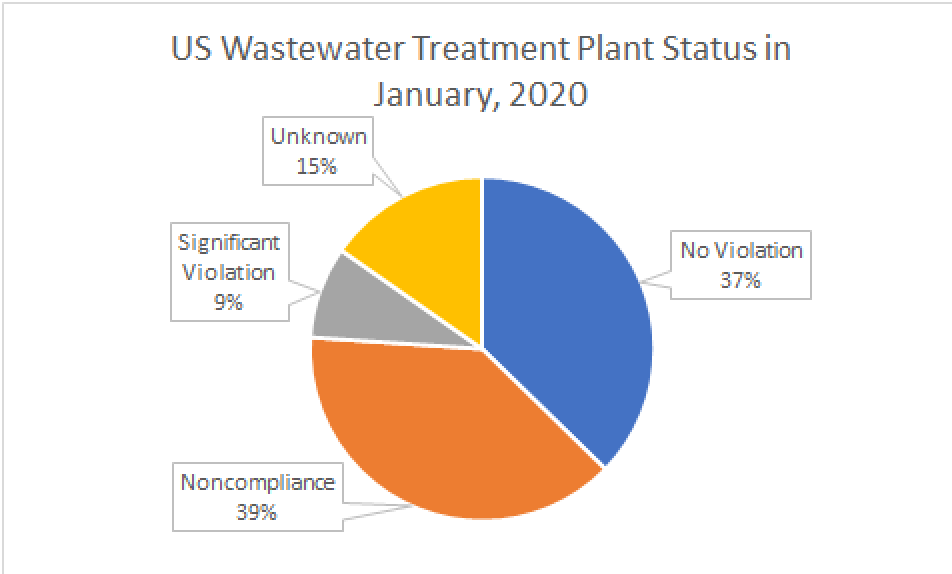

Figure 6: U.S. Wastewater Treatment Plant Status in January, 2020: Data from EPA Facilities Registry Service Database

The Municipal Utility Scale section is divided into three subsections, each discussing one of the three common methods of WWTP disinfection: chlorine, UV radiation, and Ozone. Each of these sections discusses information relating to the intervention criteria and are summarized in Table 5 at the end of the section.

Chlorine

Within wastewater treatment, chlorination is the most commonly used and well-established disinfection method which disinfects water through oxidation of an organism's cellular material (EPA, 1999a, p.1-2). If a WWTP is already using chlorination as a tertiary treatment, as this is common, the marginal cost of implementation will be zero. Chlorination leaves a residual in the water and can require dechlorination before release as it is toxic to aquatic life, which will increase costs (EPA, 1999a, p.2). Due to the corrosive nature of chlorine, it is dangerous to transport and handle and will require regulated operation and handling procedures (EPA, 1999a, p. 2). Additionally, there is the possibility that chlorine may increase antibiotic resistance (Liu et al., 2018). Estimated capital costs for a 12 MGD wastewater treatment plant using chlorination and dechlorination in 1999 is $3,385,956 with annual operating costs of $169,351 for a chlorine dose of 5 mg/L or $237,702 for a chlorine dose of 10 mg/L with capital costs at $3,385,956 (EPA, 1999a, p.6; Darby et al. 1995). This information is summarized in relation to intervention criteria below in Table 5.

UV Radiation

UV disinfection destroys cellular DNA through the use of electromagnetic energy; however, some organisms may be able to repair the damage done to DNA by this method (EPA, 1999c, p.1-2; Guo & Kong, 2019). The effectiveness of UV disinfection relies heavily on the characteristics of the water being treated, such as turbidity and total suspended solids (TSS), as well as contact time, and UV intensity (EPA, 1999c, p.1-2). UV treatment, while not requiring the transport, storage, or creation of hazardous chemicals like other methods, requires workers to clean the UV lamps with weak acids that could interact with wastewater to form harmful byproducts if not monitored appropriately (EPA, 1990c, p.4). Unlike chlorination, UV disinfection does not create any disinfection residuals and can thus be discharged directly after the treatment process (EPA, 1990c, p.2).

For UV disinfection, a medium sized UV treatment system applying a UV dose of 40mJ/cm^2 for a 12-MGD capacity is estimated by Cotton et al. (2001) to cost approximately $23,000 to $30,000 in annual operation and maintenance costs with a capital cost between 1.2 to 1.4 million dollars.

Ozone

Ozone disinfection, much like UV, generates no residual when applied at low levels, however the ozone used to oxidize cell walls must be generated on site (EPA, 1999b, p.3). This method requires complex technology with a much higher level of concern for worker safety, making it unlikely to be used for wastewater disinfection (EPA, 1999b; National Research Council, 1999, p. 234). Additionally, to reach appropriate levels of disinfection for some pathogens, excess amounts of ozone must be used, leading to an ozone residual that must be removed through a quenching process that has proven to be difficult (National Research Council, 1999, p. 234). Further, ozone is also a strong irritant and explosive in gaseous concentrations above 240g/m3 so minimizing contact and ensuring no leakages is essential with ozone disinfection (EPA, 1999b, p.3). Ozone has been shown to be effective at inactivating ARBs and ARGs as well as preventing potential regrowth which would prevent reactivation and spread of ARBs if certain conditions were met (Iakovides et al., 2019).

For ozone disinfection at 12-MGD with an ozone dosage of 2 mg/L, the capital cost for an ozone treatment system is estimated by Mundy et al. (2018) to be approximately 2.95 million dollars (p.269-270). Daily operation and maintenance costs are estimated to be $33,460 (Mundy et al., 2018, p. 270). It should be noted that cost data from Mundy et al. (2018) was published more recently than data found in the chlorine and UV sections.

Table 5: Scoring of Intervention Criteria for Municipal Utility Scale Treatment Options

Criteria	Intervention		
	Chlorine	UV Radiation	Ozone
Cost to achieve acceptable ARB removal	0	0	-
User Safety	0	0	0
Ease of Implementation	+	0	-
Sustainability	-	-	-
Adaptability	-	-	-
Maintenance	-	-	-
General Removal Efficiency	+	+	+
Residuals & Byproducts	-	+	0
Potential to Prevent Reactivation	-	-	+

2.D Recommendations

Due to conflicting literature reports, it is unclear as to which method is optimal for ARB and ARG deactivation. Charlottesville's use of UV disinfection seems to provide adequate disinfection of KPC at the wastewater treatment scale. Therefore, it is not recommended to change from the current method as more data collection and analysis would be required and the current system appears to be effective. It is also recommended that point source interventions be implemented in the UVA hospital system in order to protect patient safety. They have been proven to reduce HAI and the spread of bacteria in general in hospital settings.

In regards to handling antibiotic resistant bacteria in wastewater systems, options for treatment include:

1. Do not change the procedures already in place as they are already adequate handling the disinfection of antibiotic resistant bacteria.
    a. Municipal WWTP should test effluent water to verify that ARB are being removed as conflicting literature does not allude to an optimal disinfection method.
2. Focus on hospital level interventions, primarily at the point source, in order to decrease exposure to antibiotic resistant bacteria.

3. Focus on onsite interventions at the hospital in order to prevent ARB from entering the municipal wastewater system. This option could be used if prevention of ARB caused infections is not a priority at the hospital due to low rates of infection.

2.E Conclusions and Further Work

From Table 3, 4, and 5, several candidate interventions appear to be especially worthwhile for further evaluation. They include: physical coverings for hoppers and toilets, antimicrobial surfaces, on-site wastewater treatment, and chlorination and UV disinfection at the municipal level. In contrast, other candidate interventions such as thermal disinfection, on-site water treatment, and ozone appear to be not particularly appealing in this context.

Although UV disinfection at the utility scale appears to be sufficient in eliminating KPC before entering the environment in Charlottesville, this may not be the optimal solution for all communities. Changing the sanitation method for a wastewater treatment plant to ultimately treat a small percentage of the waste stream may not be cost effective. Installing onsite treatment in this situation may be more cost effective for the municipality but would require the hospital to incur additional costs. If there are a multitude of reasons, however, for switching from one sanitation method to another, the reduction and elimination of KPC bacteria and ARGs is a factor that should be taken into consideration.

At the point source scale, current methods developed by Mathers et al. at UVA Hospital are sufficient in decreasing the spread of KPC bacteria in hospital systems. The installation of covers and sink trap devices in hospital rooms decreased the acquisition of KPC-producing organisms in patients by roughly half (Mathers et al., 2018). For hospitals and other healthcare facilities dealing with HAIs, utilizing similar intervention methods will not only minimize the acquisition of KPC-producing organisms, they will also decrease the acquisition of HAI’s in general.

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Appendix A: Results of Total Suspended Solids and Volatile Suspended Solids from Moores Creek Samples

Table 6: VSS and TSS results from September 10th sampling.

Sampe Name	Type	TSS (g/L)	VSS (g/L)
Influent	Water	0.2	0.5
Secondary sludge	Water	7.2	4.9
Dig Effluent	Sludge	10.8	6.0
Dig Influent	Sludge	21.6	14.3
Pre-disinfection	Water	-	-
Final Effluent	Water	-	-
Upstream 1	Water	-	-
Upstream 2	Water	-	-
Upstream 3	Water	-	-
Upstream 4	Water	-	-
Upstream 5	Water	-	-
Upstream Sediment 1	Sediment	21.3	3.4
Upstream Sediment 2	Sediment	2.7	0.7
Upstream Sediment 3	Sediment	2.7	2.3
Upstream Sediment 4	Sediment	3.7	1.0
Upstream Sediment 5	Sediment	2.8	2.3
Downstream 1	Water	-	-
Downstream 2	Water	-	-
Downstream 3	Water	-	-
Downstream 4	Water	-	-
Downstream 5	Water	-	-
Downstream Sediment 1	Sediment	8.2	1.2
Downstream Sediment 2	Sediment	3.1	0.3
Downstream Sediment 3	Sediment	4.9	1.3
Downstream Sediment 4	Sediment	5.7	0.9
Downstream Sediment 5	Sediment	13.2	2.6