Correlating Residual Antibiotic Contamination in Public Water to the Drug-Resistance of *Escherichia coli*

Ashley Mulroy
Linsly School
Wheeling, WV

Table of Contents

Correlating Residual Antibiotic Contamination in Public Water to the Drug-Resistance of *Escherichia coli*

Once controlled by medical science, microbes have reemerged as agents of infectious diseases, evolving into drug-resistant pathogens that threaten to overpower all but the newest and strongest antibiotic drugs. It is not that science's allies, the antibiotics, are weakening, but that bacteria have become stronger.

Misuse and overuse of the pharmaceuticals are usually cited as culprits when acquired resistance becomes an issue. However, documentation of antibiotics present in lake waters in Berlin and other sites raises the question whether widespread environmental antibiotic contamination contributes to the development of drug-resistant pathogens.

In this study, *Correlating Residual Antibiotic Contamination in Public Water to the Drug-Resistance of Escherichia coli,* the presence of three antibiotics, Penicillin, Tetracycline and Vancomycin, were found at trace levels in five Ohio River sites, two tributary sites and in drinking water in three municipalities adjacent to the river. It was determined that filtration of the drug-contaminated water through activated charcoal removed most of the antibiotics; but filtration through sand, the most common wastewater filtration method used, did not remove any of the three antibiotics from water samples.

E. coli, a common bacteria, which was isolated from each of the seven outdoor sites, demonstrated resistance to the antibiotics with which it formerly coexisted in nature. This resistance was proportional to the antibiotic concentration present at each water site.

This issue becomes more complex as we consider that bacteria easily exchange genetic material. A relatively harmless bacteria living at the water's edge, could easily be transported—by human, animal or mechanical means—to a site into which in comes into contact with, and transfers its acquired resistance to, a potentially deadlier pathogen. The contact and transference of acquired resistance between nonpathogens and pathogens becomes more likely as drug-resistant microbes become common to our daily habitat.

The study calls for judicious distribution and use of existing, as well as new, pharmacology and points out that the failure to do so could perpetuate the condition in which pervasive, low-level antibiotic contamination would provide just the right environment in which the world's microbes actively train to outpace new antimicrobial drug development.

Correlating Residual Antibiotic Contamination in Public Water to the Drug-Resistance of Escherichia coli

Abstract

Purpose: This project was conducted to (a) determine the presence of the antibiotics Penicillin, Tetracycline and Vancomycin along a 44 km stretch of the Ohio River and two tributary streams, (b) test *in-situ* bacteria from each of the test sites for acquired drug resistance, and (c) determine the viability of selected methods of remediation.

Methods and Materials: (A) Water samples from seven outdoor locations were collected regularly, over a ten-week period, as well as single samplings of tap water from each of three municipalities adjacent to the river. Levels of antibiotic concentrations in the samples were determined utilizing gel electrophoresis, resulting in 1050 values of river and stream water data and 135 values of tap water data, upon which standard deviations and confidence levels were determined. (B) *Escherichia coli* (*E. coli*) samples isolated from test sites were subjected to the Kirby-Bauer Disk Sensitivity procedure to determine the level of bacterial resistance to Penicillin, Tetracycline and Vancomycin. In all, 28 dish cultures were studied for sensitivity to the three antibiotics, with measurable data submitted to a line of best fit statistical analysis to determine correlation coefficients. (C) Three PVC media-packed cylinders were utilized to test the efficacy of mixtures of sterile sand, *Saccharomyces cerevisiae* culture (Brewer's Yeast) and ground activated charcoal as filters for each of the three antibiotics in question. Standard deviations and confidence levels were determined for the resulting 135 values.

Observations: (A) All river and tributary sites yielded detectable amounts of the three test antibiotics in concentrations ranging from 0.7 - 5.9 parts per trillion. Lesser amounts were detected in tap water. (B) All E. coli samples cultured from river and tributary sites exhibited acquired antibiotic resistance. Analysis indicated that the greatest acquired resistance appeared in the samples containing the highest level of antibiotic contamination. (C) Water samples, which passed through packed columns of sand (the primary filtrate media used by municipalities in the mid-Atlantic region of the United States), saw no reduction in antibiotic contamination. Columns packed with a sand/activated charcoal mixture removed 93.3% of Vancomycin, 96.0% of Tetracycline and 77.1% of Penicillin concentrations.

Conclusions: The presence of antibiotic contamination in American waterways results in a progressive resistance among some bacteria to those same antibiotics that once controlled them. Some remedial filtration techniques have shown themselves to be effective, but there is further need for both study and action toward a more responsible utilization of antibiotics if these pharmaceuticals are to continue to be effective.

Consider, for a moment, the quality of life and health in the absence of pharmaceutical breakthroughs such as Penicillin, Tetracycline and Vancomycin. This research may serve as a warning that the benefits of antibiotic drugs are gradually being neutralized, with the bacteria that survive non-lethal exposures to these former wonder-drugs developing into far more powerful versions of their former incarnations. A more responsible approach to prescription and utilization of antibiotics is necessary to enable medical science to maintain control of these microbial threats to public health.

Correlating Residual Antibiotic Contamination in Public Water to the Drug-Resistance of Escherichia coli

First, do no harm. —Hippocrates

Introduction

With the 1992 discovery of the cholesterol lowering pharmaceutical, clofibric acid, in river water being monitored by German researchers for the presence of pesticides (Raloff, 1998), the first ominous note of a sobering wakeup call sounded. A host of pharmaceuticals has since been detected in European rivers and lakes, as well as the North Sea. German scientists now report that between 30-60 different drugs may be present in a typical water sample—including antibiotics, hormones, strong pain killers, tranquilizers and chemotherapy drugs (Buser, 1998).

Drugs are designed to be persistent, a property which allows them to retain their chemical properties long enough to serve a therapeutic purpose. However, 50% to 90% of a typical drug given to a human or animal is excreted, unchanged, (Montague, 1998) and unfortunately persists in the environment. With European wastewater treatment facilities equal to, or better than, those in most developed regions, would it not seem that for any population with access to an advanced system of healthcare, the potential for the same residual pharmaceutical contamination exists? The implications, in terms of water quality, is that this is not specifically a European problem. It is an issue of *global concern.*

While environmental stewards began grappling with the implications of aquatic ecosystems literally awash in a sea of pharmaceuticals, a second and louder discordant note reverberated through the medical community. In May 1996 a *Staphylococcus aureus* (*S. aureus*) infection, being treated in Japan, failed to respond to a standard course of the antibiotic Methicillin and then Vancomycin, the drug of last resort, ultimately resulting in the death of the patient (Hiramatsu, 1997). A strain of *S. aureas* resistant to Penicillin, Oxacillin, Clindamycin, Erythromycin, Ciprofloxacin, and Rifampin yielded similar results during treatment at an Illinois hospital in 1999 (Khurshid, 2000). More death reports have originated from South Africa and the United Kingdom (Levy, 1998).

Healthcare and disease-control agencies point to the overprescribing of antibiotics, the inappropriate prescribing of antibiotics for viral illnesses and the improper use of antibiotics by patients who failed to complete the entire course of medication as factors contributing to *the reemergence of microbes, once controlled by medical science, as deadly agents of infection.* (Levy, 1992) It appears to be a case in which significant medical development has become a proverbial double-edge sword.

The central theme running through this projected misuse of antibiotics is the condition in which pathogens are frequently exposed to antibiotic levels insufficient in either concentration or duration to effectively kill the entire target population. This is problematic on two levels. Pathogens may become resistant to low levels of antibiotics to which there is frequent or prolonged exposure. Or stronger antibiotics, taken for an abbreviated duration, will not entirely eradicate the disease-causing organisms. This insufficient kill handily eliminates the weaker microbes, which previously provided competition with heartier microbes for sustenance, again providing an environmental advantage to the surviving pathogens (Wistreich, 1988).

At first, it may seem a remote possibility that trace levels of antibiotics present in the world's waterways (to date found in the parts-per-million (ppm) to parts-per-trillion (ppt) range) would bear any significance to the problem of emerging strains of drug-resistant bacteria, as long as we, or the animal population, refrain from consuming these waters directly—if it were not for a biological

mechanism unique to bacterial organisms.

Bacteria are capable of, and frequently do, exchange genetic materials—including the transference of acquired drug resistance (Oram, 1994). A nonpathogenic bacteria living at the river's edge and bathed in trace levels of several antibiotics could conceivably develop resistance to those particular drugs. This resistance could be readily transferred to an opportunistic pathogen *in-situ.* Or the resistant non-pathogen may be transported by a variety of agents—human, animal, mechanical—to a site in which it comes into contact with, and transfers its resistance to, another potentially deadly microbe.

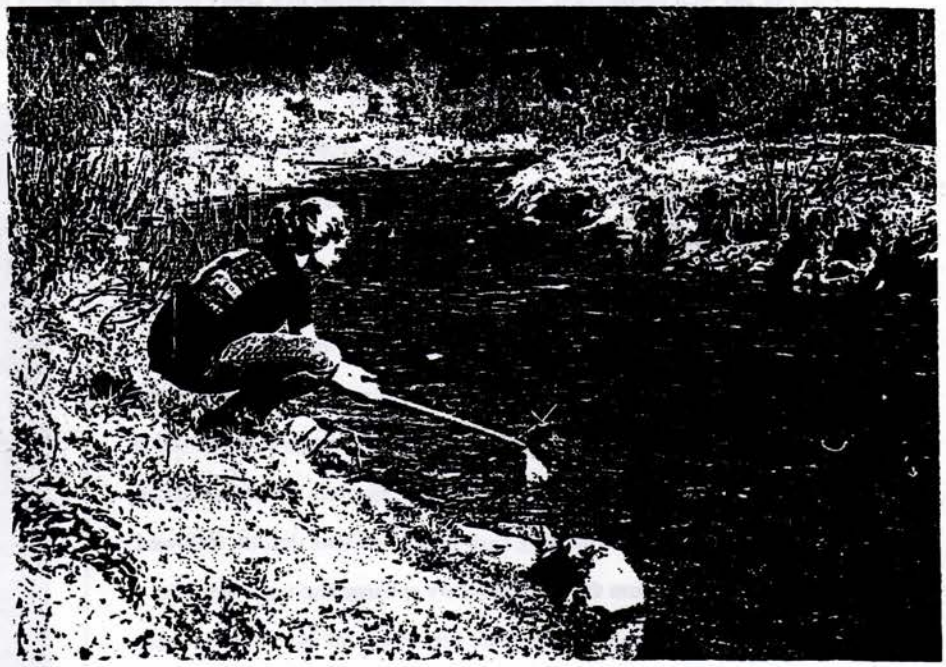
The questions : Are our waterways becoming drug-laden reservoirs which spawn pockets of antibiotic-resistant bacteria? Are our water treatment facilites generating potable water that is free of drug contaminants? Are primary filtration methods effective in removing residual drug contaminants from water, and if so, what filtration media provides the highest level of remediation?

This research addresses these questions within the regional framework of the Ohio Valley, located in the mid-Atlantic state of West Virginia in the United States.

Purpose

The specific purposes of this ten-week period of data collection are threefold:

1). The weekly monitoring, by water sample collection, of five sites on a 44 km stretch of the Ohio River and two additional tributary effluent sites, which will be analyzed for the both the presence and concentration of the antibiotics Penicillin, Tetracycline and Vancomycin. A one-time sampling of drinking water from three municipalities in three separate regions adjacent to the river will also be analyzed. All analyses will be submitted to multiple replications to permit statistical analysis.



Sample collection at tributary Site 5 on week four. Small islands of mud, field grass and rocks usually jut from the bottom of this semi-dry stream. Stream waters are expansive and swift following a week of rain and snowmelt.

2) The isolation of *in-situ* bacteria from each of the seven outdoor sites, which will be tested for acquired drug resistance utilizing the Kirby-Bauer disk sensitivity method.

3) The evaluation of packed-column filtration to determine the efficacy of sand, *Saccharomyces cerevisiae* (*S. cerevisiae*), brewer's yeast and activated charcoal in remediating Penicillin, Tetracycline and Vancomycin of known concentration levels.

Hypothesis

It is hypothesized that residual antibiotics (Penicillin, Tetracycline and Vancomycin) will be detected in water samples from the Ohio River and its tributaries, with concentration levels increasing at sites adjacent to hospitals and livestock or dairy farms. Little

or no antibiotic concentrations will be detected in samples from potable water. Additionally, *in-situ* bacteria from antibiotic-contaminated sites will exhibit resistance to those particular contaminants.

It is hypothesized that *S. cerevisiae* and activated charcoal, both of which have absorptive qualities, will prove effective in the removal of all three contaminants from water samples.

Procedures

Procedure I: Assessing Antibiotic Concentrations

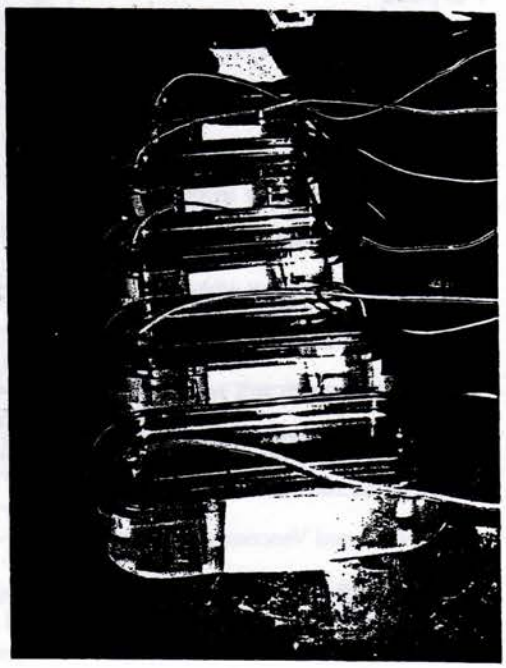
1) Water samples were collected each Sunday morning, over a ten-week period, from five locations on a 44 km length of the Ohio River and from two of its tributary streams. A onetime collection was also performed of tap water samples from three sites in regions adjacent to the river.

2) For gel well preparation, agarose gel was melted in a water bath for two hours until complete liquefaction. 50 mls of the Agarose was poured into a gel well tray in which the gel comb was placed over the negative electrode. The gel was allowed to solidify for twenty minutes and the well comb was then removed.

3) Solutions of known concentrations, ranging from 1.0 to 6.0 ppt were prepared for each test antibiotic and 1.0 ml of each concentration was loaded into five separate wells.

4) The gel was then placed into the electrolysis chamber and flooded with 300 ml of TBE buffer solution. Electrical power was supplied to the covered electrolysis chamber for a period of 20.5 hours. The chamber was then opened and the buffer removed. The gel was removed from the well, placed in a staining tray and the upper right corner of the gel removed for identification, before undergoing a 20-minute staining period. A 20-minute destain followed.

5) Each sample from the river, tributary streams and tap water was tested in this manner. Five replications were performed for each of the seven river and stream samples each week. Fifteen replications were performed for each tap water sample. Both the location and size of the bands appearing in the gel for each test sample was compared to the location and size of the bands on the original known concentrations, which served as standards for determining both the presence and the concentration of each antibiotic contaminant.



Water samples from each site are placed in gel wells to undergo gel electrophoresis.

6) The resulting 1050 values of river and stream water data and the 135 values of tap water data were studied, with standard deviations and confidence limit calculated to determine statistical significance.

Procedure II: Determining Bacterial Resistance

1) For each outdoor site sample from two selected weeks (weeks five and nine), one petri dish of tryptic soy agar was swabbed with a small amount of the sample and then incubated at 30° C for 24 hours. The 14 plates were examined, with a swab from an individual colony from each plate transferred to one of 14 new plates, for the purpose of isolating one species of organism.

2) Gram stain slides utilizing Crystal Violet, Gram's Iodine and Safarin were prepared according to standard procedures, along with wet mount slides. Under microscopic analysis, specific bacterial characteristics, such as shape and motility, identified the isolated organism as *Escherichia coli* (*E. Coli*).

3) Two separate petri dishes of isolated *E. coli* were prepared from each of the seven outdoor sites sampled on both weeks five and nine (28 dish cultures in all). Into each of these were placed three sensitivity disks, one each inoculated with Penicillin, Tetracycline and Vancomycin. Plates were then incubated for 48 hours.

4) Each incubated plate was examined and the zone of inhibition measured (in mm) around each sensitivity disk. This measurement was coordinated with the standard degree of sensitivity of the organism to each antibiotic, utilizing the Kirby-Bauer Chart. The data was submitted to a Line of Best Fit statistical analysis.

III. Assessing Filtration Efficacy

1) The bottom ends of three hollow PVC cylinders, measuring 10 cm in length by 1 cm in diameter were tightly covered with two layers of cheese cloth.

2) The first cylinder was packed with 7 ml of sterile sand; the second with 3.5 ml each of sterile sand and *S. cerevisiae* culture; the third with 3.5 ml each of sterile sand and activated charcoal ground into small particles.

3) Ten mls of river water, with known concentrations of Penicillin, Tetracycline and Vancomycin were allowed to flow freely through each filter. The filtrate, captured in a collection container following its exit from the cheesecloth, was submitted to analysis by gel electrophoresis, as described in Procedure 1.

4) Fifteen repetitions were performed for each test, resulting in 135 pieces of data (15 for each of the three test filters for each of the three antibiotics). Standard deviation and confidence limit were determined.

Data

For a summary of the 1354 pieces of collected data, please see the Appendix.

Discussion:

Ohio River and Tributary Sites

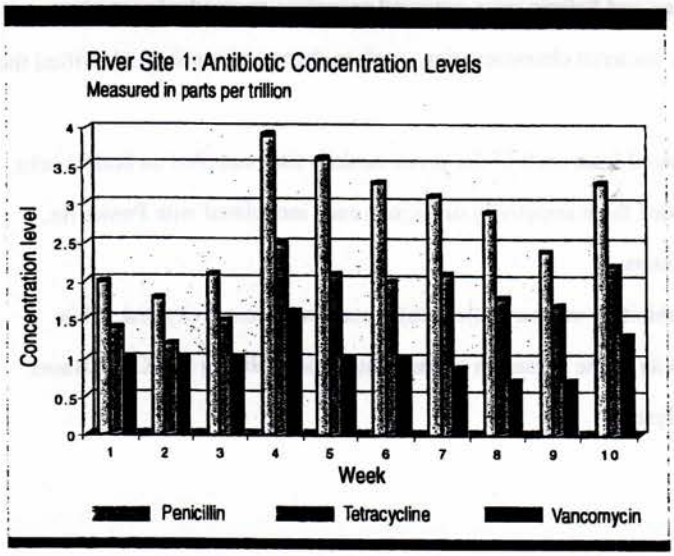
All seven river and tributary sites contained detectable amounts of the three test antibiotics, Penicillin, Tetracycline and Vancomycin.

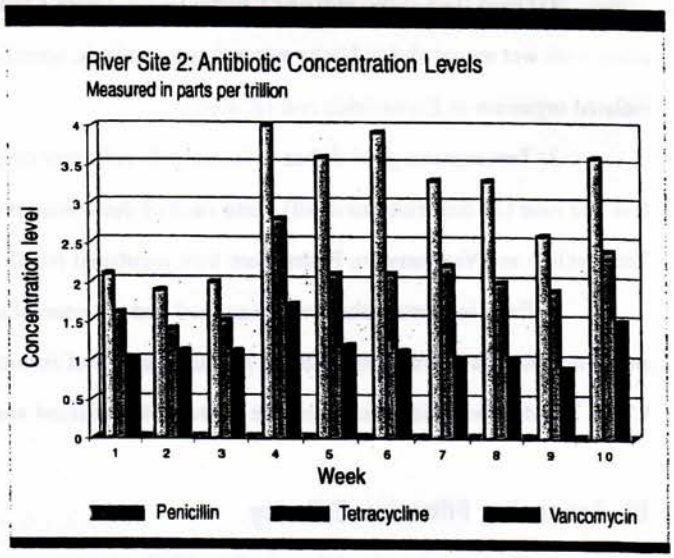
Four major trends were observed.

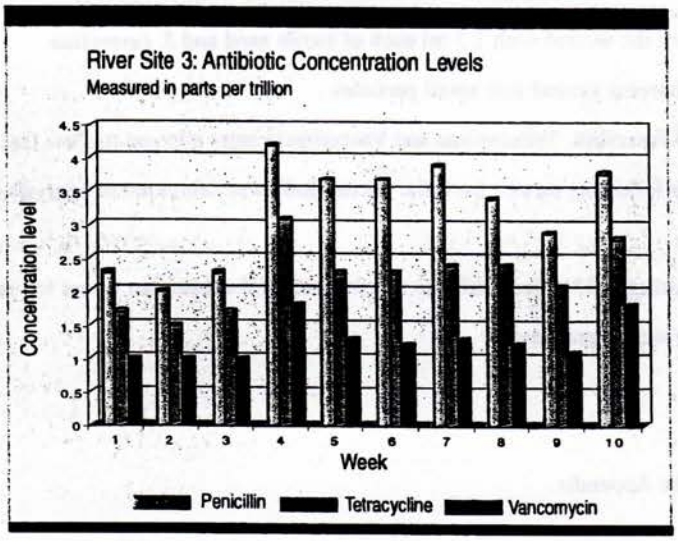
1) Concentration levels varied from week to week. A gradual increase in all three antibiotic levels developed at all seven sites following week two, and peaked at week four, which coordinated with heavy rainfall, snowmelt and flooding in the test region.

During week four, all sites experienced a dramatic increase in Penicillin levels, with concentrations increasing from the previous week's levels by 85.7%, 100.0%, 82.6%, 83.3%, 103.0%, 92.6% and 68.0% for Sites 1-7, respectively. Increases in Tetracycline were also significant: 66.7%, 86.7%, 82.3%, 94.1%, 100.0%, 116.7% and 129.4% for Sites 1-7, respectively. Increases in Vancomycin levels were by 60.0%, 54.5%, 80.0%, 57.1%, 100.0%, 81.2 % and 78.6%.

Following this peak, all sites north of the tributary streams (Sites 1-4) began a gradual tapering off, with some small weekly

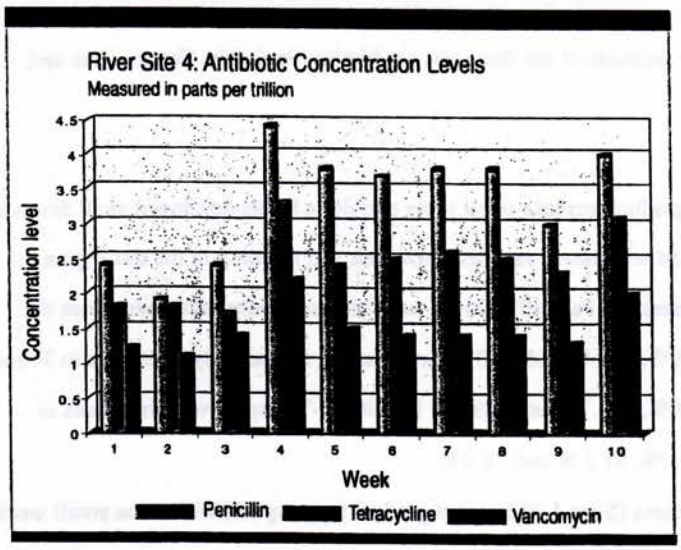


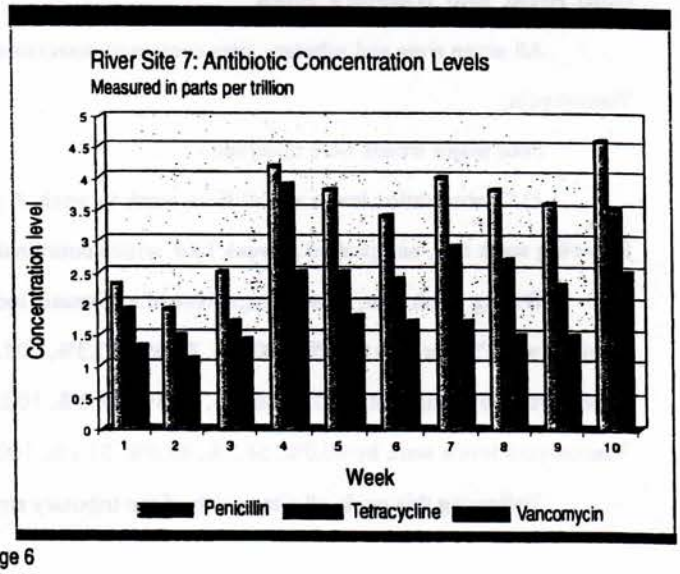


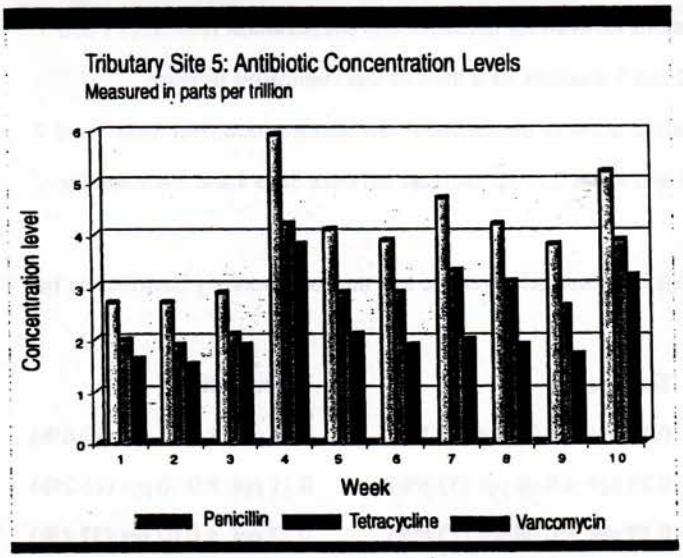


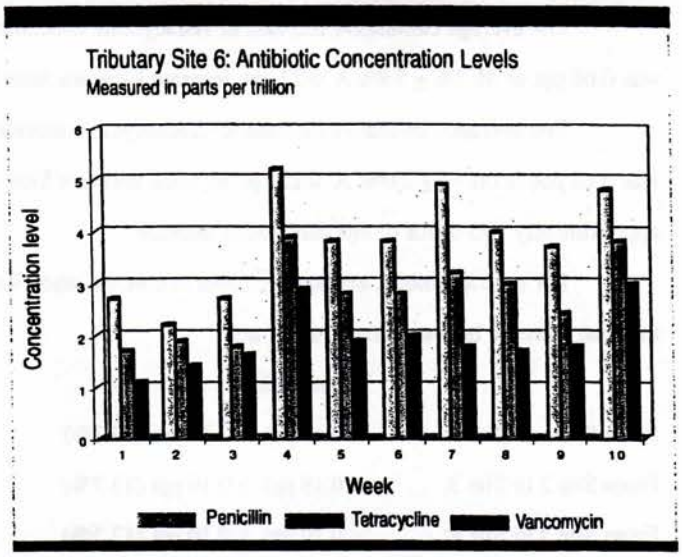
fluctuations, until week nine. Concentrations off all three drugs at these sites spiked, once again, on week ten. This again followed several days of heavy rains and flooding.

Concentration levels also began to taper off, following week four, on the tributary streams (Sites 5 & 6) and the terminal river Site 7, south of the tributaries. However, at week seven, a spike in the concentration levels of both Penicillin and Tetracycline occurred, although increases were less dramatic than those of week four and ten, Penicillin concentrations increased by 20.5% at Site 5 and 28.9% at Site 6. Downstream, river Site 7 Penicillin concentrations increased by and 17.6%. A similar pattern occurred with Tetracycline levels at these sites. Tetracycline levels increased at tributary Sites 5 and 6 by 13.7% and









14.3%. Downstream, river Site 7 showed an increase of 20.1% in Tetracycline concentrations. No weather event was associated with these increases. No Vancomycin increases were detected in week seven.

Following this increase in Penicillin and Tetracycline levels on week seven, Sites 5 through 7 followed the pattern of Sites 1-4, gradually tapering off through week nine and spiking again at week ten.

2) Concentration levels of all three antibiotics were generally higher in the two tributary streams than in the five river sites on corresponding weeks. With the exception of the week six Penicillin concentrations being equal, the Site 5 tributary stream was consistently higher in all three antibiotic concentrations than the Site 6 tributary stream.

3) On corresponding weeks, Penicillin concentration levels were consistently higher at all river and tributary test sites than Tetracycline levels, although the Tetracycline concentration came within 0.01 ppt to that of Penicillin at Site 4 on week two. In turn, Tetracycline river and tributary site concentrations were, consistently higher than Vancomycin concentrations on corresponding weeks.

Some specific parameters of the ten-week period were:

Highest Penicillin concentration, 5.9 $\pm$ 0.2 ppt at Site 5, Week 4.

Highest Tetracycline concentration, 4.2 $\pm$ 0.2 ppt at Site 5, Week 4.

Highest Vancomycin concentration, 3.8 $\pm$ 0.2 ppt at Site 5, Week 4.

Lowest Penicillin concentration, 1.8 $\pm$ 0.2 ppt at Site 1, Week 2.

Lowest Tetracycline concentration, 1.2 $\pm$ 0.2 ppt at Site 1, Week 2.

Lowest Vancomycin concentration, 0.7 $\pm$ 0.2 ppt at Site 1, Weeks 8 & 9.

4) There was an overall average increase in all antibiotic concentration levels between the northernmost river test site (Site 1) and each successive river test site downstream (Sites 2, 3, 4 and 7). (Recall that Sites 5 & 6 are tributary sites.) Average increases between each successive site ranged from 0.09 ppt to 0.19 ppt for Penicillin; 0.13 ppt and 0.23 ppt for Tetracycline; and 0.10 ppt and 0.21 ppt for Vancomycin.

The average cumulative increase in Penicillin concentrations between the northern- and southernmost river Sites 1 and 7 was 0.57 ppt or 20.1% $\pm$ 7.1%. The 0.19 ppt increase between Sites 1 and 2 and between Sites 2 and 3 accounted for two-thirds of this.

The average cumulative increase in Tetracycline concentrations between the northern- and southernmost river Sites 1 and 7 was 0.68 ppt or 36.7% ± 5.9% A 0.23 ppt increase between Sites 2 and 3 accounts for a third of this cumulative increase.

The average cumulative increase in Vancomycin concentrations between the northern- and southernmost river Sites 1 and 7 was 0.68 ppt or 66.7%± 2.0%. A 0.22 ppt increase between Sites 3 and 4 and 0.21 ppt increase between Sites 4 and 7 account for approximately two-thirds of this cumulative increase.

For each antibiotic, average increases in concentration levels between each river site and the corresponding contribution to the total increase between Sites 1 and 7 are:

	Penicillin	Tetracycline	Vancomycin
From Site 1 to Site 2:	0.19 ppt ± 0.20 ppt (33.3%)	0.15 ppt ± 0.08 ppt (22.1%)	0.14 ppt ± 0.07 ppt (20.6%)
From Site 2 to Site 3:	0.19 ppt ± 0.10 ppt (33.3%)	0.23 ppt ± 0.08 ppt (33.8%)	0.11 ppt ± 0.10 ppt (16.2%)
From Site 3 to Site 4:	0.10 ppt ± 0.10 ppt (17.5%)	0.17 ppt ± 0.08 ppt (25.0%)	0.22 ppt ± 0.07 ppt (32.4%)
From Site 4 to Site 7	0.09 ppt ± 0.20 ppt (15.8%)	0.13 ppt ± 0.20 ppt (19.1%)	0.21 ppt ± 0.10 ppt (30.9%)
	0.57 ppt (100.0%)	0.68 ppt (100.0%)	0.68 ppt (100.0%)

Some reference points may be useful while reviewing observations concerning concentration changes between sites.

- Two hospitals are located on land adjacent to the river, or to land through which a tributary flowing into the river is located, between Sites 1 and 2.
- A third hospital and a wastewater treatment facility are located between Sites 2 and 3.
- A fourth hospital and second wastewater treatment facility are located between Sites 3 and 4.
- A third treatment facility and the two tributaries are located between Sites 4 and 7. Both tributaries lie at the base of ridge lands populated by small livestock and dairy farms.

Tap Water Sites

The northernmost tap water sample (Site 8) is derived from an area served by a water treatment facility which removes its source water from the river, north of river Site 1. Antibiotics were detected in the Site 8 tap water samples at the following concentrations: Penicillin: 1.6 ± 0.2 ppt; Tetracycline: 0.8 ± 0.2 ppt; Vancomycin: 0.2 ± 0.2 ppt.

The centrally located tap water sample (Site 9) is located in the region between river Sites 3 and 4. The water source is a municipal well. Antibiotics were detected in the Site 9 tap water samples at the following concentrations: Penicillin: 1.9 ± 0.2 ppt; Tetracycline: 0.9 ± 0.2 ppt; Vancomycin: 0.2 ± 0.2 ppt.

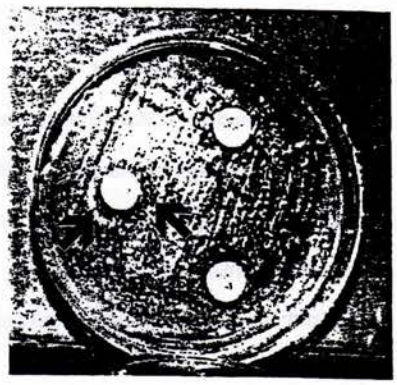
The southernmost tap water sample (Site 10) originated from a municipal well south of river Site 7. Antibiotics were detected in the Site 10 tap water samples at these concentrations: Penicillin: 1.8 ± 0.2 ppt; Tetracycline: 1.2 ± 0.2 ppt; Vancomycin: 0.4 ± 0.2 ppt.

Penicillin and Tetracycline concentrations vary only by a range of 0.3 ppt among the three sources, with the two southern municipal wellsites showing the higher concentrations. Vancomycin concentrations vary by a range of 0.2 ppt with Site 8 (river water source) and Site 9 (well water source) having equal concentrations and Site 10 (well water source) showing a 0.2 ppt elevation.

Antibiotic-Resistant *E. coli*

The degree of antibiotic resistance exhibited by a specific bacterial organism can be determined by the Kirby-Bauer Disk Sensitivity Method. Antibiotic-infused disks are placed in a live colony of bacteria. The kill zone (zone of inhibition) surrounding the disk is measured and its size compared to a predetermined zone of inhibition which would be expected for a nonresistant bacterium. The test is both organism and antibiotic specific.

All *E. coli* samples cultured from river and tributary Sites 1 through 7 exhibited acquired antibiotic resistance, in varying degrees.



Arrows point to the developing zone of inhibition surrounding a sensitivity disk in this E. coli culture.

A 21 mm zone of inhibition would be expected for nonresistant *E. coli* exposed to Penicillin. Zones of inhibition from Sites 1 through 7 ranged in diameter from a minimum of 5.5 mm to a maximum of 8.0 mm. The smallest average zone of inhibition (indicating highest antibiotic resistance) for Penicillin came from *E. coli* isolated from tributary Site 5. The largest average zone of inhibition (least resistance) for the same antibiotic came from river Site 1.

An 18 mm zone of inhibition would be expected for nonresistant *E. coli* exposed to Tetracycline. Zones of inhibition from Sites 1 through 7 ranged in diameter from a minimum of 5.0 mm to a maximum of 8.0 mm. The smallest average zone of inhibition (indicating highest antibiotic resistance) for Tetracycline also came from *E. coli* isolated from tributary Site 5. Again largest average zone of inhibition (least resistance) for the same antibiotic came from river Site 1.

The expected zone of inhibition for *E. coli* exposed to Vancomycin is 11 mm. Zones of inhibition from Sites 1 through 7 also ranged in diameter from a minimum of 5.0 mm to a maximum of 8.0 mm. *E. coli* from Sites 5 and 1 were again the most and least antibiotic resistant, respectively.

When resistance data were plotted against water sample antibiotic concentration data, and the line of best fit calculated, the correlation coefficients ranged from 0.8 to 0.9, indicating that a fair probability of a direct correlation between the bacterial resistance level and the water sample antibiotic concentration level existed. This would indicate that the greatest acquired resistance was found in the samples containing the highest level of antibiotic contamination, and the lowest resistance had developed in the bacteria from water samples with the lowest antibiotic concentrations.

Assessing Filtration Efficacy

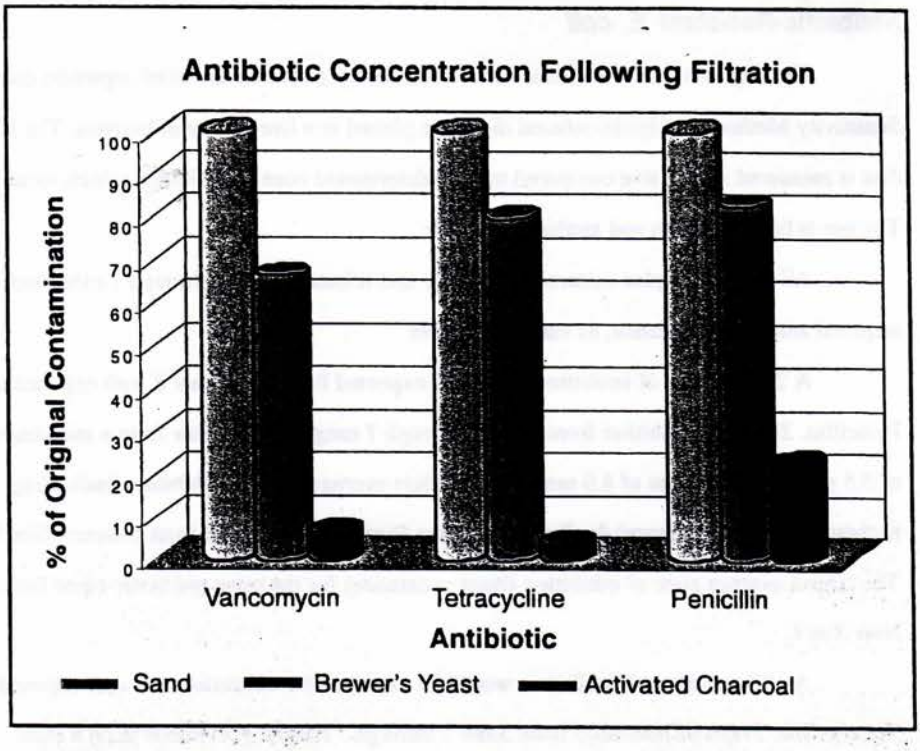
Random samples of river water were selected to test filtration materials. Samples of water containing 3.5 ppt of Penicillin, 2.5 ppt Tetracycline and 1.5 ppt Vancomycin were allowed to filter through three separate columns. Column A contained only sterile sand. Column B contained equal amounts of the same sand and *S. cerevisiae* culture (Brewer's yeast). Column C contained equal amounts of sterile sand and activated charcoal.

Filtrate leached through the column packed only with sand, showed unchanged concentration levels for all three antibiotics.

Water samples containing a 1.5 ppt concentration of Vancomycin showed an average 0.5 ppt reduction (33.3%) in concentration when filtered through the sand/yeast mixture, and an average 1.4 ppt reduction (93.3%) when filtered through the sand/activated charcoal.

Water samples containing 2.5 ppt Tetracycline also showed an average 0.5 ppt reduction (20.0%) in concentration when filtered through the sand/yeast mixture. Activated charcoal/sand filtration removed an average of 2.4 ppt (96.0%) of the Tetracycline.

Penicillin concentration was reduced an average of 0.6 ppt (17.1% of its original 3.5 ppt concentration) by yeast/sand filtration. Charcoal/sand reduced concentrations an average of 2.7 ppt (77.1%).



Conclusion

Measurable amounts of the antibiotics Penicillin, Tetracycline and Vancomycin are present in the areas of the Ohio River which pass between the northwestern border of West Virginia and southeastern border of Ohio, and in two of its tributary streams also located there. Lesser, but detectable amounts of the same antibiotics are present in tap water samples in three regions bordering these waterways. While reducing concentration levels, current wastewater treatment is ineffective at removing all traces of the antibiotics from the drinking water supply.

The presence of Penicillin, Tetracycline and Vancomycin in potable water derived from well sources indicates that these antibiotics are, not only entering our waterways via sewage systems (human sources), but are present in the groundwater as well. Groundwater contamination would seem to point to nonhuman sources and raises the question as to how the regular supplementation of animal feed with antibiotics, used as growth enhancers by many livestock and dairy farms, contributes to the pervasive existence of these pharmaceuticals in both the water and soil components of the environment. Water sampling targeting specific stream-points, both immediately above and below streams adjacent to farm sites, as well as soil sampling of the farm grounds would be necessary to positively identify or rule out these animal farms as contributors to the elevated concentrations of the two tributary streams studied, and in turn, to the river, downstream. A similar procedure could help to determine if contaminant concentration fluctuations are linked to hosptial discharges.

A primary water treatment method (of drinking water), simple filtration, can be effective in removing traces of Penicillin, Tetracycline and Vancomycin, when activated charcoal is used as the filtration medium. A wide variety of charcoal-based filters, designed for home use, are available on the market.

While filtration through sand is the primary wastewater treatment technique used by many municipalities in the mid-Atlantic region of the United States, it does not remove any of the three test antibiotics in this study. A simple change to a more effective filtration media, activated charcoal, would still allow for a gravity-driven primary treatment technique to be employed, while improving the efficacy of antibiotic contamination removal.

While remediation through simple filtration techniques is an appealing and relatively low-tech solution, its success is dependent, not only upon the correct filtration medium, but on the ability to capture and channel the contaminated water to the remediation site without its first passing into the environment. Municipal sewage systems take care of this function satisfactorily in the case of human-excreted contaminants.

But for unused antibiotics cleared from the family medicine chest and sent to the landfill or from animal-excreted antibiotics spread across pastureland and from large masses of barn wastes dumped to weather away, contaminants spread wide across the ground surface and leach deep into the soil. Remediation is then subject to the same obstacles common to the clean up of heavy metal, volatile organic compound and non-point source pollutants, such as the toxic levels of selenium seen in soils in the western United States—and that is the expense and total disruption of the biological integrity of the soil by offsite cleansing techniques, and the impracticality of methods requiring soil removal, when contamination is spread over large tracts of land.

Prevention, in these instances, is more appropriate than remediation. We can assist our physicians in not overprescribing antibiotics by not insisting on their use when symptoms indicate our illnesses to be viral in nature. While courses of prescribed antibiotics should be completed, any that are unused would best be returned to the pharmacy or prescribing physician for disposal.

We also need to address the availability of antibiotics to the dairy and livestock industry. Over-the-counter animal-grade antibiotics, such as Penicillin and Tetracycline, are readily available for purchase in farm supply stores. The amount and frequency of their administration to livestock is unregulated and left to the discretion of the farmer owner, whose interest may be driven more by profit than health or environmental concerns.

With the discovery that pockets of antibiotic-resistant *E. coli* already exist in our rivers and streams, and that evidence suggests their degree of resistance is proportional to antibiotic contaminant level, comes the realization that trace amounts of pharmaceuticals in the worlds' waterways and groundwater is much more than a minor pollution problem.

The contact and transference of acquired resistance between nonpathogens and pathogens becomes more likely as drug-resistant microbes become common to our daily habitat. We must be judicious in our distribution and use of existing, as well as new, pharmacology. Perpetuating this condition of pervasive, low-level contamination, may provide just the right environmental agar on which the world's microbes actively train to outpace new antimicrobial drug development.

Acknowledgements

• Mr. John Bisbocci, physical chemist and project advisor, assisted me in selecting gel electrophoresis as my test method, developing sampling and test procedures in accordance with the scientific method, and in evaluating the suitability of various tests of statistical significance for the project data.

• Ms. Stacy Spanovich, biology instructor at the Linsly School, instructed me in the technique of gel electrophoresis.

• Dr. David Mallow, biologist and science department chairperson of Linsly School, generously provided laboratory workspace and reviewed my statistical analysis of the project data.

• Linsly School of Wheeling, WV, USA, generously provided all laboratory equipment and chemical supplies.

References

Benson, Harold J. 1985. *Microbiological Applications.* Debuque: Wm. C. Brown Publishers.

Buser, Hans-Rudolf. 1998. Occurrence of the Pharmaceutical Drug Clofibric Acid and the Herbicide Mecoprop in Varios Swiss Lakes and in the North Sea. *Environmental Science and Technology*, Vol. 32, No. 1; pp: 188-192.

Christensen, Damaris. 2000. Keeping Bugs from Pumping Drugs. *Science News Magazine,* Vol. 157, No. 7: pp. 110-111.

Christian, Gary D., 1994. *Analytical Chemistry.* New York: John Wiley & Sons, INC.

Curtis, Helena, Sue N. Barnes. 1972. *Invitation to Biology.* New York: Worth Publishers.

Difco Laboratories. 1953. *Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures.* Detroit: Difco Laboratories.

Dobson, Roger. 1998. Rise of Super Bugs. *The Independent London,* May 3, 1998.

Harris, Daniel C. 1987. *Quantitative Chemical Analysis.* New York: W. H. Freeman and Co.

Hiramatsu, K. 1996. Reduced Susceptibility of *Staphylococcus aureas. Morbidity and Mortality Weekly Report*, Vol. 46, No. 27: pp.620

Khurshid, M. A. 2000. *Staphylococcus aureas* with Reduced Susceptibility to Vancomycin. *Morbidity and Mortality Weekly Report*, Vol. 48, No. 51 pp. 1165-1167

Levy, Stuart B. 1998. The Challenge of Antibiotic Resistance. *Scientific American.*

Levy, Stuart B. 1992. *The Antibiotic Paradox: How Miracle Drugs are Destroying the Miracle.* New York: Plenum Publishers.

Montague, Peter. 1998. Drugs in the Water. *Rachel's Environment and Health Weekly,* No. 614 Electronic Edition. http://www.moniter.net/rachel

Oram, Raymond. 1994. *The Biology of Living Systems.* Glencoe/ McGraw-Hill.

Raloff, Janet. 1998. Drugged Waters. *Science News Magazine*, Vol. 153, No. 12 pp. 187-189.

Wilson, Marion, Helen Mizer. 1974. *Microbiology in Patient Care.* New York: Macmillan Publishing Company.

Windholz, Martha, ed. 1983. *Merck Index 10th Edition.* Rahway: Merck & Co. INC.

Wistreich, George A., Max D. Lechtman. 1988. *Microbiology.* New York: Macmillan Publishing Company.

Appendix

Site Antibiotic Concentration Data

Week	Site	Drug	Conc. Avg.*
1	1	V	1.0 ± 0.0 ppt
		T	1.4 ± 0.2 ppt
		P	2.0 ± 0.0 ppt
	2	V	1.0 ± 0.0 ppt
		T	1.6 ± 0.2 ppt
		P	2.1 ± 0.2 ppt
	3	V	1.0 ± 0.0 ppt
		T	1.7 ± 0.2 ppt
		P	2.3 ± 0.2 ppt
	4	V	1.2 ± 0.2 ppt
		T	1.8 ± 0.2 ppt
		P	2.4 ± 0.2 ppt
	5	V	1.6 ± 0.2 ppt
		T	2.0 ± 0.0 ppt
		P	2.7 ± 0.2 ppt
	6	V	1.1 ± 0.2 ppt
		T	1.7 ± 0.2 ppt
		P	2.7 ± 0.2 ppt
	7	V	1.3 ± 0.2 ppt
		T	1.9 ± 0.2 ppt
		P	2.3 ± 0.2 ppt
Week	Site	Drug	Conc. Avg.*
4	1	V	1.6 ± 0.2 ppt
		T	2.5 ± 0.0 ppt
		P	3.9 ± 0.2 ppt
	2	V	1.7 ± 0.2 ppt
		T	2.8 ± 0.2 ppt
		P	4.0 ± 0.0 ppt
	3	V	1.8 ± 0.2 ppt
		T	3.1 ± 0.2 ppt
		P	4.2 ± 0.2 ppt
	4	V	2.2 ± 0.2 ppt
		T	3.3 ± 0.2 ppt
		P	4.4 ± 0.2 ppt
	5	V	3.8 ± 0.2 ppt
		T	4.2 ± 0.2 ppt
		P	5.9 ± 0.2 ppt
	6	V	2.9 ± 0.2 ppt
		T	3.9 ± 0.2 ppt
		P	5.2 ± 0.2 ppt
	7	V	2.5 ± 0.0 ppt
		T	3.9 ± 0.2 ppt
		P	4.2 ± 0.2 ppt
Week	Site	Drug	Conc. Avg.*
7	1	V	0.9 ± 0.2 ppt
		T	2.1 ± 0.2 ppt
		P	3.1 ± 0.2 ppt
	2	V	1.0 ± 0.0 ppt
		T	2.2 ± 0.2 ppt
		P	3.3 ± 0.2 ppt
	3	V	1.3 ± 0.2 ppt
		T	2.4 ± 0.2 ppt
		P	3.9 ± 0.2 ppt
	4	V	1.4 ± 0.2 ppt
		T	2.6 ± 0.2 ppt
		P	3.8 ± 0.2 ppt
	5	V	1.9 ± 0.2 ppt
		T	3.3 ± 0.2 ppt
		P	4.7 ± 0.2 ppt
	6	V	1.8 ± 0.2 ppt
		T	3.2 ± 0.2 ppt
		P	4.9 ± 0.2 ppt
	7	V	1.7 ± 0.2 ppt
		T	2.9 ± 0.2 ppt
		P	4.0 ± 0.0 ppt

Week	Site	Drug	Conc. Avg.*
2	1	V	1.0 ± 0.0 ppt
		T	1.2 ± 0.2 ppt
		P	1.8 ± 0.2 ppt
	2	V	1.1 ± 0.2 ppt
		T	1.4 ± 0.2 ppt
		P	1.9 ± 0.2 ppt
	3	V	1.0 ± 0.0 ppt
		T	1.5 ± 0.2 ppt
		P	2.0 ± 0.0 ppt
	4	V	1.1 ± 0.2 ppt
		T	1.8 ± 0.2 ppt
		P	1.9 ± 0.2 ppt
	5	V	1.5 ± 0.0 ppt
		T	1.9 ± 0.2 ppt
		P	2.7 ± 0.2 ppt
	6	V	1.4 ± 0.2 ppt
		T	1.9 ± 0.2 ppt
		P	2.2 ± 0.2 ppt
	7	V	1.1 ± 0.2 ppt
		T	1.5 ± 0.0 ppt
		P	1.9 ± 0.2 ppt
Week	Site	Drug	Conc. Avg.*
5	1	V	1.0 ± 0.0 ppt
		T	2.1 ± 0.2 ppt
		P	3.6 ± 0.2 ppt
	2	V	1.2 ± 0.2 ppt
		T	2.1 ± 0.2 ppt
		P	3.6 ± 0.2 ppt
	3	V	1.3 ± 0.2 ppt
		T	2.3 ± 0.2 ppt
		P	3.7 ± 0.2 ppt
	4	V	1.5 ± 0.0 ppt
		T	2.4 ± 0.2 ppt
		P	3.8 ± 0.2 ppt
	5	V	2.1 ± 0.2 ppt
		T	2.9 ± 0.2 ppt
		P	4.1 ± 0.2 ppt
	6	V	1.9 ± 0.2 ppt
		T	2.8 ± 0.2 ppt
		P	3.8 ± 0.2 ppt
	7	V	1.8 ± 0.2 ppt
		T	2.5 ± 0.0 ppt
		P	3.8 ± 0.2 ppt
Week	Site	Drug	Conc. Avg.*
8	1	V	0.7 ± 0.2 ppt
		T	1.8 ± 0.2 ppt
		P	2.9 ± 0.2 ppt
	2	V	1.0 ± 0.0 ppt
		T	2.0 ± 0.0 ppt
		P	3.3 ± 0.2 ppt
	3	V	1.2 ± 0.2 ppt
		T	2.4 ± 0.2 ppt
		P	3.4 ± 0.2 ppt
	4	V	1.4 ± 0.2 ppt
		T	2.5 ± 0.0 ppt
		P	3.8 ± 0.2 ppt
	5	V	2.0 ± 0.0 ppt
		T	3.1 ± 0.2 ppt
		P	4.2 ± 0.2 ppt
	6	V	1.7 ± 0.2 ppt
		T	3.0 ± 0.0 ppt
		P	4.0 ± 0.0 ppt
	7	V	1.5 ± 0.0 ppt
		T	2.7 ± 0.2 ppt
		P	3.8 ± 0.2 ppt

Week	Site	Drug	Conc. Avg.*
3	1	V	1.0 ± 0.0 ppt
		T	1.5 ± 0.0 ppt
		P	2.1 ± 0.2 ppt
	2	V	1.1 ± 0.2 ppt
		T	1.5 ± 0.0 ppt
		P	2.0 ± 0.0 ppt
	3	V	1.0 ± 0.0 ppt
		T	1.7 ± 0.2 ppt
		P	2.3 ± 0.2 ppt
	4	V	1.4 ± 0.2 ppt
		T	1.7 ± 0.2 ppt
		P	2.4 ± 0.2 ppt
	5	V	1.9 ± 0.2 ppt
		T	2.1 ± 0.2 ppt
		P	2.9 ± 0.2 ppt
	6	V	1.6 ± 0.2 ppt
		T	1.8 ± 0.2 ppt
		P	2.7 ± 0.2 ppt
	7	V	1.4 ± 0.2 ppt
		T	1.7 ± 0.2 ppt
		P	2.5 ± 0.0 ppt
Week	Site	Drug	Conc. Avg.*
6	1	V	1.0 ± 0.0 ppt
		T	2.0 ± 0.0 ppt
		P	3.3 ± 0.2 ppt
	2	V	1.1 ± 0.2 ppt
		T	2.1 ± 0.2 ppt
		P	3.9 ± 0.2 ppt
	3	V	1.2 ± 0.2 ppt
		T	2.3 ± 0.2 ppt
		P	3.7 ± 0.2 ppt
	4	V	1.4 ± 0.2 ppt
		T	2.5 ± 0.0 ppt
		P	3.7 ± 0.2 ppt
	5	V	1.9 ± 0.2 ppt
		T	2.9 ± 0.2 ppt
		P	3.9 ± 0.2 ppt
	6	V	2.0 ± 0.0 ppt
		T	2.8 ± 0.2 ppt
		P	3.8 ± 0.2 ppt
	7	V	1.7 ± 0.2 ppt
		T	2.4 ± 0.2 ppt
		P	3.4 ± 0.2 ppt
Week	Site	Drug	Conc. Avg.*
9	1	V	0.7 ± 0.2 ppt
		T	1.7 ± 0.2 ppt
		P	2.4 ± 0.2 ppt
	2	V	0.9 ± 0.2 ppt
		T	1.9 ± 0.2 ppt
		P	2.6 ± 0.2 ppt
	3	V	1.1 ± 0.2 ppt
		T	2.1 ± 0.2 ppt
		P	2.9 ± 0.2 ppt
	4	V	1.3 ± 0.2 ppt
		T	2.3 ± 0.2 ppt
		P	3.0 ± 0.0 ppt
	5	V	1.7 ± 0.2 ppt
		T	2.6 ± 0.2 ppt
		P	3.8 ± 0.2 ppt
	6	V	1.8 ± 0.2 ppt
		T	2.4 ± 0.2 ppt
		P	3.7 ± 0.2 ppt
	7	V	1.5 ± 0.0 ppt
		T	2.3 ± 0.2 ppt
		P	3.6 ± 0.2 ppt

P=Penicillin T=Tetracycline V=Vancomycin

• Each value listed under concentration average represents the mathematical averaging of the data from five test repetitions

Site Antibiotic Concentration Data, Continued

Week	Site	Drug	Conc. Avg.*
10	1	V	1.3 ± 0.2 ppt
		T	2.2 ± 0.2 ppt
		P	3.3 ± 0.2 ppt
	2	V	1.5 ± 0.0 ppt
		T	2.4 ± 0.2 ppt
		P	3.6 ± 0.2 ppt
	3	V	1.8 ± 0.2 ppt
		T	2.8 ± 0.2 ppt
		P	3.8 ± 0.2 ppt
	4	V	2.0 ± 0.0 ppt
		T	3.1 ± 0.2 ppt
		P	4.0 ± 0.0 ppt
	5	V	3.2 ± 0.2 ppt
		T	3.9 ± 0.2 ppt
		P	5.2 ± 0.2 ppt
	6	V	3.0 ± 0.0 ppt
		T	3.8 ± 0.2 ppt
		P	4.8 ± 0.2 ppt
	7	V	2.5 ± 0.0 ppt
		T	3.5 ± 0.0 ppt
		P	4.6 ± 0.2 ppt

P=Penicillin

T=Tetracycline

V=Vancomycin

* Each value listed under concentration average represents the mathematical averaging of the data from five test repetitions

Antibiotic Resistance Data

Site 1 Trial	P	T	V
1	7.0 mm	8.0 mm	7.0 mm
2	7.0 mm	8.0 mm	8.0 mm
3	7.5 mm	8.0 mm	7.5 mm
4	8.0 mm	8.5 mm	8.0 mm
Site 2 Trial	**P**	**T**	**V**
1	7.0 mm	7.0 mm	7.0 mm
2	7.5 mm	7.0 mm	7.0 mm
3	7.5 mm	7.0 mm	7.0 mm
4	7.5 mm	7.5 mm	7.5 mm
Site 3 Trial	**P**	**T**	**V**
1	6.5 mm	7.0 mm	6.5 mm
2	6.5 mm	7.0 mm	7.0 mm
3	6.5 mm	7.5 mm	7.0 mm
4	7.0 mm	7.5 mm	7.0 mm
Site 4 Trial	**P**	**T**	**V**
1	6.0 mm	6.0 mm	6.5 mm
2	6.0 mm	7.0 mm	6.5 mm
3	6.5 mm	7.5 mm	7.0 mm
4	7.0 mm	7.5 mm	7.0 mm
Site 5 Trial	**P**	**T**	**V**
1	5.5 mm	5.0 mm	5.0 mm
2	5.5mm	5.0 mm	5.0 mm
3	6.0 mm	5.0 mm	5.0 mm
4	6.0mm	5.5 mm	5.0 mm
Site 6 Trial	**P**	**T**	**V**
1	6.0 mm	5.5 mm	5.0 mm
2	6.0 mm	5.5 mm	6.0 mm
3	6.0 mm	6.0 mm	6.5 mm
4	6.5 mm	7.0 mm	6.5 mm
Site 7 Trial	**P**	**T**	**V**
1	6.5 mm	6.0 mm	7.0 mm
2	6.5 mm	6.5 mm	7.0 mm
3	6.5 mm	6.5 mm	7.0 mm
4	7.0 mm	7.0 mm	7.5 mm

P=Penicillin T=Tetracycline V=Vancomycin

Tap WaterData

Site 8

Trial	P	T	V
1	1.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.0 ± 0.0 ppt
2	1.5 ± 0.2 ppt	0.5 ± 0.2 ppt	0.0 ± 0.0 ppt
3	1.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.0 ± 0.0 ppt
4	1.5 ± 0.2 ppt	0.5 ± 0.2 ppt	0.0 ± 0.0 ppt
5	1.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
6	2.0 ± 0.2 ppt	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt
7	2.0 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
8	1.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
9	1.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.0 ± 0.0 ppt
10	1.5 ± 0.2 ppt	0.5 ± 0.2 ppt	0.0 ± 0.0 ppt
11	1.5 ± 0.2 ppt	0.5 ± 0.2 ppt	0.0 ± 0.0 ppt
12	2.0 ± 0.2 ppt	0.5 ± 0.2 ppt	0.0 ± 0.0 ppt
13	1.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.0 ± 0.0 ppt
14	1.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
15	1.5 ± 0.2 ppt	0.5 ± 0.2 ppt	0.0 ± 0.0 ppt

Site 9

Trial	P	T	V
1	2.0 ± 0.2 ppt	0.5 ± 0.2 ppt	0.0 ± 0.0 ppt
2	2.0 ± 0.2 ppt	1.0 ± 0.2 ppt	0.0 ± 0.0 ppt
3	2.0 ± 0.2 ppt	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt
4	1.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.0 ± 0.0 ppt
5	2.0 ± 0.2 ppt	1.0 ± 0.2 ppt	0.0 ± 0.0 ppt
6	2.0 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
7	2.0 ± 0.2 ppt	1.0 ± 0.2 ppt	0.0 ± 0.0 ppt
8	1.5 ± 0.2 ppt	1.5 ± 0.2 ppt	0.0 ± 0.0 ppt
9	2.0 ± 0.2 ppt	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt
10	2.0 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
11	2.0 ± 0.2 ppt	1.0 ± 0.2 ppt	0.0 ± 0.0 ppt
12	2.0 ± 0.2 ppt	0.5 ± 0.2 ppt	0.0 ± 0.0 ppt
13	2.0 ± 0.2 ppt	0.5 ± 0.2 ppt	0.0 ± 0.0 ppt
14	1.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
15	2.0 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt

Site 10

Trial	P	T	V
1	2.0 ± 0.2 ppt	1.5 ± 0.2 ppt	0.0 ± 0.0 ppt
2	2.0 ± 0.2 ppt	1.5 ± 0.2 ppt	0.5 ± 0.2 ppt
3	2.0 ± 0.2 ppt	1.5 ± 0.2 ppt	0.0 ± 0.0 ppt
4	1.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
5	1.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
6	1.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
7	2.0 ± 0.2 ppt	1.0 ± 0.2 ppt	0.0 ± 0.0 ppt
8	2.0 ± 0.2 ppt	1.5 ± 0.2 ppt	0.5 ± 0.2 ppt
9	2.0 ± 0.2 ppt	1.5 ± 0.2 ppt	0.5 ± 0.2 ppt
10	1.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
11	2.0 ± 0.2 ppt	1.0 ± 0.2 ppt	0.0 ± 0.0 ppt
12	2.0 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
13	1.5 ± 0.2 ppt	1.5 ± 0.2 ppt	0.5 ± 0.2 ppt
14	1.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
15	2.0 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt

P=Penicillin T=Tetracycline V=Vancomycin

Filtration Data

Sand Filtration Trial	P (@3.5 ppt) Concent. Reduction	T (@2.5 ppt) Concent. Reduction	V (@1.5 ppt) Concent. Reduction
1	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt
2	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt
3	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt
4	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt
5	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt
6	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt
7	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt
8	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt
9	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt
10	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt
11	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt
12	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt
13	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt
14	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt
15	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt

Yeast Filtration Trial	P (@3.5 ppt) Concent. Reduction	T (@2.5 ppt) Concent. Reduction	V (@1.5 ppt) Concent. Reduction
1	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt
2	0.5 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
3	0.5 ± 0.2 ppt	0.0 ± 0.0 ppt	0.5 ± 0.2 ppt
4	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt	1.0 ± 0.2 ppt
5	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt
6	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt
7	0.5 ± 0.2 ppt	1.0 ± 0.2 ppt	1.0 ± 0.2 ppt
8	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt
9	0.0 ± 0.0 ppt	0.0 ± 0.0 ppt	0.5 ± 0.2 ppt
10	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt	1.0 ± 0.2 ppt
11	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt
12	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt
13	0.0 ± 0.0 ppt	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt
14	1.0 ± 0.2 ppt	1.0 ± 0.2 ppt	0.5 ± 0.2 ppt
15	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt	0.5 ± 0.2 ppt

Charcoal Filtration Trial	P (@3.5 ppt) Concent. Reduction	T (@2.5 ppt) Concent. Reduction	V (@1.5 ppt) Concent. Reduction
1	2.5 ± 0.2 ppt	2.5 ± 0.2 ppt	1.5 ± 0.2 ppt
2	3.0 ± 0.2 ppt	2.0 ± 0.2 ppt	1.5 ± 0.2 ppt
3	2.5 ± 0.2 ppt	2.5 ± 0.2 ppt	1.0 ± 0.2 ppt
4	2.5 ± 0.2 ppt	2.5 ± 0.2 ppt	1.0 ± 0.2 ppt
5	2.5 ± 0.2 ppt	2.5 ± 0.2 ppt	1.5 ± 0.2 ppt
6	2.5 ± 0.2 ppt	2.5 ± 0.2 ppt	1.5 ± 0.2 ppt
7	3.0 ± 0.2 ppt	2.5 ± 0.2 ppt	1.0 ± 0.2 ppt
8	2.5 ± 0.2 ppt	2.5 ± 0.2 ppt	1.5 ± 0.2 ppt
9	3.0 ± 0.2 ppt	2.5 ± 0.2 ppt	1.5 ± 0.2 ppt
10	2.5 ± 0.2 ppt	2.5 ± 0.2 ppt	1.5 ± 0.2 ppt
11	2.5 ± 0.2 ppt	2.5 ± 0.2 ppt	1.5 ± 0.2 ppt
12	2.5 ± 0.2 ppt	2.5 ± 0.2 ppt	1.0 ± 0.2 ppt
13	3.0 ± 0.2 ppt	2.0 ± 0.2 ppt	1.5 ± 0.2 ppt
14	3.0 ± 0.2 ppt	2.5 ± 0.2 ppt	1.5 ± 0.2 ppt
15	2.5 ± 0.2 ppt	2.5 ± 0.2 ppt	1.5 ± 0.2 ppt

P=Penicillin T=Tetracycline V=Vancomycin