Determining Antibiotic Resistance in the Berks County Environmental and Clinical Settings

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Introduction

Antibiotic resistance (AR) refers to a phenomenon where pathogens, such as bacteria, become resistant to the effects of drugs and chemicals due to repeated exposure (*Antibiotic Resistance,* 2020). AR occurs as a natural process in which traits that improve survival, such as resistance to antibiotics, are selected for over time. Bacteria gain resistance through beneficial mutations, and/or the incorporation of resistant genes in their genomes (Martinez & Baquero, 2000). The overuse of antibiotics in clinical and agricultural settings accelerates this natural process of AR in bacteria (*Antibiotic Resistance,* 2020).

Implications of Antibiotic Resistance

Frieri et al., 2017 found that liberal and unnecessary use of broad-spectrum antibiotics results in AR and is associated with high morbidity and mortality When early detection of causative bacteria is lacking, infection control practices are poor, and/or the treatment regimen is left incomplete, AR in bacteria accelerates. This might be because antibiotic-resistant genes (ARG) of the pathogens are present in a reservoir called the resistome, from where pathogenic bacteria can acquire resistance via horizontal gene transfer (HGT) (Frieri et al., 2017).

In their 2016 study, Friedman et al. discussed that infections caused by antibiotic-resistant bacteria (ARB) have around a two-fold higher rate of adverse outcomes, both clinical and economic. Clinical adverse outcomes include complications, treatment failure, or death, and economic adverse outcomes include increased costs of care and longer stays in hospitals. The authors also discussed that primary reasons for treatment failure linked with infections caused by ARB include delayed treatment, lack of effective therapy, bacterial fitness, and more severe illness (Friedman et al., 2016).

Pathogens that Become Resistant

Although many pathogens can become resistant to the effects of antibiotics, certain bacteria cause human disease much more commonly than others in the environment and clinical settings. These include *Escherichia coli* (*E. coli*), *Enterococci species,* and *Staphylococcus aureus (S. aureus).* Resistance in these bacteria is discussed below:

I. *Escherichia coli (E. coli)*

In their 2013 review, Dr. Korzeniewska et al. discussed the works of many scientists who detected AR in *E. coli* strains. Feuerpfeil and Stelzer, in 1992, found that 80% of the samples from

the feces of healthy individuals consisted of bacteria that were resistant to at least some antibiotics. Silvia et al. (2007) saw a higher frequency of multiple drug-resistant bacteria in treated water than in untreated water, suggesting that wastewater treatment plants consist of a reservoir of ARB that transfers ARG to other bacteria. For example, when *E. coli* develops resistance, it is most commonly to the beta-lactam class of antibiotics (like penicillin), by producing extended-spectrum beta-lactamases (ESBLs), which are enzymes that break down beta-lactams. ESBLs are encoded by ARG.

According to the 2019 AR Threat Report released by the Center for Disease Control and Prevention (CDC), ESBL-producing *E. coli* caused 200,000 infections that cost the healthcare system $1.2 billion and led to 9,000 deaths in 2017. Increasing ever since 2012, such infections are mostly community-associated or community-onset, The CDC has labeled these infections a "serious threat" (*2019 AR Threats*, 2019), and reported that in 2019, multi-drug resistant *E. coli* were detected in 6.6% of the isolates collected from Pennsylvania, while nationally, the resistance is closer to 9.6% (*Antibiotic Resistance,* 2020).

II. *Enterococci species*

In a Chinese research study by Jia et al. (2014), resistance to many antibiotics in strains of enterococcus was found. The isolates were collected from a hospital and primarily consisted of *E. faecium* but also *E. faecalis*, *E. casseliflavus*, *E. avium*, and other *Enterococcus* species. In *E. faecium*, the resistance to antibiotics such as penicillin, ampicillin, rifampicin, ciprofloxacin, levofloxacin, fosfomycin, erythromycin, and furadantin, was detected to a significantly higher frequency than in *E. faecalis*. On the other hand, *E. faecalis* showed significantly greater resistance to chloramphenicol, quinupristin/dalfopristin, minocycline, and tetracycline than *E. faecium.* This finding suggests that different strains of bacteria have different mechanisms to fend off the effects of different drugs attributed to the β-lactamase gene *TEM, tetM, ermB, vanA, and emeA.* (Jia et al., 2014).

According to the 2019 AR Threat Report released by the CDC, Vancomycin-resistant Enterococci (VRE) caused 55,000 infections that cost the healthcare system $539 million and led to 5,400 deaths in 2017. Fortunately, effective infection control and appropriate antibiotic use have led to a decline in these infections ever since 2012. The CDC labeled these infections a "serious threat" (*2019 AR Threats,* 2019). In 2019, it was reported that VR *E. faecium* and *E. faecalis* were detected in 67.2% and 3.0% of the isolates collected from Pennsylvania, respectively, while nationally, the resistance is closer to 65.1% and 4.2%, respectively (*Antibiotic Resistance,* 2020).

III. *Staphylococcus aureus (S. aureus)*

Lakhundi & Zhang (2018) described that after the development of the synthetic antibiotic methicillin in the 1950s, methicillin-resistant *S. aureus* (MRSA) was identified in 1960. *S. aureus* has been found to quickly adapt and attain resistance to many drugs, making it a "superbug." MRSA strains produce a penicillin-binding protein (PBP) which is encoded by the *mecA* gene and degrades the ability of penicillin to bind to the bacteria. The gene is present on the mobile genetic element (MGE) that allows for it to be acquired by other, susceptible strains. One study found that 20-50% of *Staphylococcus aureus* infections in hospitals are due to MRSA strains (Lakhundi & Zhang, 2018).

According to the 2019 AR Threat Report released by the CDC, Methicillin-resistant *Staphylococcus aureus* (MRSA) caused 323,700 infections that cost the healthcare system $1.7

billion and led to 10,600 deaths in 2017. Fortunately, effective infection control and appropriate antibiotic use have led to a decline in these infections ever since 2012. The CDC labeled these infections a "serious threat" (*2019 AR Threats*, 2019), and reported that in 2019, MRSA was detected in 37.9% of the isolates collected from Pennsylvania, while nationally, the resistance is closer to 40.6% (*Antibiotic Resistance*, 2020).

Antibiotics to which Pathogens Become Resistant

In recent years, many pathogens have become resistant to the effects of drugs such as penicillin, tetracycline, and nitrofurantoin. The project focuses primarily on these antibiotics, and they will be discussed at length.

I. Tetracycline

Discovered in 1948 from Actinomycetes soil bacteria, the tetracycline (Fig. 1) family works against both Gram-positive and negative pathogens (Nelson & Levy, 2011). It was first used by Dr. Duggar in Lederle Laboratories and then later by the scientists at Pfizer (Grossman, 2016). More variations, both natural and semi-synthetic, soon followed as seen in Fig. 2.

According to Shutter & Akhondi (2022), tetracyclines are a class of broad-spectrum medications used to treat bacterial infections primarily through the mechanism of inhibiting protein synthesis by binding to the 30S ribosomal subunit. Since the drugs result in the loss of bacterial ability to replicate or grow without destroying them, tetracyclines are labeled bacteriostatic agents.

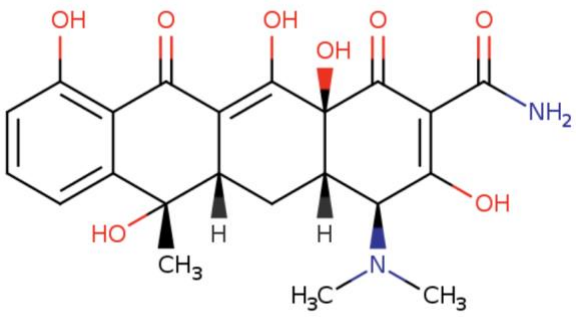

Figure 1: The basic structure of a tetracycline compound which includes polyketides with an octahydrotetracene-2-carbinoxamine skeleton structure and several hydroxy, amino, and methyl substituents ("CHEBI:26895 - Tetracyclines," 2017).

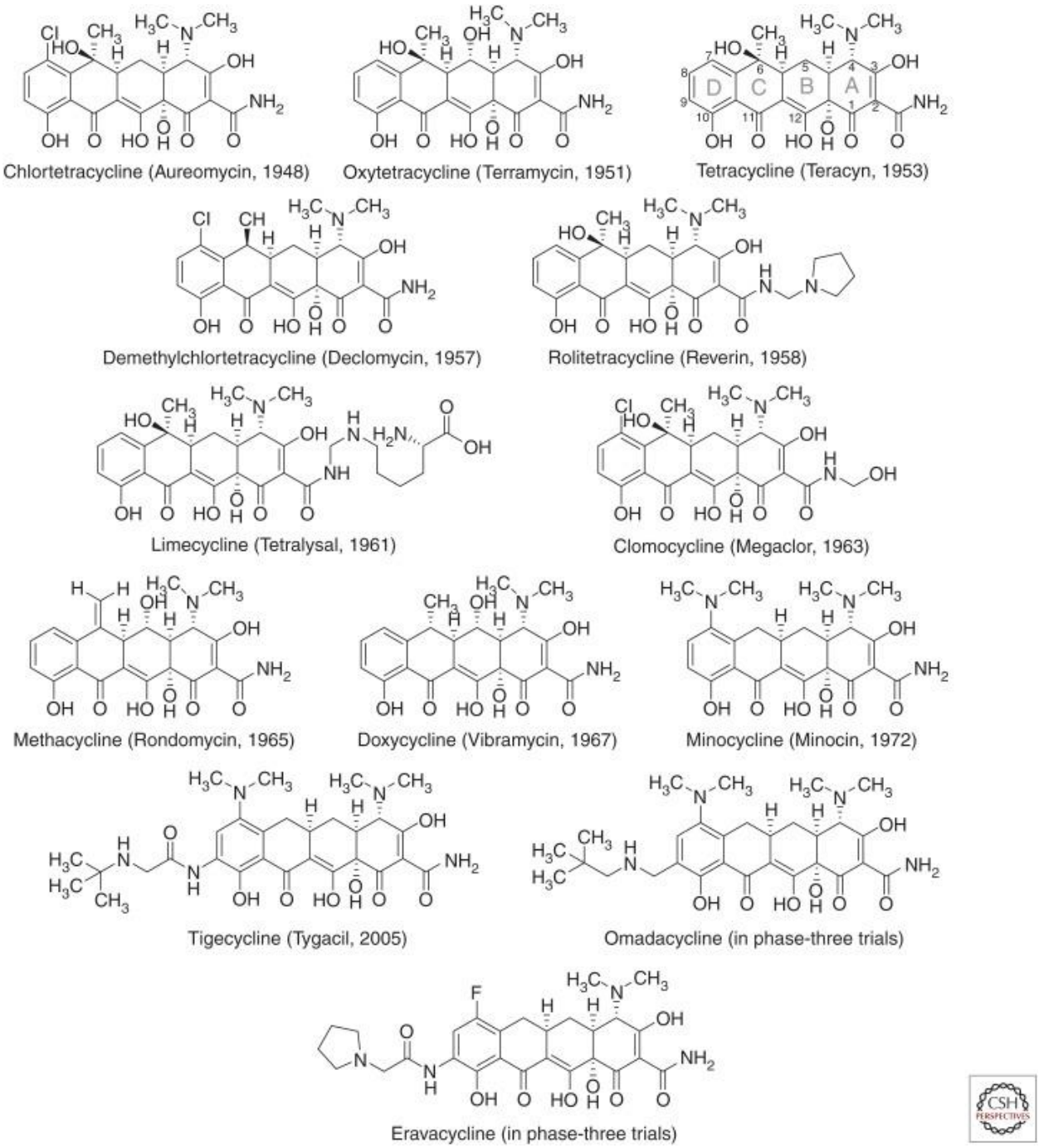

Figure 2: The structures and generic names of many clinically used and development candidates of tetracyclines (Grossman, 2016). Some of the synthetic tetracyclines were developed because resistance to naturally occurring tetracyclines emerged in bacteria, and the need for more optimal variations became a priority (Grossman, 2016).

There are a couple of mechanisms by which bacteria can develop tetracycline resistance, according to the 2016 study by Dr. Trudy H. Grossman. First, mobile genetic elements that carry tetracycline-resistance genes are exchanged between bacteria. Next, mutations within the ribosomal binding site of the bacteria could result in a loss of effectiveness of the tetracyclines.

Finally, chromosomal mutations could cause an increase in intrinsic resistance by altering the expression of key pumps/porins or activators/repressors.

II. Ampicillin

According to Raynor (1997), penicillin is one of the best-known antibiotics, and it was discovered in 1928. Ampicillin (Fig. 3) is derived from penicillin through the addition of an amino group to the benzylpenicillin molecule. This addition increased the antibacterial activity. Ampicillin exhibits a bactericidal effect, as it disrupts the synthesis of the cell wall of Gram-positive and Gram-negative bacteria. This occurs as the β-Lactam region of the drug binds to the penicillin-binding proteins (PBPs) of the bacteria, which results in the transpeptidation reaction being inhibited. The inhibition blocks the synthesis of peptidoglycans, which are crucial for cell-wall synthesis. Lacking a cell wall, the cell dies (Raynor, 1997).

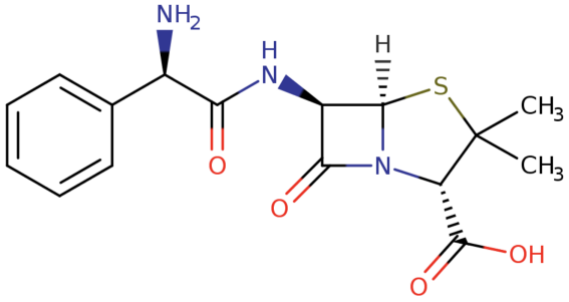

Figure 3: The basic structure of a penicillin compound has a penam ring with a 2-amino-2-phenylacetamido group at the 6th position ("CHEBI:28971 - Ampicillin," 2019).

Swami (2014) postulated that widespread use of ampicillin resulted in resistance, and such resistance occurred via three main mechanisms. First, some bacteria have reduced the access of ampicillin to the PBPs. Next, some prokaryotes have undergone a reduced binding affinity between ampicillin and PBPs. Finally, the synthesis of β-lactamase in some prokaryotes results in the degradation of the β-Lactam of ampicillin (Swami, 2014).

III. Nitrofurantoin

Huttner et al. (2015) reported that nitrofurantoin, a bactericide agent, was approved by the FDA in 1953 to treat urinary tract infection (UTI) caused by both Gram-positive and negative pathogens. Although widely prescribed for a while, its popularity declined in the 1970s because of other, more effective antibiotics. In the 2000s, with the rise of ESBL-producing bacteria, nitrofurantoin was repositioned as the first line of defense against UTIs. By binding to bacterial ribosomes and inhibiting several key enzymes, nitrofurantoin acts as an antimicrobial agent (Huttner et al., 2015).

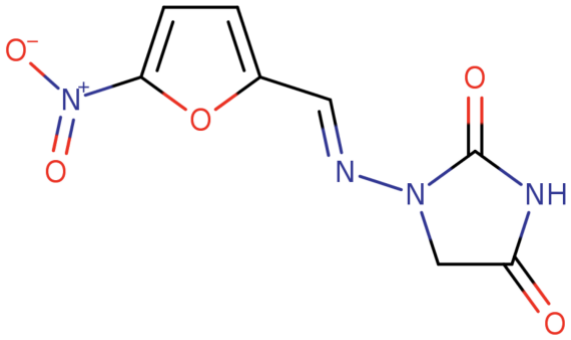

Figure 4: The basic structure of nitrofurantoin consists of an imidazolidine-2,4-dione that has a [(5-nitro-2-furyl)methylene]amino group substitution (CHEBI:71415 - Nitrofurantoin, 2017).

According to Huttner et al. (2015), the acquisition of resistance against nitrofurantoin is relatively infrequent because of the multiple modes of action. When intracellular nitroreductase activity is lost due to mutation, resistance occurs. Given its increased use recently, a rise in nitrofurantoin resistance in bacteria is likely (Huttner et al., 2015).

My Bench Research in a Microbiology Lab at PSU

I. Background

In my three years of study at Pennsylvania State University (PSU), Berks Campus, I have been engaged in a laboratory study with Dr. Tami Mysliwiec and Prof. Jill Felker. The study focuses on the presence of antibiotic resistance in bacteria found in the Blue Marsh Lake Watershed. Three sampling locations were chosen throughout the watershed. The first, henceforth called the ‘upstream’ site, is in close proximity to an agricultural area and therefore receives agricultural runoff. Next, the Blue Marsh Lake (‘lake’) is the recipient of pollutants from the human population that uses the area for recreational purposes. Finally, the ‘downstream’ site is surrounded by businesses and prone to industrial discharge. Figure 5 depicts the location of the sites relative to one another in Berks County, PA, and Figures 6-8 show images of individual sample sites.

Figure 5: The location of the upstream, lake, and downstream sites relative to one another.

Figure 6: The upstream site that is close to the agricultural region (40.422280, -76.124502). Because of its proximity to farmland, the area receives many pollutants from the agricultural runoff including fertilizers, organic matter, and nutrients.

Figure 7: The lake site that is used for recreational purposes (40.384559, -76.042395). Because humans use this site for boating, swimming, or fishing, many contaminants (chemicals, metals, and plastics) can be found here.

Figure 8: The site that is downstream from the industrial region (40.369511, -75.975224). The chemical and industrial waste from the hospitals and factories near this region often ends up at this site.

II. Methodology

Every month, water and sediment samples were collected for chemical and microbiological testing. Sampling and testing followed EPA-established protocols for recreational water. *Escherichia coli* and *Enterococci spp*. counts were collected using membrane filtration procedures, following the manufacturer's recommendations. Kirby Bauer disk diffusion methodology was used to determine the antibiotic susceptibility to six classes of antibiotics in approximately 10% of the isolated colonies from collected samples. Genomic DNA was isolated from sediment samples. Total gDNA was analyzed for antibiotic-resistant genes using PCR analysis. qPCR was employed to quantitate antibiotic-resistance genes in the microbiome of the sediment samples.

III. Results

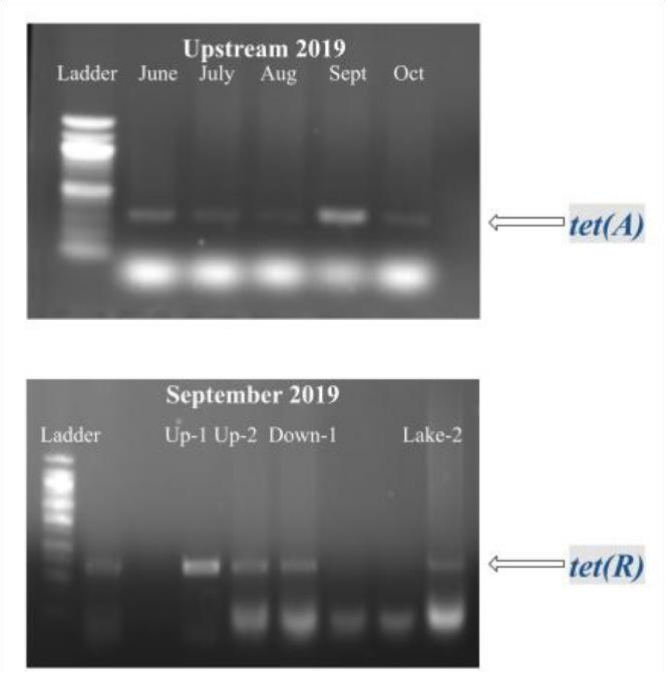

Figure 9. PCR amplification and gel electrophoresis of genomic DNA from sediment samples detected tetracycline-resistant genes. In the gDNA, the primers specific to the *tet(A)* gene identified a 405 bp fragment, and the *tet(R)* gene identified a 379 bp fragment.

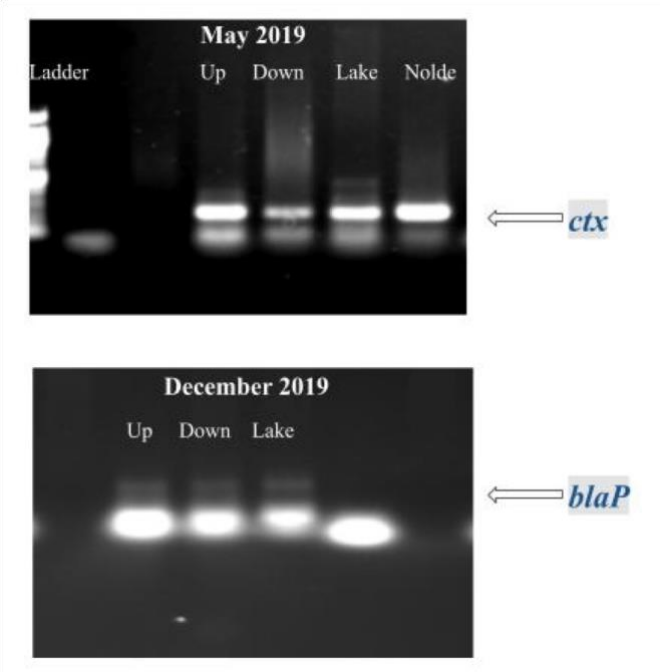

Figure 10. PCR amplification and gel electrophoresis of genomic DNA from sediment samples detected ampicillin-resistant genes. In the gDNA, the primers specific to the *ctx* gene identified an approximately 200 bp fragment, and the *blaP* gene identified an approximately 300 bp fragment.

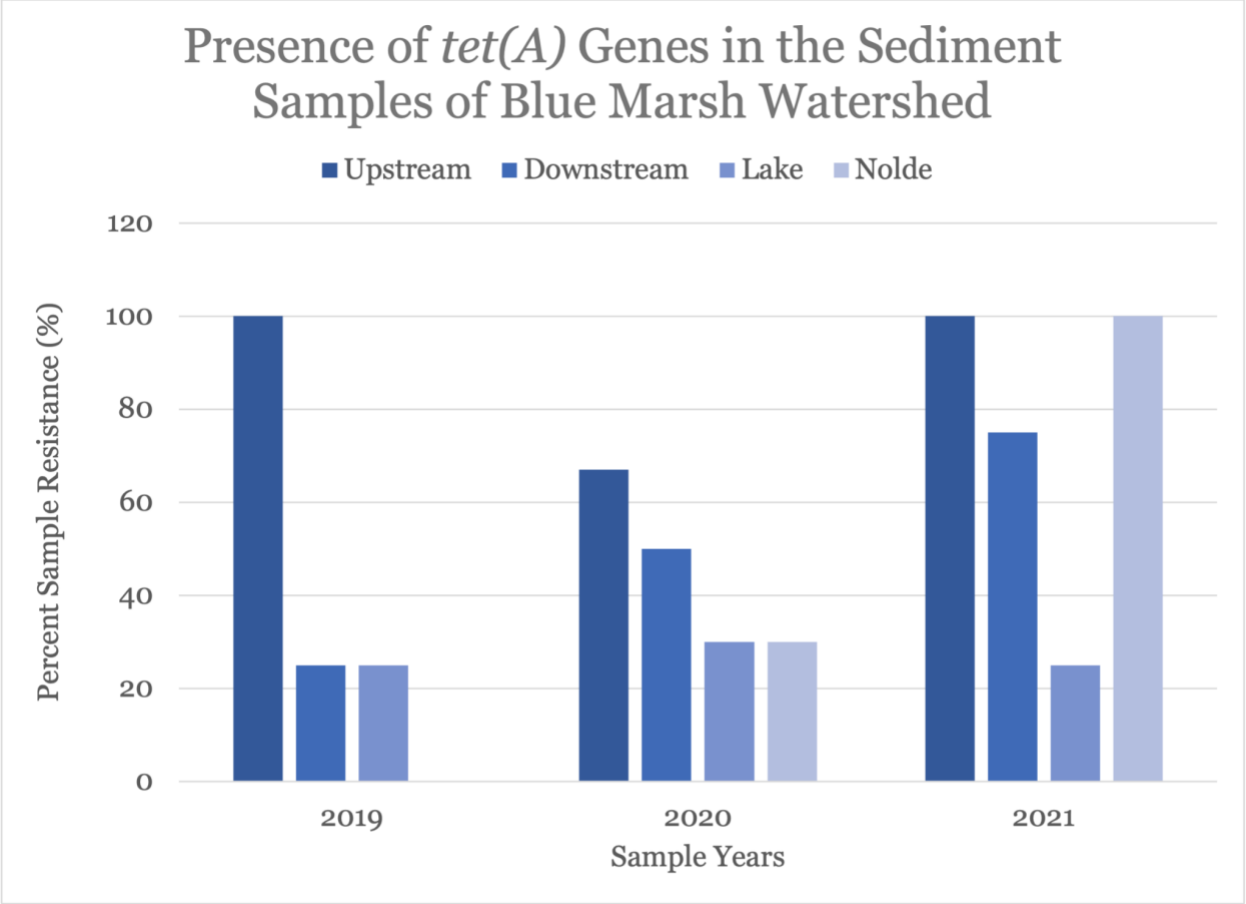

Figure 11. Quantification of the detection and prevalence of antibiotic-resistance gene *tet(A)* in the samples analyzed.

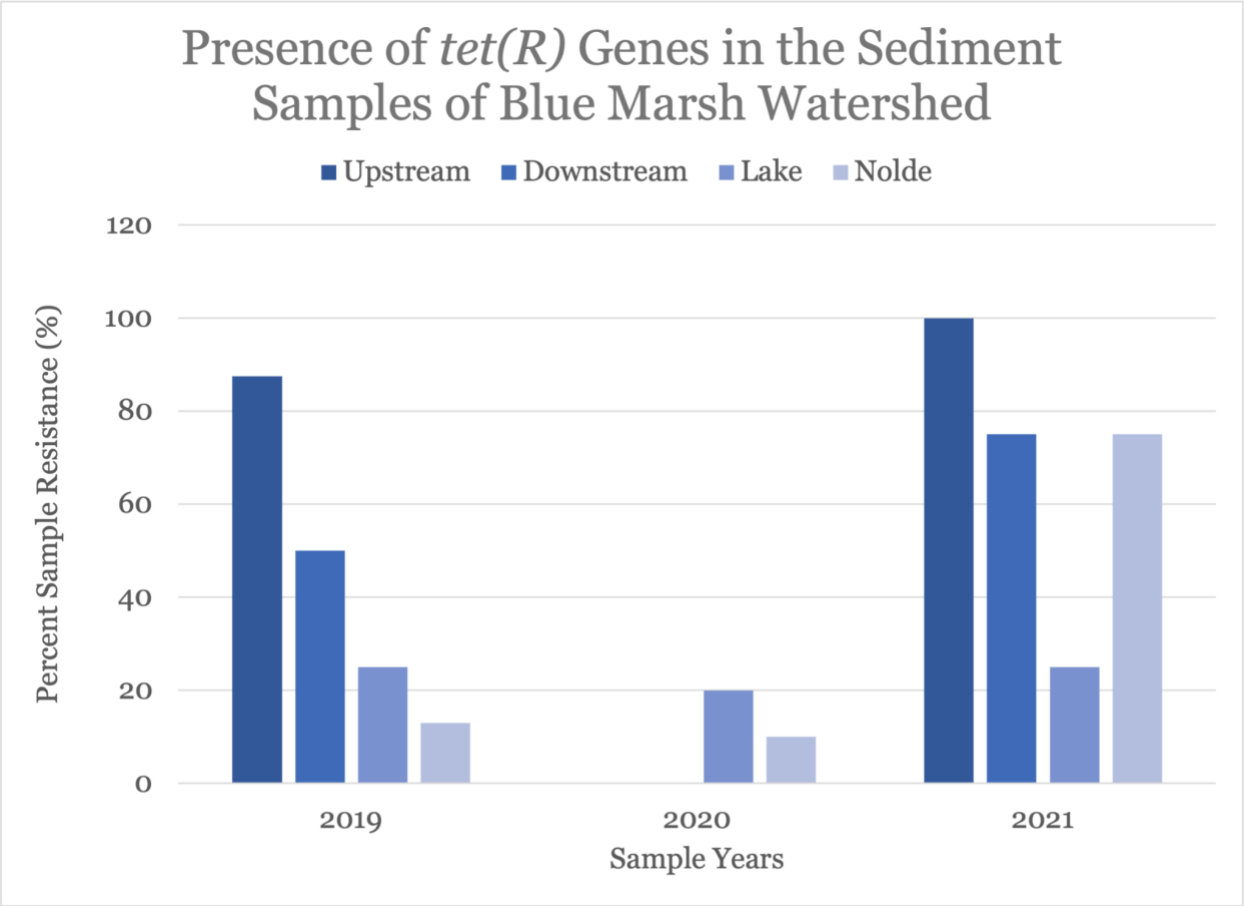

Figure 12. Quantification of the detection and prevalence of antibiotic-resistance gene *tet(R)* in the samples analyzed.

Table 1. Fold-changes in *Enterococcus* tetracycline-resistance gene *tet(A)* from 2019 to 2020.

2019-2020	Upstream	Downstream	Lake	Nolde
Winter	nd	+++	nd	nd
Spring	+	+/-	nd	nd
Summer	+	+/-	nd	+
Fall	nd	nd	+++	nd

Table 2. Fold-changes in *Enterococcus* tetracycline-resistance gene *tet(A)* from 2020 to 2021.

2020-2021	Upstream	Downstream	Nolde
Winter	+	-	+/-
Spring	+	++	nd
Summer	nd	+++	nd
Fall	nd	+/-	-

Table 3. Fold-changes in *Enterococcus* tetracycline-resistance gene *tet(R)* from 2019 to 2020.

2019-2020	Upstream	Downstream	Lake	Nolde
Winter	++	+++	++	nd
Spring	+	+/-	+++	nd
Summer	+	+	+	+
Fall	+	-	+++	+++

Table 4. Fold-changes in *Enterococcus* tetracycline-resistance gene *tet(R)* from 2020 to 2021.

2020-2021	Upstream	Downstream	Nolde

Winter	+++	+++	+
Spring	+	+	nd
Summer	+	nd	+
Fall	+/-	+	+/-

Legend:	
-	**0-1**
+/-	**1-10**
+	**10-100**
++	**100-1,000**
+++	**>1,000**
nd	**Not detected**

Figure 13: Legend to read the fold changes in Tables 1-4

IV. Conclusion

Chemical and microbial analysis of sediment samples, performed by other students in our laboratory, revealed significant seasonal variability for levels of certain pollutants. Nitrate and phosphate levels were elevated for all sites in fall and spring, and the sites had *E. coli* and *Enterococci* counts higher than the EPA recommended criteria about 36% of the time. Additionally, more than 80% of *E. coli* and 60% of *Enterococci spp*. were resistant to at least one antibiotic. PCR analysis revealed that the genome of the microorganisms in the upstream and downstream sample sites contain tetracycline-resistant genes, tet(A) and tet(R), as well as ampicillin-resistant genes, ctx, oxa and blaP. Tetracycline- and ampicillin- resistance was found—55.3% of *tet(A)*, 42.7% of *tet(R)*, and 91.7% of *ctx* resistance were found in all samples analyzed.

Antibiotic Resistance in Berks County

I. Background

The microbiology laboratory at the Reading Hospital receives specimens from in-patient and out-patient settings for microbial identification and antibiotic susceptibility testing. For the purposes of this study, Dr. Debra Powell, the infectious disease specialist at Tower Health, provided me with these data sets on antibiotic resistance found in the clinical settings locally.

II. Methodology

The following data analysis is conducted on the isolates that came from various body parts of adult patients; duplicates of isolates from the same patient were excluded. The antibiogram data from the hospital shows the percentage of isolates that are "susceptible" to specific antibiotics since identifying antibiotics likely to treat an infection successfully is the most clinically relevant information for healthcare providers. Since my bench research was focused on monitoring the presence of genes conferring antibiotic resistance, in my search for correlations with this clinical data, I derived a figure for the percent of cultured isolates categorized as resistant. I did this by subtracting the percentage of susceptible isolates from 100. The percentage of resistant isolates thus derived is inflated to some extent because it includes those isolates classified as 'intermediate' by the clinical lab. The intermediate classification is applied to isolates whose response to a specific antibiotic indicates that the organism might not be successfully treated with the antibiotic at the dosages normally used. In the hospital lab data analyzed for this study, the number of isolates classified as an intermediate was close to zero for those tested against tetracycline and ampicillin. The numbers were higher for nitrofurantoin.

III. Results

A. Ampicillin

Between the years 2016-2021, the percentage of bacterial isolates classified as resistant to ampicillin was higher compared with other antibiotics. During the same time period, an average of 0.2% of the tested isolates were resistant to ampicillin in *Enterococcus faecalis*, and an average of 24.8% of resistance was found among the isolates of Vancomycin-resistant, or VRE, strains of *Enterococcus faecalis*. In *Escherichia coli*, resistance hovered around 39.2% of the isolates. Finally, while the average resistance in *Enterococcus faecium* isolates was 53.8%, in VRE *Enterococcus faecium,* the resistance was significantly higher, with an average of 98.4%. The trends over the years of ampicillin resistance in different bacterial species are shown in Figure 14.

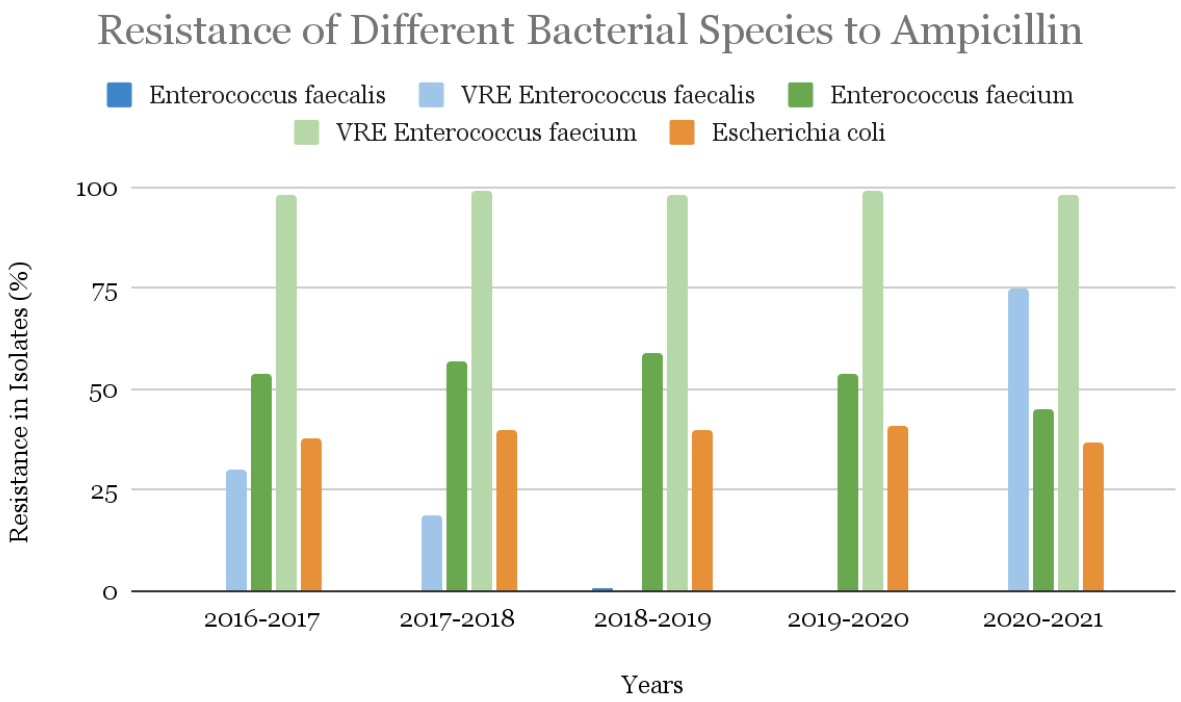

Figure 14: Ampicillin-resistance in isolates of five bacterial strains, *Enterococcus faecalis*, Vancomycin-Resistant (VRE) *Enterococcus faecalis, Escherichia coli, Enterococcus faecium,* and VRE *Enterococcus faecium* from years 2016 to 2021.

B. Tetracycline

For tetracycline, resistance in once-susceptible bacteria is high. In 21.0% of isolates of *Escherichia coli*, resistance was detected. Significantly higher resistance (in about 62.6% of the isolates) was found in Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*. Next, methicillin-resistant *Staphylococcus aureus* (MRSA) demonstrated resistance in about 5.0% of the isolates, while methicillin-sensitive *Staphylococcus aureus* (MSSA) had about 3.0% of the isolates showed resistance. The trends over the years of tetracycline resistance in these different bacterial species are shown in Fig. 15.

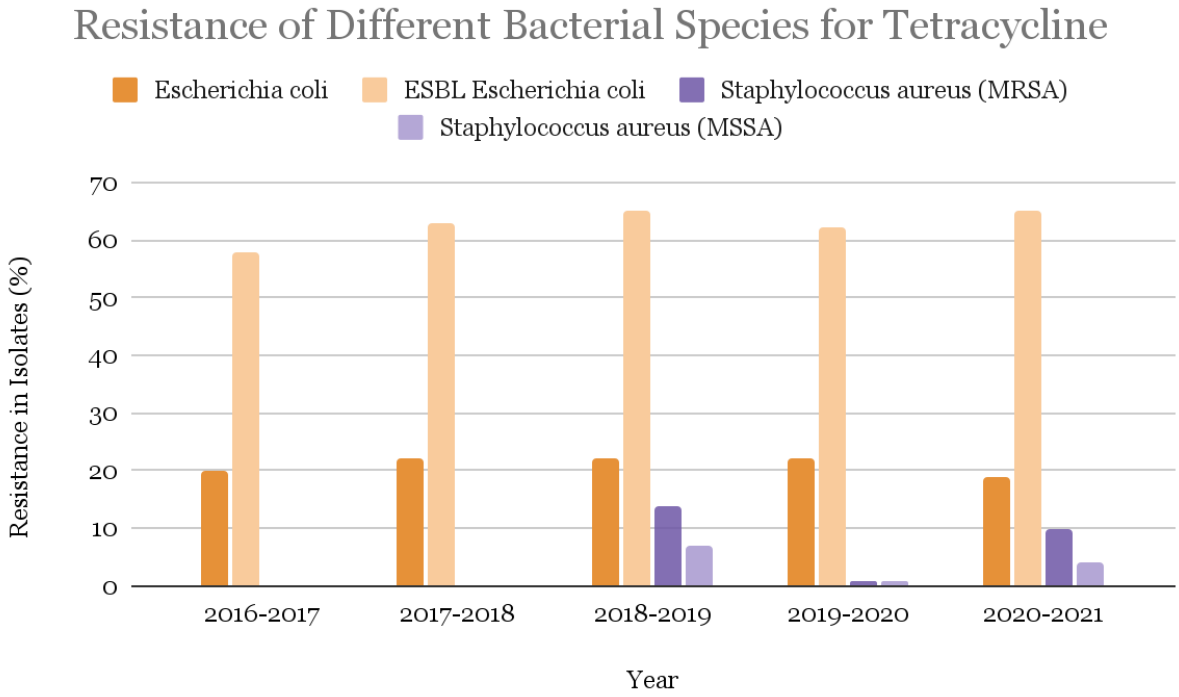

Figure 15: Tetracycline-resistance in isolates of four bacterial strains, *Escherichia coli*, Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), and Methicillin-Sensitive *Staphylococcus aureus* (MSSA) from years 2016 to 2021.

C. Nitrofurantoin

Nitrofurantoin resistance was found to be significantly lower in bacterial isolates when compared to other antibiotics. Between 2016 and 2021, an average of 1.8% of the *Escherichia coli* and 8.4% of (ESBL)-producing *Escherichia coli* showed resistance. The Methicillin-sensitive *Staphylococcus aureus* (MSSA) isolates had no resistance, while only 2.3% of the Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were found to have resistance to nitrofurantoin. Finally, *Enterococcus faecalis* and VRE *Enterococcus faecalis* had resistance in 0.3% and 7.8% of isolates, respectively. No statistical difference was found between the two. The trends over the years of nitrofurantoin resistance in these different bacterial species are shown in Fig. 16.

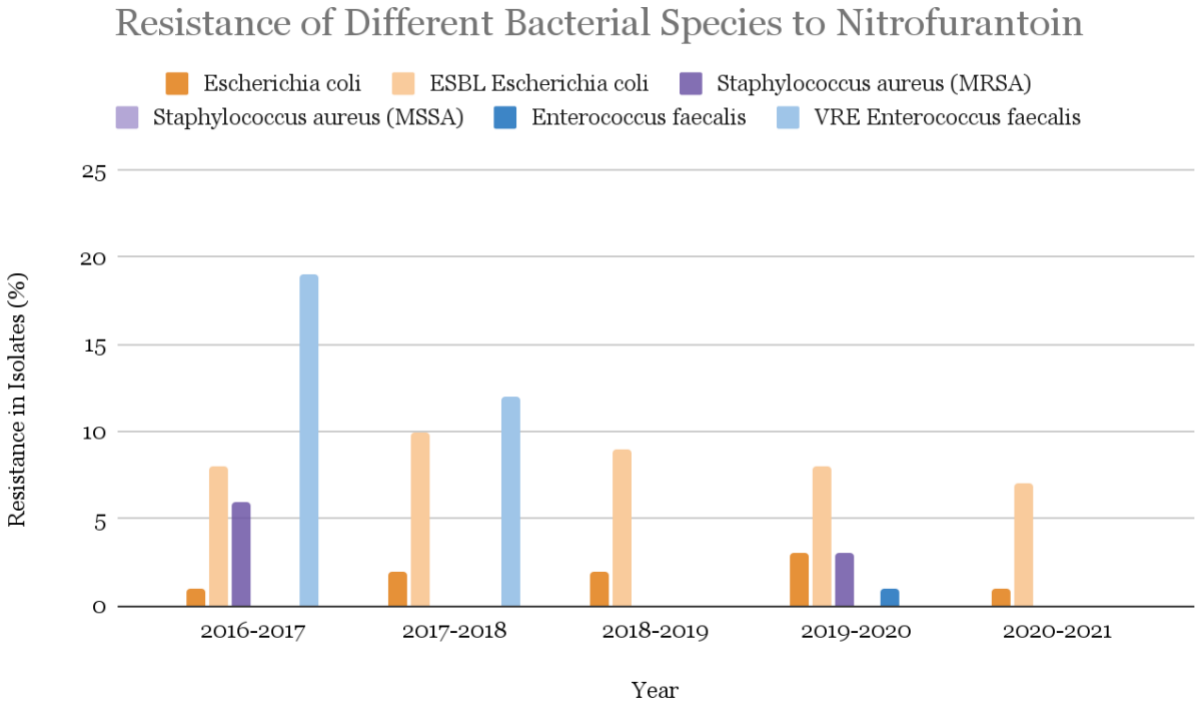

Figure 16: Nitrofurantoin-resistance in isolates of four bacterial strains, *Escherichia coli*, ESBL-producing *Escherichia coli*, *Staphylococcus aureus* (MRSA), and *Staphylococcus aureus* (MSSA), *Enterococcus faecium*, and VRE *Enterococcus faecium* from years 2016 to 2021.

IV. Conclusion

As noted earlier, the research on environmental isolates in the Blue Marsh Watershed showed an increase in the prevalence of antibiotic-resistant genes since 2019. However, susceptibility (and therefore resistance) patterns over the last few years in the clinical setting at Reading Hospital have been relatively stable. This stability suggests that the Antibiotic Stewardship Program (ASP) at the Reading Hospital is having a beneficial effect on the prevalence of resistant infections in the Reading Hospital patient population. Also, the isolates for each year are dependent upon the number of patients with bacterial infections, so the variability of resistance from year to year may be explained by the unequal number of isolates collected and tested each year.

Discussion

In conclusion, the increasing prevalence of ARB is a serious global and public-health threat. According to the World Health Organization (WHO), AR negatively impacts the ability of physicians and medical professionals to treat common infections, which in turn, leads to an increased infection rate, longer duration of illness, expensive and scarce treatment options, and poorer outcomes (*Antibiotic Resistance*, 2020). Therefore, the WHO has declared AR one of the most pressing public health issues of the 21st century. Some studies have even estimated that by 2050, over 10 million will die each year worldwide due to drug-resistant infections (Walsh, 2014).

As multidrug resistance patterns are on the rise, Frieri et al. (2017) prompted actions to devise antibiotic stewardship programs in clinical settings and the development of novel therapies that bypass the use of antibiotics altogether. Similarly, Friedman et al. (2016) proposed several changes, such as improving infection control precautions, broadening the antibacterial therapies, using combination therapy, primary source control, and expediting the microbiological test result reporting (Friedman et al., 2016). Finally, both Swami (2014) and Grossman (2016) agreed that because bacteria have increasingly acquired resistance to the older forms of these antibiotics, the only viable way to keep using tetracyclines and ampicillins is through the continued development of new synthetic variants. It is unclear how long this approach can be sustained, and therefore ways to directly disable the antibiotic-resistant mechanism need to be explored. It can be hypothesized that a reduction in the use of antibiotics in clinical and agricultural settings, without withholding treatment when appropriate, could relieve the selective pressure for mutants with resistance, resulting in the re-establishment of the sensitivity to these antibiotics (Grossman, 2016; Swami, 2014). Finally, Antibiotic Stewardship Programs, such as the one implemented at Reading Hospital, are a vital tool for controlling antibiotic resistance and should continue to be supported and promoted.

Safitaj Sindhar will graduate from Penn State Berks in December 2022 as a biology major with a minor in psychology. She is applying to medical schools this year, with a long-term goal of working internationally to improve access to quality healthcare.

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