2006

The presence, prevalence and antibiotic resistance of microbes in the Pamunkey River Basin

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Spring 2006

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This thesis has been accepted as part of the honors requirements in the Department of Biology

ABSTRACT

This project examined the presence, prevalence and antibiotic resistance of Coliforms (mainly *Escherichia coli*), *Staphloccocus aureus*, fecal *Streptococcus*, and *Enterobacteriacae* found in the water samples taken from three selected sites on the North Anna, South Anna, and Little Rivers of the Pamunkey River Basin. The diversity and concentration of the microbes isolated from these sites was evaluated in late January and late March allowing for a comparison of the occurrence of microbes at different times and between the different sites. Over the course of two months the concentration of colonies increased based on colony counts from Nutrient agar. The rate of fecal coliforms at the different sites remained low despite increase in the total concentration of coliforms/ml in March that exceeded EPA safe water guidelines. Antibiotic resistance was also evaluated using isolates from all sites and most demonstrated resistance to at least one antibiotic (as defined by growth at antibiotic concentrations greater than 8 mg/ml). There were no resistant organisms to chloramphenicol, kanamycin and tetracycline, while penicillin and erythromycin had a resistant organism at every site. The water quality of the Pamunkey River Basin should continue to be monitored based on the evidence of antibiotic resistance and pollution.

INTRODUCTION

Bacterial antibiotic resistance became a serious problem following the introduction of penicillin and other broad spectrum antibiotics in the mid-twentieth century. The current use of antibiotics in agricultural, medical, and veterinary settings can greatly exacerbate the problem when overuse causes an increase in the concentration of antibiotics and antibiotic resistant bacteria found in the environment (Kümmerer 2004).

A low level concentration of an antibiotic in water or soil can lead to resistance in a previously susceptible strain of bacteria because it stunts the propagation of susceptible isolates in the species and leaves resistant organisms to thrive under less competitive stress. In addition, the presence of antibiotic resistant organisms can lead to antibiotic resistance in a susceptible organism because bacteria share plasmids that can confer resistance during conjugation. Taken in combination, a low level of antibiotics and antibiotic resistant organisms are a real threat that exists in many streams and rivers potentially leading to an increase of resistance in environmental organisms. (Kümmerer 2004)

The readiness and ease with which bacteria can exchange plasmids not only within the same species but with widely diverse species is felt to be the larger problem in antibiotic resistance. In one study performed in Norway, researchers evaluated the ability of microorganisms from different origins (human, animal and marine) to trade R plasmids in simulated natural conditions compared to standard laboratory conditions and concluded that the different pathogenic species studied could without difficulty conjugate and share resistance elements in the environment. In one of the conditions examined, researchers found that microbes would transfer their R plasmids to others even under harsh conditions in new environments (Kruse and Sørum 1994). This means that even though *Escherichia coli* is not targeted by antibiotic regimens for feedlot cattle, resistant *E. coli* present in the cattle can easily serve as a reservoir of resistance for the bacteria that are targeted with the antibiotic regimen (Galland, et. al 2001).

In addition to the main role of resistant microbes other factors do play a role in the increase of environmental antibiotic resistance. For instance, scientists in Spain found

that while the draining of urban waste water into a local river created an increase in the antibiotic resistance in the aquatic bacteria, the ability of *Enterobacteracae,* microbes that generally have human or animal origins, and *Aeromonas* sp., naturally occurring waterborne bacteria, to exchange resistance plasmids was relatively limited. This suggests that other factors such as the presence of antibiotics that should be investigated as an important source for antibiotic resistance in aquatic environments. (Goñi-Urriza et. al 2000)

It is very important to monitor the contamination of rivers and other bodies of freshwater by sources such as human sewage and agricultural wastes due to the use of antibiotics in medicine and food production. Excess antibiotic residues and resistant bacteria in feces can increase selective pressures for antibiotic resistance in sensitive environmental strains (Kümmerer 2004) through selective forces or plasmid transfer. The observation of illegal dumping of raw sewage and the adjacent agricultural land usage (Vogelmann, et al 1998) highlight the necessity of determining the severity of fecal contamination in the Pamunkey River Basin.

To monitor this type of pollution, fecal coliforms are commonly selected as indicators of pollution because the organisms are associated with many sources from agriculture to sewage. In fact, efforts are currently focusing upon locating the actual source of these pollutants based on their pattern of antibiotic resistance. Mutliple-antibiotic resistance (MAR) profiles of *E. coli* were used to categorize the pollutants of an estuary as originating from point sources such as wastewater from cities and industries or from nonpoint sources such as agriculture (Parveen, et. al 1997). MAR profiles are not, however, the most accurate methods of classifying sources because they do not take into

account the level of resistance found in the microbes by testing different concentrations of antibiotics which this experiment will perform. In addition, the sole use of *E. coli* to determine the source of pollution is problematic because fecal coliforms are sometimes difficult to locate and isolate from potential sources for comparison. Research already conducted in Virginia utilized fecal streptococci from two rural creeks in the Page Brook basin to determine the nonpoint source (cattle, poultry, human or wildlife) it originated from by profiling the degree of resistance in the microbes and comparing it to known exemplars (Hagedorn, et. al 1999, Wiggins 1996, and Wiggins(a), et. al 1999). From this data, new land practices were established that decreased the amount of pollution in these streams dramatically (Graves, et. al 2002).

The study aims to survey the presence, prevalence and resistance of specific microbes found at three sites along the Pamunkey River Basin in order to determine what studies should be performed next. Specifically we are examining fecal coliforms, fecal *Streptococcus* and *Staphylococcus aureus*.

METHODS

Preparation of media to grow bacterial cultures.

Media used in this experiment included Nutrient, Levine EMB, Mannitol salt, MacConkey, and KF Streptococcus agar as well as Nutrient broth. All were prepared based on instructions provided by manufacturer and poured into sterile petrie dishes immediately after being removed from the autoclave. Nutrient broth was also prepared following manufacturer instructions, autoclaved and stored for use in bacteria isolation and antibiotic resistance studies.

Collection of water samples.

On January 28 and March 23 of 2005, water samples were collected in 35 ml sterile conical tubes from the North Anna, South Anna and Little Rivers (abbreviated NA, SA, and LR respectively) of the Pamunkey River Basin at intersections between the rivers and Route 1 in northern Virginia.

Enumeration of bacteria in the water samples collected.

The concentration of bacteria/ml in the water was determined by plating and counting bacterial colonies on nutrient agar. Undiluted samples (100 µl aliquots) were plated from each site in addition to a 1:10 dilution of the samples. Each dilution of the samples had three replicates. All inoculated plates were incubated for two days at 37°C. The number of colonies was counted on each plate and recorded.

Presence/Prevalence of different species in the water samples collected.

The prevalence of *Staphloccocus aureus*, fecal *Streptococcus*, and *Enterobacteriacae* was determined through the use of Mannitol Salt, KF Streptococcus, and Levine EMB and MacConkey agars respectively. One ml and 100 µl of each of the samples were plated onto a plate of each of the differential media. The plates were incubated for two days at 37°C. The number of colonies on each plate were counted and recorded.

Presence/Prevalence of fecal coliforms.

Water collected from each site (3 ml) was inoculated into the Coliscan easy gel system in replicates of three. All plates were allowed to solidify at room temperature before incubation at 37°C overnight. The number of fecal coliforms and coliforms from different sources was then counted to determine prevalence.

Isolation and visualization of selected colonies

Streak isolation was performed on select colonies from various media to ensure generation of pure cultures for later studies. Gram staining was visualized at 100X to determine form and cell wall structure of the isolates.

Antibiotic resistance spectra of isolated microbes.

During January 2006, frozen samples were streaked out onto nutrient agar plates to begin antibiotic resistance studies. Approximately half of the 44 samples preserved were considered lost after repeated failure to grow. The resistance of the microbes from each site was evaluated using Minimum Inhibitory Concentrations (MIC) to eight different antibiotics, kanamycin, erythromycin, ceftriaxone, cefotoxitin, ampicillin, penicillin, chloramephenicol, and tertracycline (abbreviated Kana, Erythro, Ceft, Cefo, Amp, Pen, Chlora, and Tet respectively). MICs test the resistance of a static concentration (approximately 1000 colonies/ml) of microbe with decreasing concentrations of an antibiotic. A colony was selected from the streak isolation plates and grown overnight at 37°C for and had an approximate concentration of 10^9 colonies per milliliter and then diluted to a final concentration of 1000 colonies/ml. This concentration (125 µl) was placed into ninety-five wells of a ninety-six well microtiter plate. (The other well is filled with nutrient broth to serve as a blank during reading.) Antibiotic solutions of 1000 µg/ml for each antibiotic were prepared and 125 µl of the antibiotic was added to the first well in a row on the plate. This creates a 1:2 dilution in the first well and from this dilution 125 µl is withdrawn and placed in the next well decreasing the antibiotic concentration by half. This process is propagated down the row until the eleventh well is reached creating a serial dilution that can be used to determine the lowest concentration

that can inhibit the bacteria selected. The twelfth row is left as a control to ensure that the organism has grown. Due to the necessity of a blank, one antibiotic (tetracycline) was diluted ten times instead eleven as the other antibiotics.

Table 1. *Enumeration of bacteria in the water samples collected.* Water samples were cultivated from three sites on the Pamunkey River Basin on nutrient agar in 1:1 and 1:10 dilutions and averaged to generate the number of colonies/ml at each site. All sites show an increase in bacteria/ml between January and March.

Location	January	March
NA	6.65	270
SA	107.5	136.67
LR	181.5	221.67

RESULTS

A primary goal of this study was to provide an understanding of the overall frequency of microbes in the Pamunkey River Basin based on the sampling of three different sites within the region at two different points in time. To that end, the concentration of microbes/ml was determined for the three sites sampled based on growth from nutrient plates (Table 1). All sites show a marked increase in the concentration of colonies in March compared to January, especially at the site along the North Anna River. While the other two sites increase around 30 colonies/ml, the NA sample increases by more the 200 colonies/ml.

Table 2. *Prevalence of Fecal* Streptococcus. Concentrations (colonies/ml) of fecal *Streptococcus* were evaluated at the three different sites. The bolded number indicates that only one plate was used in the calculation of prevalence due to uncountable growth on the other plate.

Location	January	March
NA	5	0
SA	0	0
LR	1	**0**

While modest for both months, the growth of colonies on KF *Streptococcus* and Mannitol salt agar indicate that fecal *Streptococcus* and *Staphylococcus aureus* (respectively) may be present (Tables 2 and 3). Two of the sites, NA and LR, show an increase in the concentrations of colonies grown on Mannitol salt plates, but SA shows a decrease. While all three sites grew colonies on the Mannitol salt agar during January and March, no colonies were represented for SA on KF *Streptococcus* plates at any time and NA had no colonies in March. At the other sites, fecal *Streptococcus* levels

Table 3. *Colonies/ml of bacteria grown on Mannitol Salt agar.* Two of the three sites show an increase in the amount of colonies/ml found between January and March; however, South Anna shows a decline in concentration.

Location	January	March
NA	1	6
SA	10.5	2.5
LR	1	21.5

were low with the highest concentration present in January at NA (5 colonies/ml). No fecal streptococcus was seen in March with the exception of one plate from the Little River site which could not be counted. Gram staining later confirmed the presence of fecal *Streptococcus* and *Staphylococcus aureus* at LR while Gram positive and Gram negative cocci of unknown origin were seen in isolates from NA.

Gram staining also showed that Gram negative bacilli were present at all three sites, strengthening the findings that indicate the presence of *Enterobacteriacae* (Tables 4 and 5) from all sites except NA in January. During this time, the concentrations at SA and LR were around 200 microbes/ml while no bacteria were detected at the NA site when 100 µl were plated. All sites showed growth when 1 ml of water was plated, however, the number of colonies on these plates could not be determined for any site. Both of the plates from NA and LR showed one lac + colony that covered a large portion of the plates, while the plate for SA could not be counted due to liquefying of the agar plate. Collection in March showed a decrease in total number of microbes/ml, but the presence of *lac* – bacteria was indicated in contrast to its absence of at every site in January.

Table 4. *Concentrations of Gram negative bacilli based on MacConkey medium for January.* The concentrations (in colonies/ml) of the three different sites were based on counts from plating 100 µl of the water sample because 1 ml samples were uncountable. There is no evidence that lac – Gram negative bacilli are present during January.

Location	lac +	lac -	Total
NA	0	0	0
SA	230	0	230
LR	190	0	190

Table 5. *The concentrations (colonies/ml) of Gram negative bacilli based on MacConkey agar samples for March.* The presence of lac – bacteria was noted at NA and LR at higher concentrations than lac + bacteria.

Location	lac +	lac -	Total
NA	6.5	16.5	23
SA	5	0	5
LR	25	45.5	70.1

The presence of *lac* – bacteria at NA in March was also confirmed from counts of Levine EMB agar (Tables 6 and 7). Colony counts from this agar show a rise in the overall concentration of colonies/ml from January to March at the NA and LR sites and a decrease at the SA site. The January concentration at SA was based solely on 100 µl sample due to the presence of a large *lac* + colony that obscured counting methods. In addition, the fusion of multiple colonies on the1 ml March NA and LR plates prevented counting. There were 2 colonies from the 100µl LR plate that could not be classified as either *lac* + or – due to their dark color in the March colony counts.

Table 6. *Concentrations (colonies/ml) of bacteria cultivated on Levine EMB agar for January.* No Gram negative bacilli were detected at the NA site in January and very few were seen at LR compared to SA. Bold numbers indicate concentrations based solely on one plate count due to an error in 1 ml sample.

Location	lac +	lac -	Total
NA	0	0	0
SA	**300**	**0**	**300**
LR	5	0	5

ble 7. *Concentrations (colonies/ml) of teria cultivated on Levine EMB agar for rch.* Gram negative bacilli were detected at sites; NA and LR both show a high centration of colonies/ml while SA is less h 20 colonies/ml. The presence of lac – teria was not detected at either SA or LR, ppears to be fairly abundant at NA. Bold bers indicate concentrations based solely ne plate count due to an error in the 1 ml mples.

cation	lac +	lac -	Total
A	**70**	**70**	**140**
A	19.5	0	19.5
R	**170**	**0**	***190***

One of the main functions of Levine EMB medium is to distinguish fecal *E. coli* from other sources by the production of a metallic sheen on the agar plate by the bacteria. No such colonies were observed on any of the plates. This lack of fecal origin for any of the colonies seen on the Levine EMB agar is consistent with the low concentrations of fecal coliforms found during the Coliscan plate counts (Tables 8 and 9). The overall concentrations of coliforms increased from January to March at all three sites; however

Table 8. *Prevalence of coliforms using Coliscan Easy Gel System for January.* No fecal colifroms were detected at NA while less than 2 colonies/ml were seen at either SA or LR.

Location	Fecal Coliform	Nonfecal Coliform	Total
NA	0	2.22	2.22
SA	0.44	6.66	7
LR	1.22	16.33	17.55

Table 9. *Prevalence of coliforms using Coliscan Easy Gel System for March* .Fecal colifroms were present at all 3 sites at levels less than 1 colony/ml.

Location	Fecal Coliform	Nonfecal Coliform	Total
NA	0.22	55.22	55.44
SA	0.33	22.44	22.77
LR	0.55	50.55	51

fecal coliform concentrations decreased at LR and SA.

Antibiotic resistance profiles were determined for 22 isolated microbes (Table 10) from the three different sites. Five of these microbes came from NA, 11 from SA and 6 from LR. Out of these 22 bacteria, 8 were sensitive, 2 were resistant to one antibiotic, 4 were resistant to 2 antibiotics and seven were resistant to three or more antibiotics indicating that if an microbe was resistant it was generally resistant to more than one antibiotic. The only antibiotics that all organisms were susceptible to were Chloramphenicol, Kanamycin, and Tetracycline. If one microorganism was resistant to an antibiotic at either SA or LR at least one other microorganism was resistant to the same drug.

Table 10 a, b and c. *Antibiotic Resistance Profiles.* Twenty-two isolates were tested for antibiotic resistance using MICs. Bolded concentrations (mg/ml) indicate resistance at a concentration above 8 mg/ml. The title Unknown for one isolate indicates a failure to Gram strain correctly while the question mark indicates an antibiotic whose resistance could not be determined due to an abnormality in the MIC data.

a.) Data from NA

Isolate	**Amp**	**Cefo**	**Ceft**	**Chlora**	**Erythro**	**Kana**	**Pen**	**Tet**
Staphylococcus aureus	0.488	0.488	1.95	1.9	0.488	0.488	0.488	0.97
Gram positive coccus	0.488	3.9	7.8	1.9	0.488	0.488	7.8	0.97
Gram negative bacillus	0.488	0.97	0.488	0.97	0.488	0.488	0.488	0.97
Gram variable coccus	3.9	3.9	7.8	1.9	15.6	0.488	15.6	0.97
Gram negative bacillus	0.488	7.8	15.6	1.9	0.488	0.97	0.488	0.97
Gram positive bacillus	3.9	1.9	7.8	1.9	0.488	0.488	3.9	0.97

b.) Data from SA

Isolate	Amp	Cefo	Ceft	Chlora	Erythro	Kana	Pen	Tet
Gram negative bacillus	0.97	7.8	0.488	0.97	62.5	0.488	125	0.97
Gram variable bacillus	1.9	62.5	15.6	1.9	0.488	0.488	125	0.97
Gram negative bacillus	15.6	62.5	0.488	1.9	250	0.488	over 500	0.97
Gram negative bacillus	0.488	3.9	15.6	1.9	0.488	0.488	0.97	0.97
Gram variable bacillus	0.488	1.9	3.9	3.9	0.488	0.488	0.488	0.97
Gram variable bacillus	31.25	3.9	0.97	0.97	125	0.488	over 500	0.97
Gram variable bacillus	0.488	3.9	0.488	0.97	0.488	0.488	1.9	0.97
Gram negative bacillus	0.488	3.9	3.9	1.9	0.488	0.488	0.97	0.97
Gram negative bacillus	15.6	125	0.488	0.97	125	0.488	62.5	0.97
Unknown	1.9	15.6	0.488	3.9	125	0.488	over 500	0.97
Gram negative bacillus	15.6	7.8	3.9	7.8	125	0.488	over 500	3.9

c.) Data from LR

Isolate	Amp	Cefo	Ceft	Chlora	Erythro	Kana	Pen	Tet
Gram negative coccus	0.488	1.9	0.488	0.488	0.488	0.488	0.97	0.97
Gram negative bacillus	7.8	15.6	1.9	1.9	125	?	125	1.9
Gram negative bacillus	0.488	3.9	0.488	0.97	62.5	0.488	62.5	0.97
Gram positive bacillus	3.9	0.97	0.488	1.9	31.25	0.488	31.25	1.9
Gram negative bacillus	0.488	3.9	7.8	3.9	0.488	0.488	0.488	0.97
Gram negative bacillus	1.9	31.25	0.488	1.9	250	0.488	125	0.97

DISCUSION

This project evaluated the number of microbes found at sites in the Pamunkey River Basin including the South Anna River which is known to have adjacent agricultural land usage, the North Anna River, and the Little River. The presence of fecal coliforms, fecal *Streptococcus*, and *Staphylococcus aureus* was also screened for within the vast biodiversity of these three rivers. In addition, the antibiotic resistance of isolated

microbes from each location was evaluated to explore resistance in these river environments.

With the data obtained by nutrient agar counts, concentrations of bacteria were shown to increase with the rising temperatures over the two month period. During collections at both time points, freezing precipitation occurred either during or the day before water samples were acquired. This would have lowered the temperature of the water and could have lead to artificially low concentrations in some or all of the samples taken. The most suspicious data point is for the concentration of microbes in the NA during January at 6.65 colonies/ml. In approximately 2 months this concentration must increase more than 40 times the original reading to reach the concentration of colonies reported in March while the other two sites see around a 30 colonies/ml increase. It is possible that a mistake was made during the plating of the NA samples in January. If this was the case, it would also account for the extremely low concentration of colonies/ml of NA compared to the other two sites. It could also be that NA simply does not have that many colonies/ml in January as supported by the consistently low concentration in all other prevalence studies in January.

Screening for the Gram + cocci, *Staphylococcus aureus* and fecal *Streptococcus* was accomplished through the use of the selective media Mannitol salt and KF *Streptococcus* correspondingly. The colonies present on the Mannitol Salt medium are indicative of *Staphylococcus aureus*; however, more studies should be used to confirm this preliminary finding. Currently, Gram staining confirmed the presence of *Staphylococcus aureus* at LR in March, staining of two isolated organisms indicate that at least one bacillus species from SA will grow on the medium. While a common and

normally harmless species some strains of *Staphylococcus aureus* are quite harmful. A few bacterial pneumonias for instance are caused by *S. aureus* and the organism is one of the most common nosocomial infections. Over time, strains that inhabit different hospitals have become resistant to many of the antibiotics used to treat bacterial infections including methicillin leading to Methicillin Resistant *Staphylococcus aureus* (MRSA) which presents a serious treatment problem in medicine today. (Centers for Disease Control and Prevention) Therefore, it is always prudent to check the levels of *S. aureus* present in the environment and to evaluate the level of resistance in the organism.

Fecal *Streptococcus* is an important bacterium to look for in water assessment studies. In fact, the EPA tests the level of fecal *Streptococcus* when assessing water quality. While the results in this study may look insignificant, the concentrations of fecal *Streptococcus* during January for NA and LR exceed the limits defined for safe recreational water at .33 colonies/ml (Childers and Schulz). It is also probable that if the uncountable LR plate could have been included in the results, LR would have exceeded the limits both months.

While the levels for fecal *Streptococcus* are higher than recreational water limits, all fecal coliform counts from coliscan plates are within the limits for safe recreational water (at 2 colonies/ml). The limit on fecal coliforms in clean water standards is understandable since the occurrence of *Salmonella* increases with the number of fecal coliforms/ml. Despite falling below the concentrations of fecal coliforms for safe water, the total concentration of coliforms/ml is above the 20 microbes/ml limit set by the EPA for all of the March samples. (Childers and Schulz) Concentrations for both fecal

Streptococcus and fecal coliforms will probably continue to rise at the different sites as the water becomes warmer and animals become drawn to the rivers (Graves, et. al 2002).

The level of contamination at these sites based on fecal *Streptococcus* and coliform concentrations are unacceptable based upon criteria defined by the EPA. To aid in addressing this problem, the location of the pollution should be identified. This could be done through the use of antibiotic resistance profiles as in previous Virginia watersheds (Hagedorn, et. al 1999, Wiggins 1996, and Wiggins(a), et. al 1999) but these procedures are cost and resource prohibitive due to the need for a local source library. The best hope for driving these costs down is the creation of multiwatershed library as Wiggins and colleagues have already attempted with some success (Wiggins(b), et. al 2003).

Further evidence affirming the presence of *Enterobacteriacae* is the presence of colonies on both MacConkey and Levine EMB media. One of the most interesting features of the two media is the total absence of *lac* – bacteria are not present during January, but are detectable in March. Further study could focus on reaffirming the absence of *lac* – bacteria in January and explaining why they are not present. Are they simply cold-sensitive or is there another factor to account for that is creating a harsher environment?

The prevalence of the bacteria screened for in this study is prone to error due to the limited number of replicates with plates using MaConkey, Levine EMB, KF *Streptococcus*, or Mannitol Salt agars. In order address this issue further elucidate the temporal framework of these environments and ecosystems, a longitudinal study of at least two years that utilized more replicates than this survey could be done to provide a

better understanding of how temperature changes over time affect what bacteria are present at any given concentration in an environment and how these changes affect the resistance profiles of the organisms.

The antibiotic resistance profiles are perhaps the most intriguing of the results presented in this study. It is important to note that at least three of the antibiotics, erythromycin, cefotoxitin, and penicillin are used in agricultural settings and that the site, SA, with the highest number of antibiotics that are no longer effective has been identified with agricultural land usage. More sites along the SA could be sampled and profiled to determine how this land usage has affected the river. Most organisms exhibiting resistance were resistant to more than one antibiotic; this finding by itself is not surprising since all of the antibiotics (except erythromycin) that organisms were resistant to belonged to the same class of antibiotic, β-lactams. The genes that code for the enzymes that can cleave the lactam bond on these antibiotics either are plasmid linked or chromosomal. (US General Accounting Office 1999) The presence of multiple microbe resistance at one site for an antibiotic would seem to indicate the transfer of plasmids, but it does not have to mean that at all. The number of isolates, 21, examined is quite small to definitively support plasmid or chromosomal resistance. Plasmid isolation may be used to determine if susceptible organism would develop the same resistance as the original colony, but it's a very difficult procedure to perform on wild bacteria compared to genetically engineered bacteria in the lab. In addition to attempting to discover the nature of the gene coding for resistance, the water at a given site could be examined to see if it may cause antibiotic resistance in susceptible organisms. The selective preference for resistance in background antibiotic levels has so far been explored very marginally in

antibiotic resistance studies. In this case, induction studies could test the ability of site water to induce resistance in susceptible lab bacteria.

It is important to continue to evaluate the presence, prevalence and resistance of organisms found in this basin to further elucidate the effects of land usage, climate, and time on these dynamic populations of microbes.

ACKNOWLEDGEMENTS

Dr. Paula Lessem for all her patience, perseverance and guidance, Michaela Clemence and Richard for all of their support.

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