

Impact of Agricultural and Waste Water Treatment Facility Runoff on the Incidence of Antibiotic Resistant Bacteria in Streams

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ABSTRACT

Due to increased usage of antibiotic drugs over the past few decades, researchers are finding increasing proportions of bacteria in the environment that are resistant to antibiotics. Areas that are especially affected include streams that receive runoff from farms utilizing antibiotic drugs in their animal feed and from waste water treatment facilities. The goal of this study was to determine if these types of pollution are causing an increase in populations of antibiotic resistant bacteria in streams. Water was collected from three points along a stream receiving runoff from agricultural areas and from points above, at, and below the outflow pipe of a waste water treatment facility. Water was also collected from a location geographically removed from these pollution sources. Bacteria filtered from the water samples were plated on media selective for the growth of coliforms or media selective for the growth of *Acinetobacter*. Colonies picked from these plates were grown on media containing ampicillin, chloramphenicol, norfloxacin, streptomycin, or tetracycline, or no antibiotic. Susceptibility or resistance to antibiotics was determined by comparing the percentage of colonies that grew on media with and without antibiotic. The number of coliform bacteria resistant to ampicillin was significantly higher at the waste water treatment facility outflow pipe than upstream of the outflow. Greater numbers of coliforms and *Acinetobacter* resistant to chloramphenicol, streptomycin, and tetracycline were also found at and below the outflow compared to upstream. Agricultural runoff seems to contribute to an increase in the number of coliform bacteria resistant to ampicillin, streptomycin, and tetracycline, and to the number of *Acinetobacter* resistant to tetracycline. These results appear to indicate that the use of antibiotics in both agriculture and in humans is increasing the incidence of antibiotic resistant bacteria in lotic environments.

INTRODUCTION

Antibiotics are a vital part of our medical care system and save countless lives every day from infections that were untreatable before the introduction of these drugs. Unfortunately, antibiotics also select for mutant bacteria that are resistant to concentrations of previously effective drugs. Antibiotics have become so overused in clinical settings and as prophylactics and growth stimulators in farm animals that many bacteria have developed resistances to such an extent that new antibiotics and higher concentrations of older drugs, which approach levels toxic to the human system, are necessary to treat infections (Houndt and Ochman, 2000). Recent studies suggest a connection between antibiotics used in agriculture and antibiotic-resistant infections in humans (Chee-Sanford et al, 2001). The development of antibiotic resistant bacterial strains can lead to serious human health problems, both from life-threatening infections and from toxic levels of drugs.

A wide range of antibiotic resistant bacteria have been isolated from polluted environmental samples including *Acinetobacter* spp. (Guardabassi et al, 1998, 1999 and 2000) and members of the family Enterobacteriaceae (Freney et al, 1988, Parveen et al, 1997, Mazel et al, 2000, and Goñi-Urriza et al, 2000). *Acinetobacter* are gram-negative

bacteria that occur in many different environments, including soil, water, sewage, food stuffs and on human skin (Guardabassi et al, 1999). They are typically resistant to β -lactam and aminoglycosidic antimicrobics and are possibly a reservoir of resistance genes in hospital environments, as well as being an increasingly common source of nosocomial infections (Jawad et al, 1994). *Acinetobacter* were chosen for examination in this study as a gauge of antibiotic resistance in both polluted and unpolluted water samples because of their ubiquity and ability to acquire resistance genes, as well as their clinical importance (Guardabassi et al, 2000).

The Enterobacteriaceae encompasses a number of genera of bacteria isolated from humans and animals including *Klebsiella*, *Enterobacter*, *Serratia*, *Yersinia*, and *Escherichia*. Also included are the coliforms, which are gram-negative, lactose-fermenting bacteria that live in the intestines of humans and warm-blooded animals. Like *Acinetobacter* spp., members of the Enterobacteriaceae are common causes of nosocomial infections and some of these bacteria can pass resistance genes across species through plasmid exchange either in the gastrointestinal tract of their human hosts or in sewage systems (Parveen et al, 1997). The Enterobacteriaceae were chosen for this study because they are commonly found in polluted aquatic environments (Parveen et al, 1997) and for their importance as a cause of nosocomial infections (Freney et al, 1988).

The introduction of antibiotics into the environment causes a selective pressure which results in an increase in the proportion of bacteria that are resistant to antibiotics (Houndt and Ochman, 2000). Increased resistance to a wide variety of antibiotics has been found in bacteria located in streams receiving runoff from agricultural areas (Chee-Sanford et al, 2001) and higher frequencies of resistant bacteria have been found in streams receiving waste water from hospitals (Guardabassi et al, 1998) and sewage from cities (Ogan and Nwiika, 1993, Goñi-Urriza et al, 2000, and Parveen et al, 1997). Previous studies have shown that aquatic isolates of *Acinetobacter* collected at or downstream from the discharge point of waste water treatment facilities are resistant to chloramphenicol and oxytetracycline (Guardabassi et al, 1998 and 1999). A study of fecal coliforms collected from similarly polluted areas found bacteria resistant to ampicillin, chloramphenicol, tetracycline, and penicillin (Ogan and Nwiika, 1993) and another study found higher rates of multiple antibiotic resistant (MAR) bacteria in waters polluted with sewage treatment facility effluent (Parveen et al, 1997). This study aimed to investigate whether agricultural runoff and waste water treatment facility effluent are causing an increase in the proportions of antibiotic resistant bacteria in the Oneonta area. Three locations were studied, including an agricultural area of Charlotte Creek, the Susquehanna River above and below the waste water treatment facility, and Shelley Brook. These streams are at the headwaters of the Chesapeake Bay watershed and could be important contributors to reservoirs of antibiotic resistant bacteria in this watershed.

MATERIALS AND METHODS

Study Sites

Shelley Brook represents an area that should not have any introduced pollution. It is located on the upper-tract of Hartwick College's Pine Lake Environmental Campus in West Davenport, NY and due to this remote location it is not near any residences and has no other streams flowing into it. The only potential impact is from foot traffic from a hiking trail which crosses it. Charlotte Creek is impacted by non-point sources of pollution. Agricultural runoff is entering the stream, but it is difficult to tell exactly where this input

occurs, and how much effluent enters the water. Conversely, the Susquehanna River is impacted by a point source of pollution located at the outflow pipe from the waste water treatment facility. The outflow pipe is a specific point in the river where pollution enters the water. The exact volume of effluent and what is contained in it can be measured, and the impact downstream of this point can be compared to the conditions above the point source of pollution.

Water Sampling

Water samples were collected in 1 liter Nalgene bottles from each of seven sites. One sample was collected from Shelley Brook where it crosses the Jaycee Henderson trail on the Pine Lake Environmental Campus. Three samples were collected from Charlotte Creek along Charlotte Creek Road in Davenport, NY, each 50 meters apart. The final three samples were taken from the Susquehanna River in Oneonta, NY, one from 50 meters above the outflow pipe of the waste water treatment facility, one from the outflow pipe, and one from 50 meters below the outflow pipe. Samples were refrigerated over night and processed the following day.

Bacteria Selection

Bacteria were collected from the water samples by filtering through 0.45 μm pore filters. For the Shelley Brook samples 10 ml, 5 ml, and 1 ml volumes of the sample were filtered. For the Charlotte Creek and Susquehanna River water samples 1 ml of undiluted sample and 1 ml each of 10^{-1} and 10^{-2} dilutions were filtered. Higher volumes were filtered for Shelley Brook because it is an unpolluted stream and has fewer bacteria than Charlotte Creek or the Susquehanna River, for which smaller volumes resulted in similar numbers of colony forming units. The filters were placed on selective media and the plates were incubated over night at 37°C for coliforms and 25°C for *Acinetobacter*. MacConkey agar was used for the selection of coliforms, and Leed's *Acinetobacter* media (Jawad et al, 1994) was used for the selection of *Acinetobacter*.

Isolated colonies picked from the selection plates with a sterile toothpick were each transferred to one well of a 96-well plate containing 150 μl of Luria-Bertani broth. Only 48 wells of each 96 well plate were inoculated. The plates were incubated over night at 37°C . The following day cultures were prepared for freezing by addition of a drop of sterile glycerol to each well. Each plate was taped shut, placed into a Ziploc storage bag, and stored at -70°C .

Resistance Testing

Frozen stock cultures were used as the inoculum for antibiotic resistance testing. A sterile 48-prong replica-plater was dipped into the frozen stock cultures and then into the wells of a plate, each containing 150 μl of Luria-Bertani broth. The newly inoculated plates were incubated over night at 37°C . In order to dilute the cultures to an amount that would result in distinct colonies when plated, 5 μl of freshly grown culture was transferred from each well of the plates to 95 μl of sterile water in the corresponding well of a new plate. The diluted isolates were plated on Mueller-Hinton agar containing one of the five antibiotics at one of the three concentrations. The antibiotic plates were arranged in five rows by antibiotic. In each row was a control plate, three antibiotic plates in order of increasing concentration and a second control plate, for a total of twenty-five plates. The 48-prong replica-plater was dipped in the wells and stamped on each plate in succession to transfer the diluted inoculum from the microwell plate to the antibiotic and control plates. The replica-plater was re-inoculated between plates and sterilized between rows. The

process was repeated three times for a total of three replicates of each antibiotic set or seventy-five plates. The antibiotic plates were then incubated over night at 37°C. The antibiotics used were ampicillin (10, 30, and 50 µg/ml), chloramphenicol (10, 30, and 50 µg/ml), norfloxacin (20, 40, and 60 µg/ml), streptomycin (20, 30, and 50 µg/ml), and tetracycline (10, 30, and 50 µg/ml).

The proportion of resistant colonies was calculated as the number of colonies on an antibiotic plate divided by the number of colonies on the control plates. The data were analyzed using ANOVA and post-hoc tests in SPSS.

RESULTS

Resistances of Coliform Bacteria

At the Susquehanna River sites there was a significant effect of the waste water treatment facility effluent on the proportions of coliform bacteria that were resistant to ampicillin ($p < .001$), chloramphenicol ($p < .001$), streptomycin ($p < .001$), and tetracycline ($p < .001$) (Table 1). Increases in the proportions of resistant bacteria were found both at the outflow pipe and downstream of the outflow. The increase in the proportion of resistant bacteria was particularly striking for ampicillin (Figure 1). Upstream of the outflow 62% of the coliform bacteria were resistant to ampicillin at the lowest concentration and 23% were resistant at higher concentrations. At and below the outflow pipe over 95% of the coliform bacteria were resistant to all three concentrations of ampicillin. For chloramphenicol, streptomycin, and tetracycline no coliforms were resistant at the upstream site. At the outflow and downstream of the effluent input an increase of between 2% and 38% for chloramphenicol, between 4% and 26% for streptomycin, and between 2% and 30% for tetracycline was seen in the proportions of resistant bacteria. No coliforms were resistant to norfloxacin.

Between 23% and 58% of the coliform bacteria in Shelley Brook were resistant to ampicillin, depending on the concentration. The proportion of resistant coliforms was even higher at the Charlotte Creek sites where 26% to 92% were resistant to ampicillin (Figure 2). The proportion of coliforms resistant to chloramphenicol, streptomycin, and tetracycline was low across the Shelley Brook and Charlotte Creek sites.

The Susquehanna River site upstream of the waste water treatment facility outflow pipe was chosen for comparison to the Charlotte Creek sites in order to gauge the difference in the proportion of resistant bacteria between a river before a point of effluent impact and a stream impacted by non-point sources of pollution. It was found that the two upstream Charlotte Creek sites had significantly higher proportions of coliforms resistant to ampicillin than were present above the outflow in the Susquehanna River ($p < .001$, Figure 3). The proportion of resistant coliform bacteria in the Susquehanna River was between 23% and 62%, whereas the proportion resistant at the two Charlotte Creek sites was between 51% and 92%. The proportion of coliforms resistant to streptomycin was significantly higher at the downstream site of Charlotte Creek than at the upstream site of the Susquehanna River ($p < .001$, Figure 4). No coliform bacteria were resistant to streptomycin in the Susquehanna River site, but at the Charlotte Creek site between 6% and 12% of the coliforms were resistant to streptomycin.

Resistances of Acinetobacter

Almost 100% of the *Acinetobacter* in the Susquehanna River were resistant to ampicillin. Between 37% and 65% of the *Acinetobacter* were resistant to streptomycin, and only about 2% of the bacteria were resistant to norfloxacin. Significant increases in the proportion of *Acinetobacter* resistant to chloramphenicol ($p < .001$, Figure 5) and tetracycline ($p = .014$) were seen in the outflow and downstream sites of the Susquehanna River.

One hundred percent of the *Acinetobacter* in Shelley Brook were resistant to ampicillin. Between 82% and 96% of the *Acinetobacter* were resistant to chloramphenicol and between 84% and 96% were resistant to streptomycin. Lower proportions of these bacteria were resistant to norfloxacin and tetracycline. In Charlotte Creek between 97% and 100% of the *Acinetobacter* were resistant to ampicillin, between 81% and 95% were resistant to chloramphenicol, and between 47% and 71% were resistant to streptomycin. Lower proportions of *Acinetobacter* were resistant to norfloxacin and tetracycline. Shelley Brook was used as a basis for comparison to Charlotte Creek because it is a more pristine area that should not be heavily impacted by agricultural pollution. In Charlotte Creek only resistance to tetracycline was significantly different from Shelley Brook ($p < .001$).

When the Charlotte Creek sites were compared to the upstream site of the Susquehanna River in the same manner as was done for the coliforms, significantly higher proportions of *Acinetobacter* resistant to both chloramphenicol and streptomycin were found in Charlotte Creek. For chloramphenicol, all three Charlotte Creek sites had higher proportions of resistant *Acinetobacter* ($p < .001$, Figure 6) and for streptomycin, the two Charlotte Creek sites farthest upstream had higher proportions of resistant bacteria ($p < .001$ and $p = .006$, respectively, Figure 7).

Table 1. Results of a univariate ANOVA used to compare the percentage of resistant coliform bacteria between the Susquehanna River sites, and between the Shelley Brook and Charlotte Creek sites.

<u>Antibiotic</u>	<u>Susquehanna River</u>	<u>N</u>	<u>F-value</u>	<u>P-value</u>
Ampicillin	Concentration	27	303.648	P < .001
	Site	27	4987.324	P < .001
	Site x concentration	27	227.296	P < .001
Chloramphenicol	Concentration	27	34.527	P < .001
	Site	27	122.124	P < .001
	Site x concentration	27	9.330	P < .001
Streptomycin	Concentration	27	17.913	P < .001
	Site	27	204.272	P < .001
	Site x concentration	27	5.163	P = .006
Tetracycline	Concentration	27	35.091	P < .001
	Site	27	58.635	P < .001
	Site x concentration	27	20.152	P < .001
<u>Antibiotic</u>	<u>Shelley Brook and Charlotte Creek</u>	<u>N</u>	<u>F-value</u>	<u>P-value</u>
Ampicillin	Concentration	36	21.858	P < .001
	Site	36	23.209	P < .001
	Site x concentration	36	1.347	P = .276
Chloramphenicol	Concentration	36	163.905	P < .001
	Site	36	33.530	P < .001
	Site x concentration	36	35.791	P < .001
Streptomycin	Concentration	36	9.988	P = .001
	Site	36	25.363	P < .001
	Site x concentration	36	2.971	P = .026
Tetracycline	Concentration	36	34.843	P < .001
	Site	36	217.569	P < .001
	Site x concentration	36	21.667	P < .001

Table 2. Results of a univariate ANOVA used to compare the percentage of resistant *Acinetobacter* between the Susquehanna River sites, and between the Shelley Brook and Charlotte Creek sites.

<u>Antibiotic</u>	<u>Susquehanna River</u>	<u>N</u>	<u>F-value</u>	<u>P-value</u>
Ampicillin	Concentration	27	1.00	P = .387
	Site	27	1.00	P = .387
	Site x concentration	27	1.00	P = .433
Chloramphenicol	Concentration	27	28.526	P < .001
	Site	27	273.186	P < .001
	Site x concentration	27	7.247	P = .001
Norfloxacin	Concentration	27	7.000	P = .006
	Site	27	16.000	P < .001
	Site x concentration	27	7.000	P = .001
Streptomycin	Concentration	27	45.786	P < .001
	Site	27	11.622	P = .001
	Site x concentration	27	2.721	P = .062
Tetracycline	Concentration	27	427.491	P < .001
	Site	27	5.410	P = .014
	Site x concentration	27	28.802	P < .001
<u>Antibiotic</u>	<u>Shelley Brook and Charlotte Creek</u>	<u>N</u>	<u>F-value</u>	<u>P-value</u>
Ampicillin	Concentration	36	2.545	P = .099
	Site	36	4.212	P = .016
	Site x concentration	36	1.576	P = .197
Chloramphenicol	Concentration	36	40.778	P < .001
	Site	36	159.333	P < .001
	Site x concentration	36	3.444	P = .013
Norfloxacin	Concentration	36	25.333	P < .001
	Site	36	42.667	P < .001
	Site x concentration	36	12.000	P < .001
Streptomycin	Concentration	36	62.284	P < .001
	Site	36	171.309	P < .001
	Site x concentration	36	1.152	P = .364
Tetracycline	Concentration	36	455.671	P < .001
	Site	36	80.889	P < .001
	Site x concentration	36	55.285	P < .001

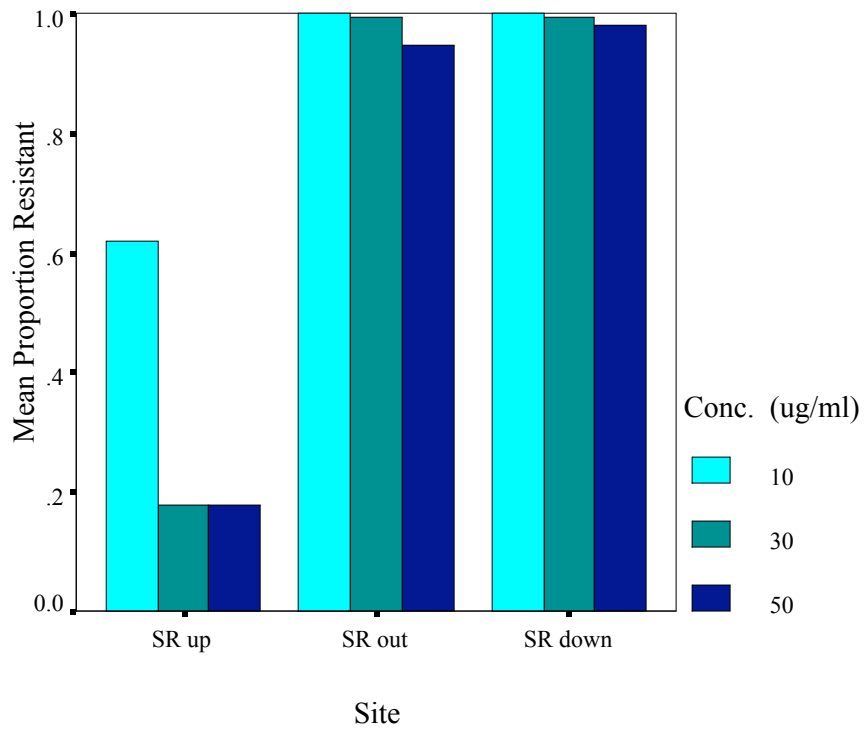


Figure 1. The proportions of coliform bacteria resistant to ampicillin at the Susquehanna River sites.

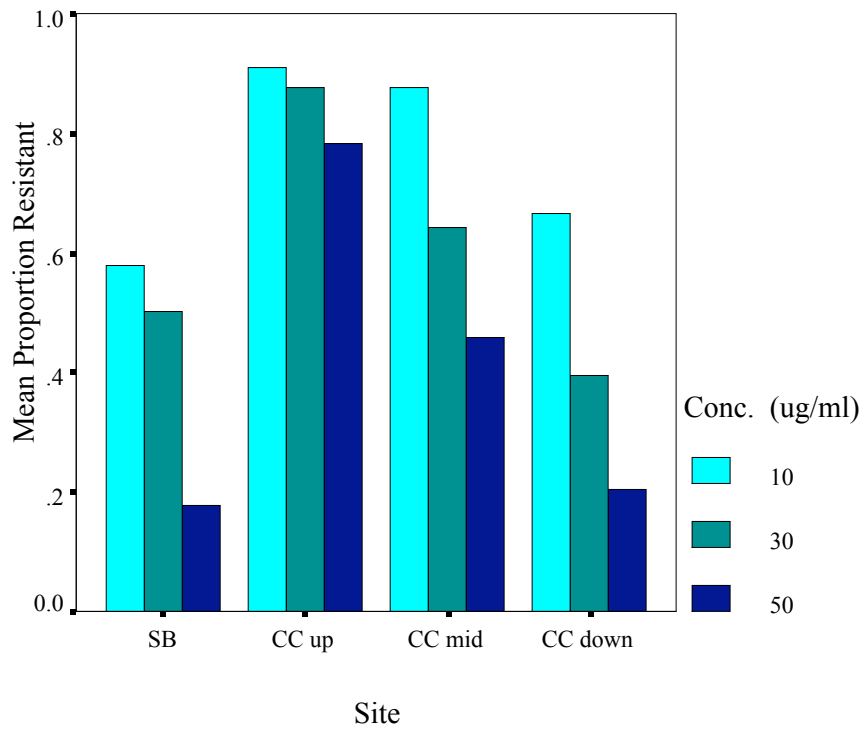


Figure 2. The proportions of coliform bacteria resistant to ampicillin at the Shelley Brook (SB) and Charlotte Creek (CC) sites.

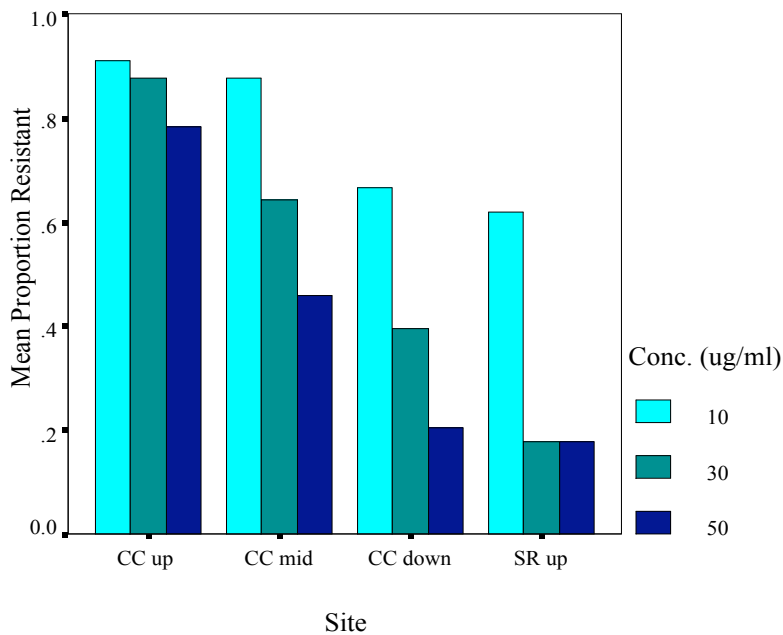


Figure 3. The proportions of coliform bacteria resistant to ampicillin in Charlotte Creek (CC) compared to the upstream site of the Susquehanna River (SR). The upstream and middle sites of Charlotte Creek were significantly different from the upstream site of the Susquehanna River ($p < .001$).

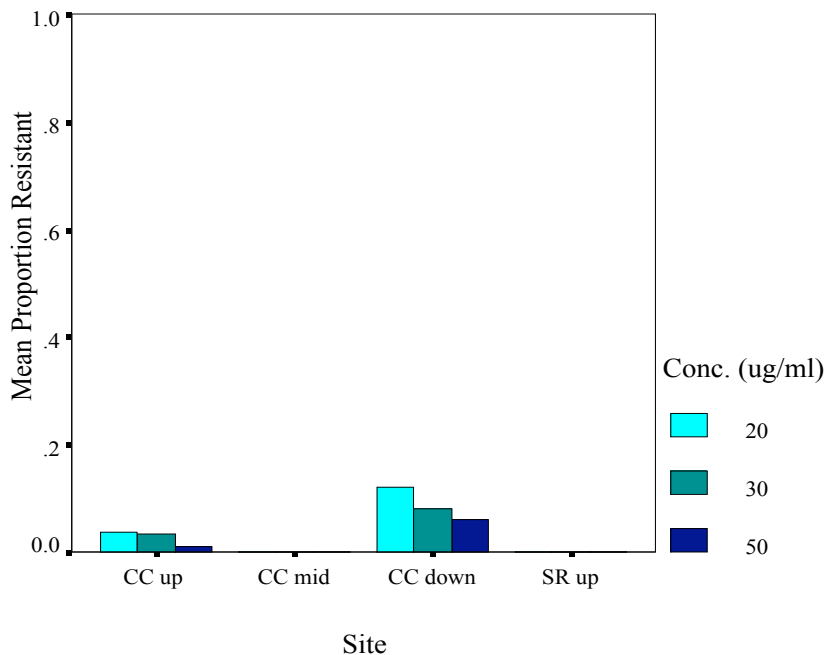


Figure 4. The proportions of coliform bacteria resistant to streptomycin in Charlotte Creek (CC) compared to the upstream site of the Susquehanna River (SR). The downstream site of Charlotte Creek was significantly different from the upstream site of the Susquehanna River ($p < .001$).

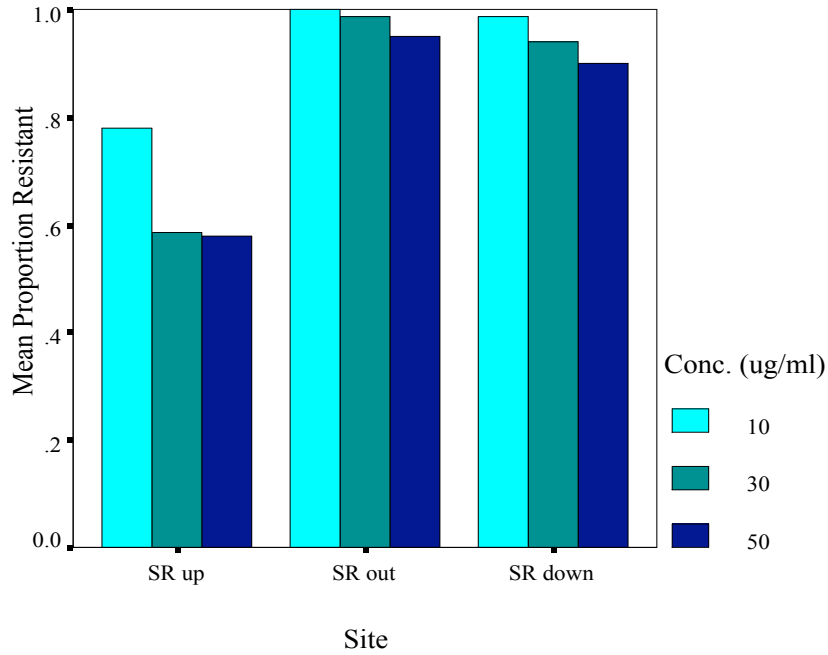


Figure 5. The proportions of *Acinetobacter* resistant to chloramphenicol at the Susquehanna River (SR) sites.

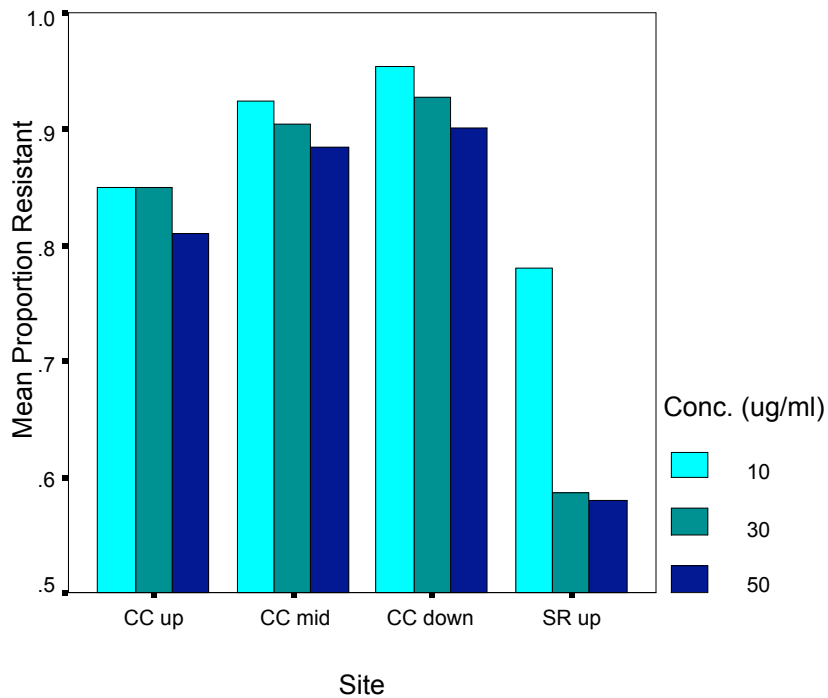


Figure 6. The proportions of *Acinetobacter* resistant to chloramphenicol in Charlotte Creek (CC) compared to the upstream site of the Susquehanna River (SR). All three sites had a significantly higher proportion of resistant bacteria than in the Susquehanna River site ($p < .001$).

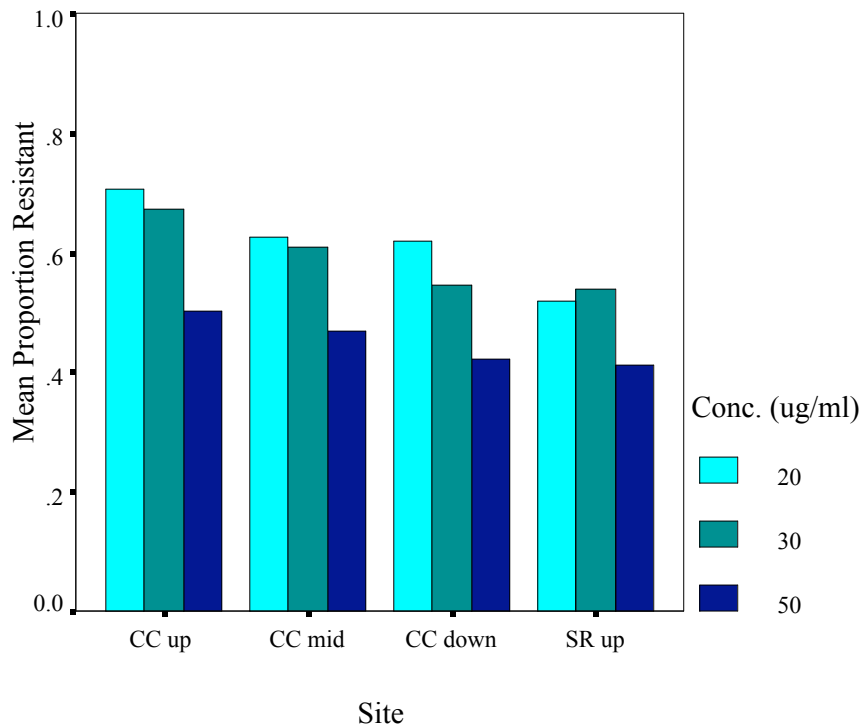


Figure 7. The proportions of *Acinetobacter* resistant to streptomycin in Charlotte Creek (CC) compared to the upstream site of the Susquehanna River (SR). The upstream and middle sites of Charlotte Creek had a significantly higher proportion of resistant bacteria than the Susquehanna River site ($p < .001$ and $p = .006$, respectively).

DISCUSSION

The results of this study support the hypothesis that effluent from waste water treatment facilities impacts populations of antibiotic resistant bacteria in the Susquehanna River, and additionally that there is some effect of antibiotic use in agriculture or seepage from private septic systems on the pools of resistant bacteria in streams.

Significant increases in the proportions of coliform bacteria resistant to ampicillin, chloramphenicol, streptomycin, and tetracycline were found at and below the outflow pipe of a waste water treatment facility. There are two possible mechanisms for these increases. The effluent may contain antibiotics that are selecting for bacteria in the river that are resistant to antibiotics. Any population of bacteria may contain mutants that are naturally resistant to antibiotics and exposure to these drugs will kill the normal bacteria in the population while selecting for the resistant mutant strains. The data for streptomycin resistance supports this possibility, as the proportion of resistance is highest at the outflow pipe and then decreases fifty meters downstream, possibly as the selective pressure of the antibiotic becomes more diluted (Chee-Sanford et al, 2001). The clinical use of streptomycin is likely the source of this selective pressure in the Susquehanna River. An alternative but not exclusionary hypothesis is that effluent may contain resistant bacteria that are multiplying and potentially spreading those resistance genes to other bacteria in the river environment (McArthur and Tuckfield, 2000). The water entering the waste water treatment facility comes from private homes and A.O. Fox hospital, where the use of antibiotics causes

a selective pressure in the human system that will lead to an increase in the proportion of resistant mutants in the sewage water. The effluent from the waste water treatment facility most likely contains some of these resistant mutants.

Interestingly, virtually all of the *Acinetobacter* isolates were resistant to ampicillin, even at the highest concentrations. *Acinetobacter* are commonly resistant to β -lactams (Jawad et al, 1994), which most likely explains this phenomenon. Other than resistance to ampicillin, only resistance to chloramphenicol and tetracycline showed a significant increase at the outflow and downstream sites of the Susquehanna River, indicating that the effluent may be selecting for resistant strains of *Acinetobacter* in the river, or that clinical isolates of *Acinetobacter* with evolved resistance to these drugs are contained in the effluent.

The proportions of resistant coliform bacteria in Shelley Brook were higher than expected and this is most likely due to the production of natural antimicrobics such as small-polypeptide defensins by bacteria competing in the environment (Houndt and Ochman, 2000). These interactions may also be occurring at the Charlotte Creek sites, but to a lesser extent because the bottom of the creek has more silt and less decaying organic matter than Shelley Brook. Because Charlotte Creek is impacted by non-point sources of pollution such as septic tank seepage and agricultural runoff it is much more difficult to assess the impact of this pollution on the numbers of resistant bacteria. The percentage of coliforms resistant to ampicillin was significantly higher than that found in Shelley Brook, and at two of the Charlotte Creek sites it was higher than that found above the outflow point of the Susquehanna River. This suggests that agricultural runoff or additionally private septic tank seepage is impacting the levels of ampicillin resistant coliforms found in these and similar streams that are influenced by non-point sources of pollution. The levels of resistance in Charlotte Creek to streptomycin were higher than was found at the upstream Susquehanna River site and similar to those found at the Susquehanna River site downstream of the outflow. Streptomycin is often used on apples and pears (Walsh, 292), so it is possible that this is a source of pollution in Charlotte Creek.

For the *Acinetobacter*, virtually 100% of the isolates were resistant to ampicillin. This was unexpected at the Shelley Brook site where the resistance is theoretically naturally occurring, but again this is likely due to the natural β -lactam resistance of *Acinetobacter*. Very high levels of resistance to chloramphenicol were also found in Shelley Brook and at all three Charlotte Creek sites, suggesting that there may be competitive microbial interactions occurring, or that *Acinetobacter* isolates are naturally resistant to chloramphenicol. Additionally, a very high proportion of *Acinetobacter* in Shelley Brook, and only a slightly lesser proportion in Charlotte Creek, were resistant to streptomycin. This is most likely due to the presence of *Streptomyces*, the natural source of streptomycin (Demain and Lancini, 1991), in the soil in the streambed and along the shores of Shelley Brook and Charlotte Creek. The Susquehanna River is a larger and faster-flowing body of water, so the bacterial community living in it may be less impacted by the presence of *Streptomyces*.

The proportion of *Acinetobacter* resistant to chloramphenicol at all three of the Charlotte Creek sites was higher than that found above the outflow site of the Susquehanna River. This suggests that there is some use of chloramphenicol in the area that drains into Charlotte Creek that is impacting environmental isolates of *Acinetobacter* in a way comparable to how the use of chloramphenicol in medicine is impacting clinical isolates of *Acinetobacter*. Additionally, the proportion of *Acinetobacter* resistant to streptomycin was higher at two of the Charlotte Creek sites than at the upstream site in the Susquehanna River.

This may be due to naturally occurring resistance that can develop in response to interactions with *Streptomyces* as was discussed above.

It is very important that we gain a better understanding of how the use of antibiotics in both agriculture and medicine is impacting both environmental and clinical isolates of bacteria. Antibiotics not only select for resistant strains of bacteria in clinical settings, but they are also poorly absorbed in both humans and animals, and waste in the form of agricultural runoff into streams and waste water treatment facility effluent often contains antibiotics that continue to select for resistant strains in the environment. The higher levels of coliforms resistant to ampicillin, chloramphenicol, and tetracycline found at the downstream site of the Susquehanna River indicate that the resistant bacteria may not only be multiplying, but could also be transferring resistance genes to other bacteria in the environment (Kelch and Lee, 1978). This results in reservoirs of resistant bacteria that can pass genes for resistance on to other bacteria ultimately leading to the faster spread of resistance genes to not only bacteria within the same species, but also to other more diverse types of bacteria. As genes conferring resistance to common antibiotics spread it becomes increasingly likely that an individual will become infected with a resistant strain of a bacteria that may cause an infection which could be very difficult to treat. Understanding how our use of antibiotics impacts bacteria in environmental settings may help reduce the spread of these resistance genes. Charlotte Creek flows into the Susquehanna River, and this river lies at the headwaters of the Chesapeake Bay watershed. Any reservoirs of resistant bacteria that are contained in this water will only be compounded as more streams and rivers polluted by agricultural runoff and waste water treatment facility effluent contribute to the watershed.

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