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Characterization of Aquatic Sediment Bacteria Resistant to Triclosan and Antibiotics Above and Below the Town Branch Wastewater Treatment Plant

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Abstract

Characterization of Aquatic Sediment Bacteria Resistant to Triclosan and Antibiotics Above and Below the Town Branch Wastewater Treatment Plant

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Wastewater treatment plants appear to play a role in releasing antimicrobial compounds and resistant genes into the environment. The phenomenon of bacterial resistance to multiple antimicrobial compounds is not well understood with regards to public health. Studies have examined cross-resistance to triclosan and various antibiotics in bacteria. These resistance mechanisms: target site modification, membrane resistance, and efflux pumps indicate possible ways in which bacteria have adapted to antibiotics as well as triclosan. In addition, antibiotic resistance genes occur naturally in the environment and are passed from species to species through horizontal gene transfer. Sediment samples were collected from above and below the Town Branch wastewater treatment plant in Lexington, KY. Bacteria capable of growing in the presence of triclosan were subcultured on media containing antibiotics. Isolates were then identified through sequencing of the 16S rRNA. The three most prevalent genera identified were *Pseudomonas*, *Enterobacter*, and *Bacillus*. All of the isolates were resistant to β-lactams and several other antibiotics. Moreover, all of the isolates were sensitive to tetracycline, doxycycline, and ciprofloxacin. Therefore, isolates potentially exhibit cross-resistance to triclosan and various antibiotics. Efflux pumps are a mechanism that may allow these

isolates to target triclosan and antibiotics by pumping them out across the cell membrane back into the environment. However, the impact of triclosan on the spread of antibiotic resistance genes in the environment is not well understood. Therefore, the relevance of this co-resistance remains unclear.

Keywords: wastewater treatment plants, triclosan, cross-resistance, antibiotic resistance genes

Table of Contents

List of Figures

List of Tables

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Introduction

Triclosan (TCS) is a diphenyl ether derivative (2, 4, 4'-trichloro-2'-hydroxydiphenyl ether); referred to as a synthetic antimicrobial that is commonly found in many consumer products, such as detergent, toothpaste, shampoo, hand soap and mouthwash. Since these consumer products are so widely used, and TCS has been detected in surface water, wastewater treatment plants, sediments, and drinking water across the country as well as in Europe, there is increasing concern about its ecological effects (Carey & McNamara, 2015; Drury, Scott, Rosi-Marshall, & Kelly, 2013).

Approximately 100 tons of the triclosan enter wastewater treatment plants per year (Carey et al., 2015). Wastewater treatment plants (WWTPs) obtain triclosan through daily human activities, such as washing ones hands or clothes. Given the fact that TCS is also found in many oral products, it is commonly found in human urine, which then enters WWTPs. According to a survey conducted on a relatively small population (157

subjects) of multiethnic pregnant women in Brooklyn, NY, TCS was found in all the participants' urine samples (Pycke et al., 2014). Although WWTPs remove the majority of TCS, there is still a small percentage present in the treated effluent. An estimated 50-56% of the total annual loading of TCS into surface waters comes from WWTP effluent (Drury et al., 2013), which is estimated to release 0.24 kg/day of triclosan (Lozano, Rice, Ramirez, & Torrents, 2013). TCS tends to accumulate in sediments near the WWTPs effluent due to its hydrophobic property. Several studies have shown that triclosan can remain in sediments up to 50 years (Anger et al., 2013; Buth et al., 2010; Miller & Heidler, 2008). Another possible way TCS gains entry into aquatic environments is through leaky sewer pipes. This untreated wastewater naturally contains a higher concentration of TCS. However, the majority of triclosan released into the environment comes from WWTPs sludge (5.37 kg/day), also known as the treated remaining solids (Lozano et al., 2013). These treated solids are then spread over fields as fertilizers.

The effect TCS has on public health remains unknown; however, studies have investigated the possibility of this chemical compound being a disruptor of normal growth and development in frogs. Veldhoen et al. (2006) examined how releasing TCS into the environment could affect the thyroid hormone-mediated process of metamorphosis in the North American frogs, *Rana catesbeiana*. There are three developmental stages that the tadpole undergoes: premetamorphosis, postmetamorphosis, and metamorphic climax (Dodd & Dodd, 1976; Gosner, 1960). During premetamorphosis, thyroid hormone is not present. This is characterized by minimal development in the hindlimb buds (Veldhoen et al, 2006). Thyroid hormone levels then increase during the second phase, prometamorphosis, which accounts for the extensive

developmental changes in the hindlimbs. When the thyroid hormone levels peak, the tadpole undergoes major morphological changes. This is known as the metamorphic climax; the third and final developmental stage for the tadpole. Thyroid hormone action targets tadpole tissues in a variety of ways from tail loss and limb formation to remodeling of the brain (Shi, 2000). TCS and thyroid hormone are structurally similar, which causes concern for animals and humans that utilize thyroid hormone action. According to Veldhoen et al. (2006), early onset of metamorphosis in the tadpoles indicates that TCS at low concentrations found in the effluent released from WWTPs into aquatic environments affects thyroid hormone-mediated development. Further, TCS at low concentrations can cause alternations in thyroid hormone-regulated gene expression at a critical life stage for *Rana catesbeiana*. While undergoing prometamorphosis the tadpole's thyroid hormone levels increase, which could heighten its sensitive to TCS.

Instead of remaining sensitive to TCS many bacteria are showing resistance to the man-made antimicrobial. TCS is toxic to bacterial fatty acid biosynthesis because it inhibits the fabI enzyme; thus, preventing the last reaction in fatty acid elongation (Heath et al., 1999; McMurry, Oethinger, & Levy, 1998). Fatty acids form the cell membrane which allows bacteria to regulate substances entering and exiting the cells (Escalada, Harwood, Maillard, & Ochs, 2005). In order to combat triclosan, bacteria, such as *Pseudomonas aeruginosa* possess various defense mechanisms. Three of the most common resistance mechanisms are target site modification, membrane resistance, and efflux pumps (Carey et al., 2015).

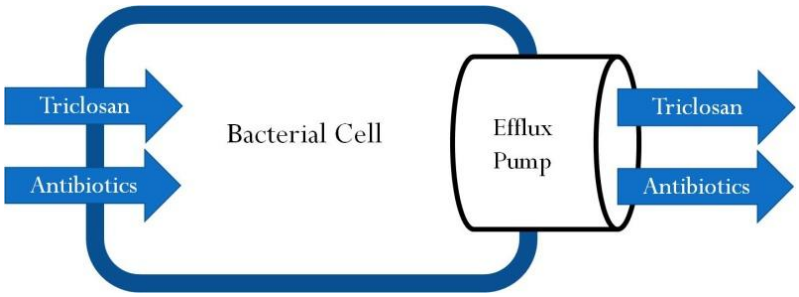
Target site modification involves changes in single or multiple amino acids in the *fabI* gene, which creates resistant *fabI* proteins (Brenwald & Fraise, 2003; Yu et al.,

2010). By increasing the concentration of the *fabI* protein through up-regulation or locating an allele on a mobile genetic element (Ciusa et al., 2012), bacteria can overcome the effects of TCS. When bacteria up-regulate *fabI*, it can also target many other genes in response to TCS. In addition, triclosan resistant bacteria have been found to contain enoyl-acyl carrier protein reductase isoenzymes, which function similarly to *fabI* (Zhu, Lin, Ma, Cronan, Wang, 2010). Enoyl-acyl carrier protein reductase catalyzes the final reaction of elongation in the fatty acid synthesis (Massengo- Tiassé & Cronan, 2009). There have been several studies that have shown these isoenzymes replacing *fabI*. For instance, Zhu et al. (2010) discovered FabV, a particular isoenzyme, replaces *fabI*, which greatly increases resistance to triclosan in *P. aeruginosa*. Heath et al. (2000) found different isoenzymes, FabK, which replaces *fabI* in *Streptococcus pneuononia*, resulting in increased tolerance to TCS, and FabL, which enhances resistance to TCS in *Bacillus subtilis*.

Membrane resistance could be another potential mechanism for cross-resistance to antibiotics. The outer membranes of some bacteria have properties that allow resistance to hydrophobic compounds, in this case TCS and antibiotics. According to Tkachenko et al. (2007), TCS exposure seems to possibly increase the concentration of fatty acids present in the cell membrane of *Staphylococcus aureus*. Therefore, TCS cannot pass through the cell membrane, rendering the chemical compound impermeable. This prevents disruptions within the cell. Bacteria may be using this mechanism for cross-resistance between triclosan and antibiotics in the environment due to the non-specific rejection of hydrophobic chemicals.

A variety of bacteria utilize efflux pumps to rid substances from their intracellular spaces. Efflux pumps function by excreting the substance across the cell membrane and back into the environment (Figure 1). Kern et al. (2000) and Levy (2002) suggest that this mechanism is effective against TCS as well as several other antimicrobials and antibiotics. In particular, it is typically the non-specific multidrug efflux pumps that confer resistance to triclosan and antibiotics (Chuanchuen et al., 2001; Pycke et al., 2010). These efflux pumps function by expelling the originally exposed substance as well as other substances. This mode of action facilitates the explanation of how cross-resistance occurs in culturable bacteria.

Figure 1. Mechanism for an efflux pump.



Several studies have examined cross-resistance to triclosan and various antibiotics in bacteria. Suller and Russell (2000) discovered that *Staphylococcus aureus* not only becomes resistant to triclosan and other antibiotics, but also this bacterium passes on the cross-resistance gene to the following generations. This process suggests that the presence of triclosan may cause the selection for the cross resistance gene, which would mean survival for bacteria containing this gene. In the presence of antibiotics, the same is true in regards to natural selection for the cross resistance gene. Furthermore, this trait

can be transferred from one species to another species, which explains the concern of triclosan and cross-resistance for public health.

The mechanism attributed to cross-resistance is efflux systems. In *P. aeruginosa* up-regulation of efflux pumps increased the minimum inhibitory concentration of triclosan by more than sixfold, whereas efflux pumps in *Escherichia coli* increased the minimum inhibitory of triclosan by only twofold (Chuanchuen et al., 2001). Additionally, Chuanchuen et al. (2001) discovered that bacteria exposed to triclosan can select for multidrug resistance, in particular an antipseudomonas drug. Multidrug resistance in *P. aeruginosa* is concerning in hospital settings because this pathogen causes serious life-threatening infections for immunodeficient individuals. *P. aeruginosa* has an intrinsic resistance to many antibiotics (van Delden & Iglewski, 1998). Therefore, the infection becomes chronic due to the inability of several antibiotics to kill the bacteria.

Antibiotic producing bacteria occur naturally in the environment. They need antibiotic resistance genes to protect themselves (Hopwood, 2007; Martin and Liras, 1989; Tahlan et al., 2007). Antibiotic resistance genes are typically found on plasmids, which means these genes can be moved and transferred to other bacteria through horizontal gene transfer (Zhang et al., 2011). Horizontal gene transfer allows the bacteria to transmit the antibiotic resistance genes and pass them to other bacteria through means other than reproduction. Bacteria can obtain genes from the environment through this process (Thomas & Nielsen, 2005). Studies have shown that soil contains environmental bacteria with antibiotic resistance genes independent of human influences (Allen et al., 2010), which suggests bacteria are in fact naturally acquiring antibiotic resistance genes

from the environment. Even before the use of antibiotics, evidence has shown that antibiotic resistance genes existed on plasmids and were common in nature.

For isolates that do not produce antibiotics, mutations occur, which inhibit antibiotic action (Allen et al., 2010). In environmental bacteria, these types of mutations have been frequently observed. This reveals that various selective pressures have lead to the extensive presence of antibiotic resistant strains in nature. Some selection pressures in the environment are generated by agricultural antibiotics that promote growth and improve the effectiveness of feed (Sarmah, Meyer, & Boxall, 2006); fish farming, which uses preventative antibiotics to maintain healthy populations (Cabello, 2006); and antibiotics that occur naturally apply selective pressure on neighboring organisms. These prophylactic antibiotics used in feed and water select for antibiotic resistance. Antibiotics from agricultural sources enter into soil and aquatic environments, and these compounds undergo selective pressure that may in turn directly affect treatment of human pathogens (Segura, Francois, & Sauve, 2009; Thiele-Bruhn, 2003).

Antibiotic resistance genes released by anthropogenic activities can enter the environment through different means (Carey et al., 2015). Antibiotics released in feces and urine from hospitals enters the hospital WWTPs. Further, the hospital wastewater effluent may release antibiotic resistance genes. Households also contribute to the discharge of antibiotics into WWTPs through individuals taking prescribed antibiotics for bacterial infections. Once antibiotics enter the WWTPs, they can end up in sludge, also known as left-over solids, and spread over fields as fertilizer (Berglund, 2015). This dispersal of antibiotics likely results in resistant bacteria following the same route, which results in environments where there is a mixture of antibiotics, antibiotic resistance genes,

resistant bacteria, and environmental bacteria that could naturally contain antibiotic resistance genes (Baquero, Martinez, & Cantón, 2008). These environments allow for the creation of new resistant strains through horizontal gene transfer between the same or different species of bacteria. Humans encounter these bacteria through a variety of ways, such as consuming contaminated crop that have been fertilized with sludge and drinking contaminated ground or surface water due to fish farms or animal feces (Berglund, 2015). By ingesting these resistant bacteria, they will likely transfer their antibiotic resistance genes to other bacteria within the human microbiome through horizontal gene transfer (Wellington et al., 2013).

There are three methods of horizontal gene transfer: conjugation, transduction, and transformation in which bacteria can acquire an antibiotic resistance gene from the environment. Conjugation allows for the transfer of DNA among a wide range of species (Smillie, Garcillán-Barcia, Francia, Rocha, & de la Cruz, 2010). This mode of transfer has occurred from bacterial cells to eukaryotic cells as well as in many different environments, in particular sewage wastewater and activated sludge (Bates, Cashmore, & Wilkins, 1998; Davison, 1999). Plasmids are the most important mobile genetic elements capable of being transmitted through conjugation (Smillie, Garcillán-Barcia, Francia, Rocha, & de la Cruz, 2010). Another process of horizontal gene transfer, transduction, uses bacteriophages to attach to a cell and transfer its DNA. This method encompasses a broad range of hosts. Bacteriophages are able to infect different classes of bacteria (Jensen et al., 1998). These diverse host ranges allow bacteriophages to transfer genes between environmental bacteria and human microbiomes (Muniesa, Colomer-Lluch, & Jofre, 2013). The third process, transformation, involves the uptake and incorporation of

fragments of DNA from the surroundings through the cell membrane. In a study on a river basin in China, the concentration of extracellular DNA was found to be higher than the concentration of intracellular DNA (Mao et al., 2014). This suggests that extracellular DNA serves as an important way to access and transfer genes through transformation.

Wastewater treatment plants greatly reduce the total number of bacteria in the released effluent; however the treatment does not remove antibiotic resistance genes. Human activities and naturally occurring antibiotic resistance genes in the environment come together in WWTPs. This may contribute to horizontal gene transfer of resistance among both clinically and environmentally diverse microorganisms (LaPara & Burch, 2012). Therefore, WWTPs could be connected to the increased prevalence and scope of antibiotic resistance genes as well as antibiotic resistant bacteria.

Triclosan and antibiotics enter WWTPs simultaneously. Several studies have observed these compounds exhibit cross-resistance. Suller and Russell (2000) conducted a clinical study on *Staphylococcus aureus*. The isolates that had a minimal inhibitory concentration to TCS between 0.025 and 1 mg/L were resistant to multiple antibiotics. Some of the TCS resistant isolates were grown in media that did not contain TCS for 10 days. As a result the isolates lost TCS resistance, indicating TCS selects for resistance that is not normally expressed in these strains. This implies that removing TCS from consumer products or improving the wastewater treatment process could decrease TCS resistance in the environment. In turn, limiting or removing TCS could decrease antibiotic resistance genes present in nature because of cross-resistance. However, these are only speculations. Many studies have investigated the effects of TCS on culturable bacteria, which revealed cross-resistance between TCS and antibiotics. Nevertheless,

culturable bacteria make up a small percentage of the total bacteria in the environment. As a result, Carey and McNamara (2015) raised the following questions in their recent review: How does triclosan impact antibiotic resistance genes in the environment? Will limiting triclosan decrease antibiotic resistance genes?.

To better understand the effects of TCS and WWTPs, bacteria ought to be classified. Identification occurs by sequencing the 16S ribosomal ribonucleic acid (rRNA). Almost all bacteria contain the 16S rRNA gene and the gene is composed of thousands of base pair, making it large enough for identification purposes. Universal primers bind to the gene at two different domains. Weisburg, Barns, Pelletier, and Lane (1991) discovered a primer pair, 27F and 1492R, that was capable of amplifying nearly all of 16S rRNA gene. This primer pair targets the region that differs from bacteria to bacteria, allowing for the identification of bacteria to be possible. The objectives of this project include identification of bacteria resistant to TCS above and below the Town Branch WWTP in Lexington, KY as well as characterizing cross-resistance among triclosan and various antibiotics in these bacteria.

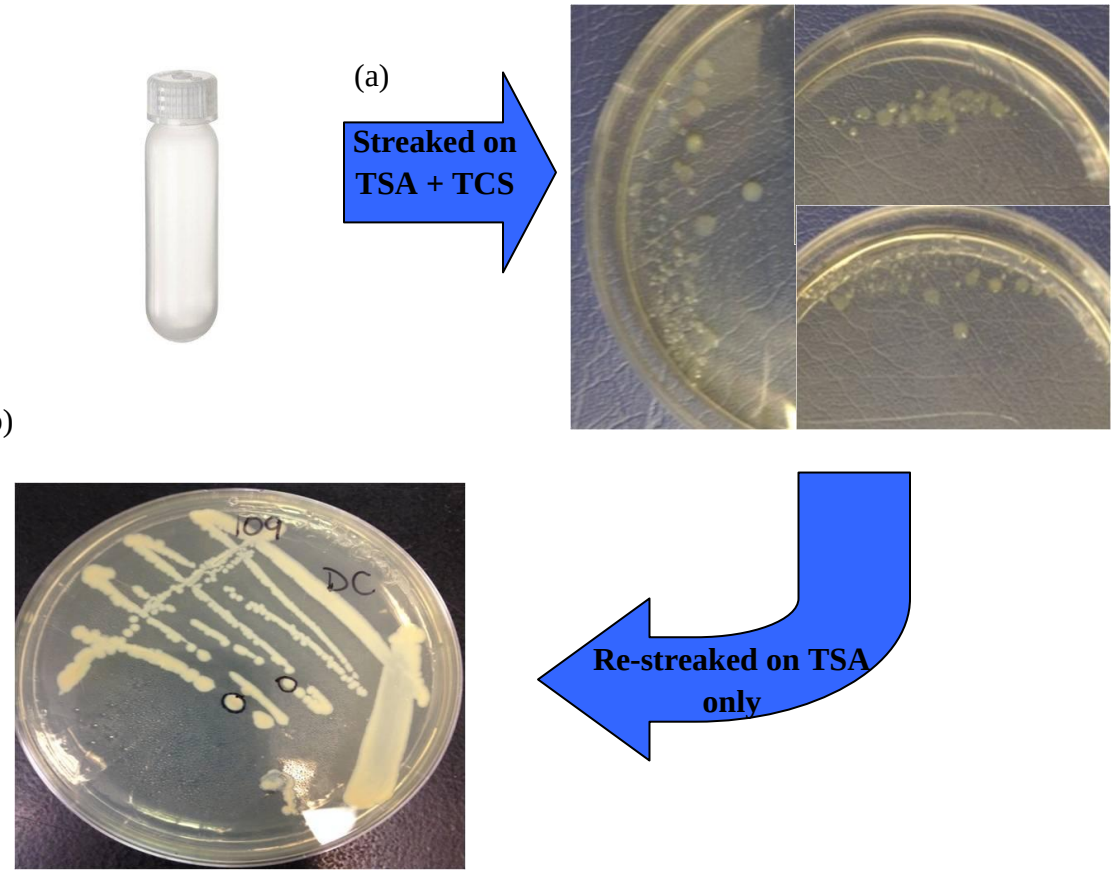
Materials and Methods

Isolation of Bacteria

Sediment samples were collected from downstream and upstream of where the effluent was released from the Town Branch WWTP in Lexington, KY. These sediment samples were then transferred to 50 mL test tubes with 20 mL of potassium phosphate (K_2PO_4) buffer. The samples were shaken at 150 rpm for a 24-hour period. The suspension was then streaked onto plates containing tryptic soy agar (TSA) and TCS

(0.1% w/v; Figure 2a). Individual colonies were then re-streaked onto TSA only to obtain a pure culture (Figure 2b).

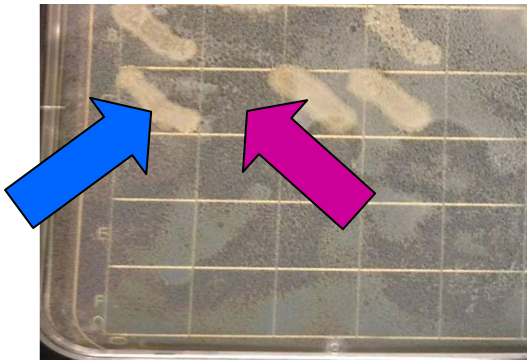
Figure 2. (a) Sediment samples were streaked on plates with TSA and TCS. Diverse colonies grew differing in color, shape, and size. (b) Pure cultures were obtained. Isolates were re-streaked onto TSA only.



Spotting on Antibiotic Plates

The pure cultures were grew overnight in tryptic soy broth (37˚C, 150 rpm). Cultures were then spotted on TSA supplemented with: penicillin, ampicillin, oxacillin, amoxicillin, cefdinir, tetracycline, doxycycline, erythromycin, sulfamethoxazole, trimethoprim, or ciprofloxacin (100 µg/mL). The bacteria were identified as either resistant or sensitive (Figure 3).

Figure 3. An example of our results, showing two types of bacteria either resistant or sensitive. The blue arrow is showing resistant bacteria, whereas the purple arrow is indicating sensitive bacteria.



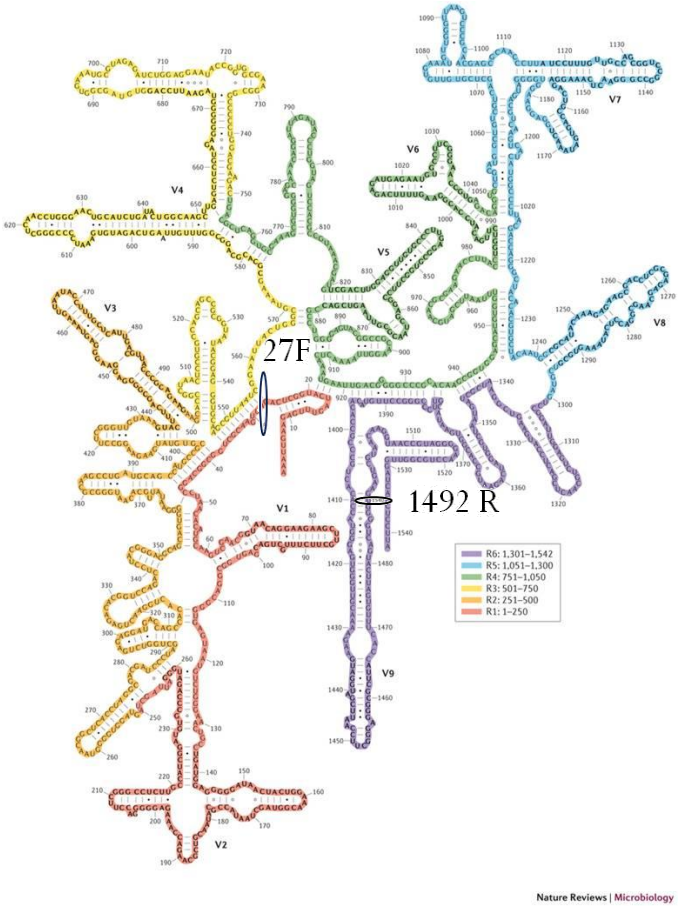
Isolation of DNA from Bacteria

DNA was isolated with the Promega Wizard Genomic DNA kit, which treated all samples as Gram-positive.

Polymerase Chain Reaction of 16S rRNA gene

Polymerase Chain Reaction (PCR) was performed using the universal primers, 27F and 1492R, targeting 16S rRNA (Figure 4). PCR was run for 25 cycles (denaturation 60 s at 94˚C, annealing 30 s at 48˚C, and elongation 60 s at 72˚C; Lane, 1991). Agarose gel electrophoresis was used to confirm the presence of PCR amplicons prior to sequencing.

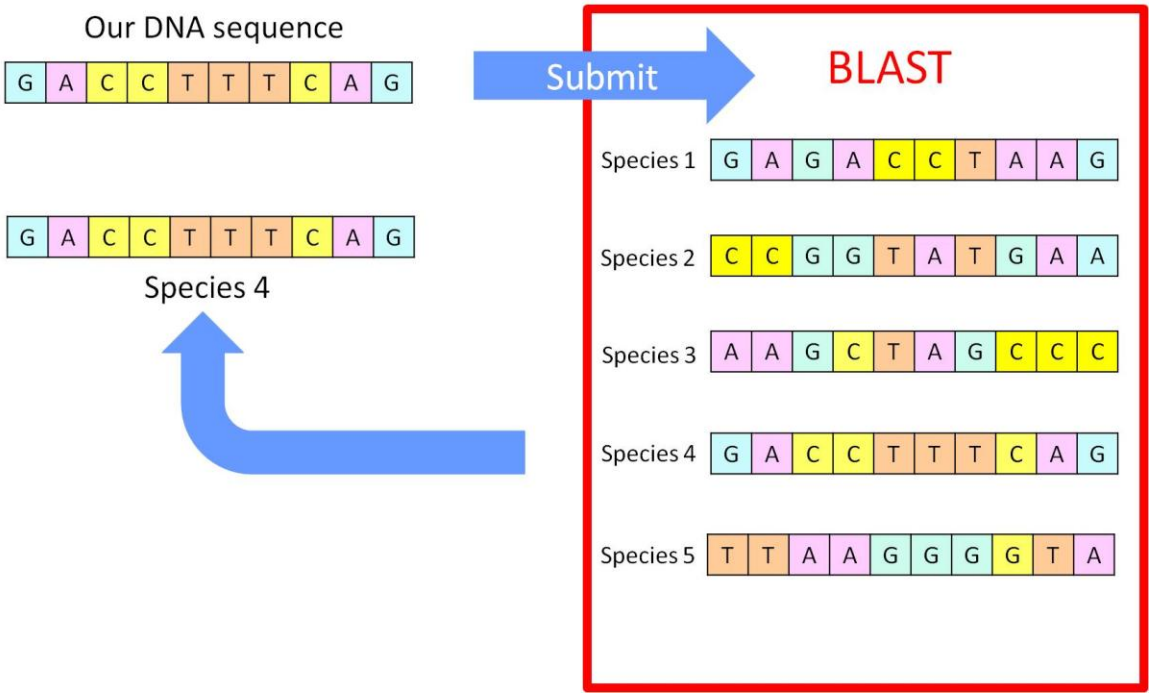
Figure 4. The universal primer pair, 27F and 1492R, bound to their appropriate domains on the 16S rRNA, indicates the identification sequence.



Sequence Analysis

The PCR fragments were sequenced at University of Kentucky Advanced Genetic Technologies Center (UK-AGTC). The sequences were identified using nucleotide Basic Log Alignment Search Tool (BLAST; Figure 5).

Figure 5. BLAST works by searching through a database of DNA sequences to find statistically significant matches. In this case, BLAST found an exact match (100%) to the DNA sequence submitted, which means the isolate is identified as species 4.



Results

The three most prevalent genera identified were *Pseudomonas* (n=5), *Enterobacter* (n=3), and *Bacillus* (n=5; Table 1). From the data collected, common tends were observed. All the isolates were resistant to β-lactam antibiotics as well as several other antibiotics. Further, all isolates were sensitive to tetracycline and doxycycline, which are closely related, as well as ciprofloxacin.

Table 1. Preliminary identification of triclosan resistant isolates. *Green indicates resistant bacteria and blue indicates sensitive bacteria.

Location	Preliminary Identification	Pencillin	Ampicillin	Oxacillin	Amoxicillin	Cefdinir	Tetracydine	Doxycycline	Erythromycin	Sulfamethoxazole	Trimethoprim	Ciprofloxacin
Upstream	Pseudomonas sp. (Sample 3)		N/A									
Upstream	Pseudomonas sp. (Sample 30)		N/A									
Upstream	Pseudomonas sp. (Sample 32)		N/A									
Upstream	Pseudomonas sp. (Sample 43)		N/A									
Upstream	Pseudomonas sp. (Sample 49)		N/A									
Upstream	Enterobacter sp. (Sample 7)		N/A									
Upstream	Enterobacter sp. (Sample 28)		N/A									
Upstream	Enterobacter sp. (Sample 31)		N/A									
Upstream	Aeromonas sp. (Sample 4)		N/A									
Upstream	Pontibacillus sp.(Sample 27)		N/A									
Upstream	Brevibacillus sp. (Sample 29)		N/A									
Upstream	Citrobacter sp. (Sample 50)		N/A									
Downstream	Bacillus sp. (Sample 102)											
Downstream	Bacillus sp. (Sample 104)											
Downstream	Bacillus sp. (Sample 105)											
Downstream	Bacillus sp. (Sample 106)											
Downstream	Bacillus sp. (Sample 107)											

Discussion

Pseudomonads are ubiquitous in nature and resistant to multiple antibiotics. These bacteria utilize efflux pumps as a way to obtain resistance. One type of pseudomonad, *P. aeruginosa* is a serious pathogen for cystic fibrosis patients, burn victims, as well as ICU patients (Centers for Disease Control and Prevention [CDC], 2014). Cystic fibrosis patients are high risk for life-threatening effects caused by chronic *P. aeruginosa* infections (van Delden et al., 1998). Treatment failures are due to multidrug resistant bacteria (Pier, 1998). Four types of efflux systems: MexAB-OprM (Poole, Krebes, McNally, & Neshat, 1993), MexCD-OprJ (Poole et al., 1996), MexEF-OprN (Köhler et al., 1997), and MexXY (Aires, Köhler, Nikaido, & Plesiat, 1999; Mine, Morita, Kataoka, Mizushima, & Tsuchiya, 1999; Westbrock-Wadman et al., 1999) have been characterized in *P. aeruginosa*, which provide intrinsic resistance to antibiotics as well as triclosan. According to Chuanchuen et al. (2001), triclosan is not only a substrate for several efflux pumps in *P. aeruginosa*, but also selects for multidrug resistant bacteria. Therefore, since triclosan puts selective pressure on the development of resistance, Chuanchuen et al. (2001) concludes that continual, unregulated use of this antimicrobial may assist in the selection of multidrug resistant bacteria which in turn affects antibiotic resistance.

Enterobacter are typically found in fecal matter and cause infections of the skin and soft- tissue, lower respiratory tract, and urinary tract (Fraser, 2015). There has been an increase in the spread of resistance in *Enterobacter* which is problematic for treating these infections. *Enterobacter* shows resistance to the narrow-spectrum β-lactams, such as penicillin, ampicillin, and amoxicillin. In addition, resistance to trimethoprim and sulfamethoxazole is common in *Enterobacter*; however, resistance to ciprofloxacin is

rare (Fraser et al., 2015). Our results align with the known resistance present in *Enterobacter*. While several *Bacillus* species were identified, none are known human pathogens, like *Bacillus cereus* which causes food poisoning (Bottone, 2012).

Antibiotic resistance genes protect bacteria through different mechanisms (Table 2). β-lactams inhibit cell wall synthesis. With this is mind, it is plausible that the isolates may have outer membranes that confers resistance to hydrophobic compounds, such as TCS and antibiotics. This membrane resistance could occur in the form of biofilm, which would function by blocking antibiotics and TCS from entering the bacterial cell. Membrane resistance is a potential mechanism for triclosan and cross-resistance to antibiotics. Other antibiotics, such as sulfamethoxazole and trimethoprim inhibit folic acid synthesis. Although humans acquire folic acid from their diets, bacteria must produce folic acid in order to survive and grow. Erythromycin, tetracycline, and doxycycline target protein synthesis in bacteria. Ciprofloxacin also effects protein synthesis by inhibiting DNA replication by targeting DNA gyrase (Collin, Karkare, & Maxwell, 2011). This enzyme is used to unwind the double helix during DNA replication. As the data indicated, all the sediment isolates were greatly affected by the antibiotics that impact protein synthesis (i.e. tetracycline, doxycycline, and ciprofloxacin).

Previous studies have investigated cross-resistance between triclosan and antibiotics in culturable bacteria. Cottell et al. (2009) found triclosan-resistant strains of *Staphylococcus aureus*, *E. coli* and *Acinetobacter johnsonii* were sensitive to tetracycline, which is consistent with our results. We had predicted some of the isolates would show resistance to tetracycline due to its abundance in nature (Daghrir & Drogui, 2013). Such

cross-resistance was observed by Chuanchuen et al. (2001). Cottell et al. (2001) also discovered that the strains mentioned above were resistant to ciprofloxacin, which mirrors our results. In contrast, their strains exhibited sensitivity to β-lactams, whereas our strains were resistant.

A limitation to our study was only culturable bacteria were characterized, which make up a very small percentage of all bacteria (Ling et al., 2015). Therefore, the direct role of TCS on antibiotic resistance genes in nature and the impact on human health is unknown. It has not been defined if TCS causes cross-resistance to antibiotics in the environment. Meyer and Cookson (2010) suggest that cross-resistance in bacteria caused by exposure to triclosan is case-dependent. There are several variables, such as type of bacteria, concentrations of triclosan, and the mechanism bacteria use in different situations that factor into the phenomenon of cross-resistance. With this in mind, TCS does not always cause cross-resistance to antibiotics, but rather this phenomenon occurs occasionally (Saleh, Haddadin, Baillie, & Collier, 2010).

By identifying the impact of triclosan resistance in the environment, there would be a better understanding of how resistance genes impact human health. WWTPs contain a high density of bacteria, which facilitates the optimal conditions for proliferation and exchange of resistance genes (Carey et al., 2015). It is thought that TCS increases the abundance of resistance genes through selective pressure in these communities. Moreover, the transfer of resistance genes in the environment after the effluent is released from WWTPs is not well known. Transmission of resistance genes can occur through transformation, transduction, and conjugation. Transformation is the direct uptake of DNA; transduction used bacteriophages to cause viral infection; and conjugation utilizes

plasmids to transfer DNA. Understanding the rate of transfer among naturally derived antibiotic resistance genes and its threat on human health is necessary to determine if TCS plays a role on public health.

In conclusion, this is an ongoing study and 200 more isolates need to be characterized to provide a more compensative picture of cross-resistance around wastewater treatment plants.

Table 2. Antibiotic resistance genes found in bacteria and the corresponding resistance mechanisms.

Antibiotic	Mode of Action	Example Resistance Gene	Resistance Mechanism	References
Tetracycline	Inhibits protein synthesis	tetA	An efflux pump expels tetracycline across the membrane back into the environment.	(Schnappinger & Hillen, 1996)
Erythromycin	Inhibits protein synthesis	ErmB	Codes for different methylases which result in erythromycin resistance	(Seppälä, Skurnik, Soini, Roberts,& Pentti, 1998)
β-lactams	Inhibits cell wall synthesis	blaSHV	Codes for β-lactamases which disrupt the four-membered β-lactam ring	(Cheong, Ho, Choo, & Chew, 2014; Farlex, 2012)
Ciprofloxacin	Inhibits DNA replication by targeting DNA gyrase	gyrA	Point mutations result in amino acid changes	(Schmitz et al., 1998)
Sulfamethoxazole	Inhibits dihydropteroate synthase	*sul 1*	Codes for dihydropteroate synthase which is resistant to sulfonamides	(Heuer & Smalla, 2007)
Trimethoprim	Inhibits dihydrofolate reductase	*dfrA*17	Codes for dihydrofolate reductase which is not sensitive to trimethoprim	(Lee et al., 2001)

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