8-1997

A profile of antibiotic resistant bacteria in the James River

Keith Allen Boisvert

A Profile of Antibiotic Resistant Bacteria in the James River

By

Keith Allen Boisvert

A thesis for the degree of

Master of Science

University of Richmond

August, 1997

Thesis Director

Dr. Lisa Muehlstein

A Profile of Antibiotic Resistant Bacteria in the James River

By

Keith Allen Boisvert

B.S., Worcester State College, 1991

A Thesis

submitted to the Graduate Faculty

of the University of Richmond

in Candidacy

for the degree of

MASTER OF SCIENCE

in

Biology

August, 1997

Richmond, Virginia

Abstract

Spatial and temporal variability in the antibiotic resistance of bacterial assemblages in the lower portion of the James River was examined. The antibiotic resistance of culturable coliforms was compared to the resistance in the microbial community. Antibiotic resistance was determined via colony formation on media containing antibiotics. The antibiotics ampicillin, chloramphenicol, streptomycin and tetracycline were used at concentrations ranging from 10 to 500 ug/ml. Statistically significant differences in the mean number of antibiotic resistant isolates were observed among both sample sites and dates. The sampling site at river mile 99 just below Richmond showed both increased antibiotic resistance and bacterial density making this site a possible public health risk. It is possible that increased salinity in the lower portion of the river resulted in a significant decrease in bacterial density and antibiotic resistance. The percentage of antibiotic resistant coliform bacteria was significantly greater than those isolates representing the microbial community as a whole, except when tested against streptomycin at 10 ug/ml.

Approval Page

I certify that I have read this thesis and find that, in scope and quality, it satisfies the requirements for the degree of Master of Science.

Dr. Lisa Muehlstein

Dr. John Bishop

Dr. Brad Goodner

Acknowledgements

I would like to thank Dr. Lisa Muehlstein and thesis committee members Dr. John Bishop and Dr. Brad Goodner for their assistance, guidance and time.

Thanks also to Dr. Jim Beets, Linda M. Hines and Mr. Mark Schwitzerlett for their help with statistical analysis and computer support. I would also like to thank Mr. Mark Bushing of the Virginia Department of Environmental Quality.

Table of Contents

Introduction

L. Pasteur and J.F. Joubert recognized the clinical potential of antibiosis in 1877. The modern era of antimicrobial therapy began some 50 years later in the mid 1930s with the discovery of prontosil. Since the 1940s thousands of antibiotics have been isolated, identified and used to combat infectious disease (Davies, 1994). Bacteria have been evolving, developing and exchanging mechanisms to resist the effects of these antimicrobial substances (Davies, 1994). This resistance could bring about a medical disaster that would return physicians to the pre-penicillin days when even seemingly small infections could turn lethal for lack of effective drugs (Travis, 1994).

The antibiotics used in this study to detect resistance were ampicillin, chloramphenicol, streptomycin and tetracycline. These antibiotics were used in previous studies to determine antibiotic and multiple antibiotic resistance (MAR) in aquatic environments (Murray et al., 1984; Zelibor et al., 1987; DeVincente et al., 1989; Ogan & Nwiika, 1992; Wnorowski et al., 1993). Ampicillin inhibits cell wall synthesis (Hof, 1991). Chloramphenicol, streptomycin and tetracycline have different modes of inhibiting protein synthesis (Roberts, 1992). However, strains of bacteria resistant to antibiotics are a serious health problem (Murray et al. 1984; Travis, 1994).

There are two main types of bacterial antibiotic resistance: innate and acquired. Innate resistance is when all members of a bacterial species are resistant to an antibiotic. This

resistance usually is due to the group or species physiologic or biochemical makeup. A bacterial group, such as mollicutes, might lack an antibiotic binding site or target. Mollicutes are the smallest type of bacteria and lack cell walls. Mollicutes are innately resistant to penicillin and cephalosporin because they lack cell walls and the corresponding penicillin or cephalosporin binding sites found in those walls (Roberts, 1992). Access to an antibiotic target site might be reduced via membrane permeability (Roberts, 1992). An example of this would be Gram-negative bacteria such as Escherichia coli. Gram-negative bacteria are surrounded by a lipopolysaccharide (LPS) bilayer that acts as a barrier against the diffusion of many antibiotics into the cell (Nikaido, 1994). However, hydrophilic antibiotic molecules readily penetrate this layer (Nikaido, 1994). Other bacteria can secrete enzymes that destroy certain antibiotics (Davies, 1994). The best example of this would be the bacterial enzyme penicillinase. Penicillinase hydrolyzes the Beta-lactam ring of penicillin which interferes with penicillin therapy (Davies, 1994). This type of resistance may also be acquired.

Acquired resistance is a decline in the therapeutic value of a drug due to the emergence of resistant organisms. This kind of resistance often results from prolonged clinical use of the drug. Resistance may result from 1) a spontaneous mutation in the infecting organism (Roberts, 1992; Davies, 1994) 2) the selection of preexisting resistant organisms from within a larger pool of sensitive organisms, (Walter & Vennes, 1985), 3)

resistance via gene acquisition. Plasmids are the most common genetic elements that carry resistance genes among bacteria (Mach & Grimes, 1982). If a plasmid carries genes that code for resistance to one or more antibiotics the plasmid is considered a Resistance or R-factor (Davies, 1994).

Various factors and processes may contribute to increased bacterial antibiotic resistance. These include bacterial exposure to antibiotics, heavy metals, low nutrient levels in aquatic environments and wastewater treatment (Murray et al., 1984; Pathak et al., 1993; Summers,, 1993).

Wastewater treatment increases the transfer of antibiotic resistance (Bell, 1978; Bell et al., 1981; Mach & Grimes, 1982; Murray et al., 1984; Walter & Vennes, 1985). Processes like chlorination lower the total number of bacteria in sewage but may substantially increase the proportions of antibiotic resistant and potentially pathogenic organisms. It is not clear whether chlorination selects for or induces antibiotic resistance and no physiologic link between chlorine and antibiotic resistance presents itself (Murray et al., 1984). Wastewater treatments have also been found to increase the proportion of bacteria carrying R-factors (Murray et al., 1984). Conventional sewage treatment systems favor the transfer of R-factors via conjugation (Bell, 1978; Bell et al., 1981; Meckes, 1982). Nonpathogenic strains can transfer resistance to other strains via the transfer of R-factors (Murray et al., 1984). This may also increase the incidence of bacteria resistant to more than one antibiotic.

Resistance acquired in this manner is usually not just to one drug but to several at a time (Baldry, 1976).

Bacteria resistant to two or more antibiotics are known as multiple antibiotic resistant (MAR) bacteria. MAR bacteria are significant because if their resistance genes are carried on a plasmid, the genes are genetically linked and this multiple resistance capacity can be transferred, en bloc, to other strains (Baldry, 1976; Davies, 1994). Also significant is the difficulty in treating infections caused by (MAR) pathogens. A good example of this is the bacterium Mycobacterium tuberculosis which causes tuberculosis. The rise of MAR strains of M. tuberculosis has resulted in many cases of marginally treatable, often fatal tuberculosis (Iseman, 1993). Another example is Salmonella which causes enteric fever (Alam et al., 1995). Salmonella typhi and S. paratyphi are particularly frequent in sewage polluted fresh water (Rheinheimer, 1992).

Information about the temporal and spatial distribution of resistant and MAR bacteria would be helpful in deciding which areas of the James River might be potential health risks. This information is significant because approximately two million people, one third of Virginia's population, live in the James River watershed and use its waters. By the year 2000 this number is projected to be 2.3 million (Sevebeck et al., 1989). There are now 21 significant municipal dischargers on the river. Most urban development and industrial activity is concentrated at or below the fall line in Richmond, Petersburg, Hopewell and Hampton

Roads. Pollution by nutrients just below Richmond is a growing concern. The James River receives the highest nutrient input of any river in Virginia (Gregory et al., 1994). These nutrients come from sewage treatment plants, industrial discharges, urban runoff and agriculture.

A major problem currently being addressed in Richmond is the sewer system. About 11,000 acres of Richmond's land area are served by sewer pipes, which use one pipe to carry both sewer and storm water to sewage treatment plants. During heavy rains the volume of water is too great for plants to handle and excess water goes directly into the river. This water carries large quantities of raw sewage (Gregory et al., 1994). During a large rainfall Richmond's combined sewers contribute more fecal bacteria than all other sources on the river combined (Gregory et al., 1994). This is significant because sewage polluted water may play a role in the spread of bacteria carrying R-factors (Bell et al., 1981). Sewage contaminated water also increases the incidence of antibiotic resistant aquatic bacteria (Pathak et al., 1993). Antibiotic resistant bacteria in both drinking and wastewater pose an important potential health problem (Murray et al., 1984; Al-Jebouri, 1985; DeVincente et al., 1989). Increased use of antimicrobials and dumping of wastewater is creating a reservoir of resistance genes in surface waters (Papapetropoulou, 1996). Surface waters also provide a medium for the spread of antibiotic resistant genes via transduction, conjugation and transformation (Papapetropoulou, 1996).

A contributor to this reservoir of resistance are coliform bacteria. Coliform bacteria also were isolated and their antibiotic resistance profiles determined. The density of coliform bacteria is used to measure water quality. Two types of coliform were studied fecal and total. Fecal coliforms originate from fecal matter and total coliforms may include bacteria that were not fecal in origin. Coliform bacteria are not necessarily pathogenic but the density of these bacteria may indicate the presence of pathogens.

It was hypothesized that antibiotic resistance in the river would be more pronounced in coliform bacteria than non-coliform. A typical coliform would be more likely to be exposed to antibiotics or other antibiotic resistance inducers and exhibit corresponding resistance. One example is antibiotics being selective for R+ plasmid coliforms in the alimentary canals of humans and animals (Walter et al., 1985; Bell et al., 1983). It has also been well documented that many wastewater treatment plants increase the incidence of MAR coliforms by facilitating R plasmid transfer (Mach, 1982; Bell et al., 1983; Murray et al., 1984; Walter et al., 1985). Factors such as these would be reflected in the antibiotic resistant capacities of James River coliforms.

The research described in this thesis focused on the occurrence of single and multiple antibiotic resistance in the microbial community of the James River east of the fall line. Two null hypotheses were tested: 1) there was no spatial

difference in the distribution of antibiotic resistant and MAR bacteria in the lower 110 miles of the James River and, 2) there was no temporal difference in the distribution of antibiotic resistant and MAR bacteria in the lower 110 miles of the James River from January 1995 to May 1995.

In regard to coliform bacteria three null hypotheses were tested: 1) Coliform bacteria in the James River were not significantly more-antibiotic resistant than those bacteria which comprise the aquatic community. Rejection of this null would establish coliform bacteria as a pollutant contributing to increased antibiotic resistance in the James river. 2) There was no spatial difference in the distribution of antibiotic resistant and MAR coliform bacteria in the lower 110 miles of the James River. 3) There was no temporal difference in the distribution of antibiotic resistant and MAR coliform bacteria in the lower James.

Materials and Methods

The study site for these experiments was the section of the James River between river mile 110.34 to approximately river mile 5.72 (Figure 1). This research was conducted from December 1994 to May 1995. One pilot study and six trials were performed. The pilot study was done to determine the types, and concentrations of antibiotics to be used in testing and finalize testing locations along the river. Trial dates were; 1/26/95, 1/29/95, 3/16/95, 4/20/95, 5/16/95 and 5/29/95. Trial dates are referred to as trials 1-6, respectively. A trial consisted of two individual water samples being taken at each of seven sampling sites along the river. Bacteria were isolated from these water samples and their antibiotic resistant capabilities tested.

Test Sites

Seven water sampling or test sites were chosen for their relative equidistance from one another and their correspondence to established Virginia Commonwealth Department of Environmental Quality (DEQ) water quality and or biological monitoring stations located on the river (Figure 1). These water monitoring stations are tested monthly for fecal coliform and salinity levels.

Salinity at sites 6 and 7 was significantly greater than at sites 1-5. Sites 6 & 7 had mean salinity levels of 9.5 and 18 parts per thousand (ppt), respectively. Salinity ranged from 8 to 21 ppt at these sites. Both sites 6 and 7 had statistically significantly higher salinity levels than sites 1-5 (Table 1). Site 7 had a salinity level significantly greater than site 6.

Water Sampling

Approximately 200 milliliter water samples were taken in sterile polypropylene bottles 10 centimeters below the surface of the water and approximately 2 meters from the north shore. At each site two samples were taken approximately 10 meters apart, per trial. Water samples were kept refrigerated for no more than 24 hours before the isolation and enumeration of bacteria began.

Isolation and Enumeration of Bacteria

Bacteria were isolated from each water sample via the spread plate technique. Volumes of 0.01, 0.1, 0.5 and 1 ml, of water sample, or a corresponding serial dilution were transferred to the center of Tryptic Soy Agar (TSA) plates and spread evenly over the surface with a sterile bent glass rod. The TSA plates were incubated at 25 degrees Celsius for 30 hours. The number of resulting visible colonies equaled the number of viable organisms able to grow on the specified media in the presence of oxygen at 25 degrees Celsius. By counting these colonies, the number of viable bacterial cells in the original sample, able to grow under the specified conditions, was determined (Rump & Krist, 1988).

Determination of Bacterial Antibiotic Resistance

Antibiotic resistance was determined by aseptically transferring approximately 40 of the isolated bacterial colonies onto a sterile TSA plate. Only non-contiguous colonies were chosen. The TSA plate receiving these colonies then became the 'Master Plate'. Since there were two water samples taken at a site there were two 'Master Plates' per site, in a trial, each

with approximately 40 bacterial colonies. These colonies were transferred, via replica plating, to TSA plates. These TSA plates had predetermined concentrations of antibiotic incorporated in the medium. Four different antibiotics at three different concentrations each were tested. They were, ampicillin at 10, 100 and 500 ug/ml, chloramphenicol at 10, 25 and 50 ug/ml, streptomycin at 10, 75 and 100 ug/ml, tetracycline at 5, 20 and 50 ug/ml. Antibiotics were added to cooling agar from sterile liquid stock solutions. Stock solutions ranged from 15 to 100 mg/ml (Maniatis et al., 1982).

The inoculated plates containing antibiotic were then incubated for 24 hours at 25 degrees Celsius. After 24 hours, inoculated plates containing antibiotic were compared to control plates, inoculated plates containing no antibiotic. Bacterial colonies growing on both control and test TSA plates were designated resistant to that antibiotic at that concentration. Bacterial colonies that only grew on the control plate and not in the presence of antibiotic were considered sensitive to that antibiotic and concentration.

Determination of MAR

To determine MAR, antibiotics were grouped by concentration. A colony was considered MAR if it was resistant to two or more antibiotics at the lowest test concentrations, middle concentration level or highest level (Gedebou& Tassew, 1980; French et al., 1987). A colony was not deemed MAR if it were resistant to an antibiotic at the lowest test concentration and a

different antibiotic at the highest test concentration. Antibiotics were grouped in this way to more clearly show decreased MAR with increased antibiotic concentration.

Isolation of Coliform Bacteria

The membrane filter technique was used to isolate coliform bacteria. Water samples of 1, 5, and 15 milliliters were filtered through a Gelman 47 mm 0.45 um metricel membrane filter. Bacteria were trapped in the filter. The filter was then placed on EMB levine agar which selects for coliform bacteria and incubated for 24 hours at 37 degrees Celsius. Total coliform bacteria trapped on the filter appeared as red colonies with a metallic sheen (Rump & Krist, 1988).

Determination of Coliform Antibiotic Resistance

Isolation and antibiotic screening of total coliform bacteria were performed via the same methods used to determine bacterial antibiotic resistance above. Coliform isolation and determination of antibiotic resistance were only performed in trials 4, 5 and 6. Atleast 10 coliform colonies were isolated from every water sample taken in trials 4-6.

Coliform colonies were transferred, aseptically, to sterile TSA plates. However, due to the often small number of coliform bacteria isolated at a site as few as 10 coliform bacteria per water sample were tested for antibiotic resistance.

Results

Antibiotic Resistance in the Microbial Community

A total of approximately 3820 bacterial colonies were isolated and their antibiotic resistance profiles determined. The antibiotics ampicillin, chloramphenicol, streptomycin and tetracycline at 3 varying concentrations were used in testing bacterial antibiotic resistance. Tetracycline at 50 ug/ml was the most effective antibiotic and concentration, ampicillin at 10 ug/ml was the least effective (Table 2). This table is significant in that it shows which antibiotics were the most effective against bacteria in the James River.

In order to determine if an increase in antibiotic concentration yielded a significant decrease in the mean number of resistant isolates Tukey's Pairwise Comparisons (ANOVA) was performed (Table 2). There was no significant decrease in the mean number of resistant isolates corresponding to increases in streptomycin from 75 to 100 ug/ml and tetracycline from 20 to 50 ug/ml. Every other increase in antibiotic concentration yielded a significant decrease in the mean number of resistant isolates.

Temporal and Spatial Differences in Resistance

Resistance often was lowest at sites 6 and 7 (Table 3). The best example of this can be seen when bacterial isolates were tested against chloramphenicol (Figure 2). Tukey's Pairwise Comparison was again utilized to determine if observed differences in antibiotic resistance differed significantly

between sites. This analysis was run for every antibiotic and concentration.

Bacterial antibiotic resistance rarely differed between sites 1-5 (Table 3). Often, higher numbers of resistant bacteria occurred at site 2 than other sites. More isolates from site 2 were resistant to streptomycin at 75 and 100 ug/ml and tetracycline at 50 ug/ml than site 5 (Table 3). Isolates at site 3 were resistant to tetracycline at 50 ug/ml more often than isolates from sites 1, 4 and 5. These were the only statistically significant differences between the microbial communities of sites 1-5. Spatial differences were more prevalent when bacterial isolates were being tested against antibiotics with the highest bactericidal effects.

Statistically significant differences in the mean number of resistant bacteria isolated between trial dates occurred most often when those isolates were tested against ampicillin and streptomycin at 10 ug/ml and tetracycline at 5 ug/ml (Table 4). Temporal differences in all resistant bacteria isolated are shown in Table 4. Trials 5 and 6 exhibited increased amounts of resistant bacteria towards ampicillin and streptomycin. No, increase in resistance was observed towards chloramphenicol and tetracycline for trials 5 and 6.

There was no relationship between antibiotic resistance and municipal discharges (Figure 1, Table 3). There was no relationship between non-point sources pollution and antibiotic resistance. Changes in mean numbers of antibiotic resistant

bacteria could not be attributed to either of these variables.

MAR Bacteria

The proportion of bacteria that were MAR at sites 6 and 7were often significantly lower than that of sites 1-5 (Table 5 (Figure 3A)). There were no significant differences in the mean amounts of MAR bacteria isolated between trials (Figure 3B).

Coliform Isolates

Over 500 coliform bacteria were isolated and their antibiotic resistance profiles determined. When countable numbers were achieved, fecal and total coliform bacteria only comprised 0.1 and 0.7 percent, respectively, of the microbial community (Tables 6A & 6B). The microbial community at sites 2 and 3 had a significantly greater amount of colony forming units per milliliter (CFU's/ml) than most other sites (Table 6A). The microbial community during trial 5 also had a significantly greater amount of colony forming units per ml (CFU's/ml) than any other trial (Table 6A). However, there was no significant difference between sites or trials in the amounts fecal coliforms per milliliter (Table 6B). Total coliform numbers were not analyzed statistically.

For every increase in antibiotic concentration a significant decrease in the amount of resistant coliforms was not always observed (Table 7). The effectiveness of test antibiotics and concentrations on coliform isolates are summarized in (Table 7). Ampicillin at 10 ug/ml was the least effective and tetracycline at 50 ug/ml the most effective antibiotic against coliform

isolates. The percentage of antibiotic resistant coliform bacteria was significantly greater than those isolates representing the microbial community as a whole, except when tested against streptomycin at 10 ug/ml (Table 8).

Temporal and Spatial Differences in Resistance

Statistically significant differences in the amounts of resistant coliform isolates between sites are shown in Table 9. Spatial patterns of resistant coliform isolates were similar to those patterns exhibited by the isolates representing the entire microbial community. Sites 6 and 7 exhibited reduced amounts of drug resistant isolates.

Tukey's Comparison determined that of the isolated coliforms the mean resistant in trial 5 was significantly greater than the mean resistant of trial 6 when tested against chloramphenicol at 50 ug/ml, streptomycin at 10 ug/ml, tetracycline 5 and 20 ug/ml. Trial 5 isolates were also significantly more resistant than those of trial 4 when tested against chloramphenicol at 50 ug/ml (data not shown).

MAR Coliforms

Probability dictated that increased numbers of resistant isolates yielded a corresponding increase in the amount of MAR coliform bacteria (Figure 4). The difference in the mean amounts MAR coliform bacteria between sites 1-5 and 6, 7 is not as drastic as the difference observed for isolates representing the entire microbial community. Tukey's Pairwise Comparison revealed no significant temporal or spatial differences in the mean amount

of MAR coliforms isolated. Coliforms were significantly more often MAR than colonies representing the microbial community.

Discussion

This study was aimed at determining the temporal and spatial incidences of culturable antibiotic resistant bacteria in the James River. Spatial differences in the mean number of antibiotic resistant bacteria were consistently seen. Areas of increased resistance could be a potential human health risk and warrant further investigation. Temporal differences in resistance were not cosistently revealed. Also of value was information gathered about the bactericidal effects of antibiotics and concentrations. This information could be applied toward a more detailed study of antibiotic resistant bacteria in the James River.

Increased antibiotic concentration did not always yield a significant decrease in the total number of resistant isolates tested. There was no significant decrease in the mean number of resistant isolates for all 84 replicates when tetracycline was increased from 20 to 50 ug/ml. However, this did not mean that there would be no significant differences in the amounts of resistant bacterial isolates between sites for tetracycline at concentrations of 20 and 50 ug/ml. Tetracycline at 50 ug/ml exposed significant differences in the mean amounts of resistant bacteria between sites. These differences were not seen when these same colonies were tested against tetracycline at 20 ug/ml. These highly resistant colonies may be the result antibiotic over use or innately resistant, but were only exposed at high

antibiotic concentrations.

Of the antibiotics and concentrations tested in this study ampicillin was the least bactericidal. Similar findings were observed in other studies located in India and Canada (Pathak et al., 1993; Murray et al., 1984). This suggests that ampicillin resistance is widely distributed around the world and not just a phenomenon equated with the James River. Ampicillin resistance may result from the proportion of Gram- bacteria in a sample, as ampicillin is more effective against Gram+ species.

When focusing on samples from the James River, spatial differences in the amounts of resistant bacteria were more prominent when assemblages were tested against the most bactericidal antibiotics and concentrations. Tetracycline at 50 ug/ml and streptomycin at 75 ug/ml and 100 ug/ml were the most effective antibiotics and revealed spatial differences in the mean amounts of antibiotic resistant bacteria. This indicates that some sites on the river are either receiving inputs of bacteria with elevated resistance or the microbial community in these areas are comprised of bacteria innately resistant to certain antibiotics. This may be the result of factors related to urbanization like dense human population, use of antibiotics and sewage treatment plants. These factors have been shown to increase antibiotic resistance and MAR in fecal coliform bacteria (Bell et al., 1983). It is conceivable that these same factors are affecting localized areas of the microbial community in the James River. However, no relationship between human

density, urbanization or municipal discharges and the mean number of antibiotic resistant bacteria was determined.

An environmental factor that occurred naturally and may have influenced the amounts of antibiotic resistant bacteria in the river was salinity. Relatively few microorganisms in lakes and rivers are salt tolerant and will not grow, under natural conditions, in waters with more than 1 ppt salt (Rheinheimer, 1992). Of course, different bacteria are effected differently. For instance, Aeromonads are more sensitive to salinity and have a higher mortality rate than fecal coliforms (Monfort & Baleux, 1991). It has been shown that, under laboratory conditions, antibiotic resistant coliform isolates survived longer in sea water than sensitive organisms (Qureshi & Qureshi, 1991). In the James River there was no selective advantage displayed by antibiotic resistant organisms towards salinity.

Statistically significant differences in antibiotic resistant bacterial assemblages were observed. Differences between sites 1-5 and 6,7 were common. It is not known whether isolated bacteria at sites 6 and 7 represented the microbial community. It is possible that marine bacteria were unable to grow due to a lack of sodium in the culture media. It is also possible that fresh water bacteria were unable to grow after exposure to increased salinity.

It is likely that sites 6 and 7 increased salinity levels prompted a change in the bacterial assemblages yielding a decrease in both colony forming units and antibiotic resistant

isolates. Growth of freshwater bacteria in the St. Lawrence River were reduced by 15 and 50% after exposure to salinities of 10 and 20 ppt, respectively (Painchaud et al., 1995). These salinity measurements are comparable to those at sites 6 and 7. The fact remains that the proportion of antibiotic resistant isolates at sites 6 and 7 was less than that of sites 1-5.

Bacterial assemblages were also tested for significant differences in MAR. Increased numbers of isolates resistant to antibiotics prompted a corresponding increase in MAR isolates. However, this increase in MAR isolates was not always statistically significant.

Temporal differences in antibiotic resistance, over the six-month testing period, were most often revealed when isolates were tested against the least bactericidal antibiotics and concentrations, ampicillin at 10, streptomycin at 10 and tetracycline at 5 ug/ml. Temporal patterns of antibiotic use have been reflected in both gram- enteric bacteria (Martinez et al. 1994) and in pathogens (Nowak, 1994). However, no information was found about a correlation between antibiotic use and resistance in aquatic microbial communities.

Other factors, possibly environmental, may have accounted for these differences. The best examples of these factors would be increased water temperature and rain water runoff. Water temperature is a survival factor for some bacteria (Flint 1987). Increased rainfall results in increased water runoff and increased coliform bacteria levels in the James River (Gregory et

al, 1994). Temperature and dissolved oxygen levels did not vary significantly between sites. Carbon and phosphorous levels, in the river, varied for both sites and trials. Over the duration of the study, water temperature steadily increased while the dissolved oxygen level decreased. Due to the interaction of various environmental factors it is impossible to attribute changes in antibiotic resistance to a single environmental factor. However, there does seem to be a correlation between increased salinity and decreased bacterial antibiotic resistance at sites 6 and 7. Sites 6 and 7 showed both decreased amounts of antibiotic resistant bacteria and CFU's/ml.

Sites 2 and 3 had significantly greater amounts of CFU's/ml than most other sites. Trial 5 had significantly more CFU's/ml than any other trial. With increased bacterial density genetic exchange increases. This may result in the exchange of R-factors, virulence genes or both. This bacterial density and genetic exchange could be a public health risk.

The density of fecal coliform bacteria are used to measure water quality. Fecal coliform bacteria were enumerated by the DEQ but not tested for antibiotic resistance. The percentage of fecal coliforms in the microbial community tended to be low. Fecal bacteria are often of little numerical importance in many freshwater systems (Ogan & Nwiika, 1993). Fecal coliforms, on average, represented only 0.1 percent of the total microbial community in the James River. Coliform bacteria are still worth studying as they pose a health risk (Murray, 1984; Al-Jebouri, et

al., 1985 Pathak et al., 1993).

Total coliform bacteria are also used to measure water quality and differ from fecal coliforms in that not all total coliforms are fecal in origin. Total coliforms were isolated at sites 1-7 in trials 4, 5 and 6. It was hypothesized that humanity's contribution to antibiotic resistance in the river would be more pronounced in coliform bacteria. A typical coliform would be more likely to be exposed to antibiotics or other antibiotic resistance inducers and exhibit corresponding resistance. One example is antibiotics being selective for R+ plasmid coliforms in the alimentary canals of humans and animals (Walter & Vennes, 1985; Bell et al., 1983). It has also been well documented that many waste water treatment plants increase the incidence of MAR coliforms by facilitating R plasmid transfer (Walter & Vennes, 1985; Bell et al., 1983; Murray et al., 1984; Mach & Grimes, 1982). Factors such as these would be reflected in the antibiotic resistant capacities of James River coliforms.

Once introduced to an aquatic environment many factors reduce the density of coliform bacteria, specifically Escherichia coli. These factors include ultraviolet radiation, temperature, salinity, availability of nutrients and competition with other microbes (Flint, 1987).

Total coliform bacteria often were more resistant to test antibiotics than colonies representing the microbial community. They were also more often MAR. However, significant temporal and spatial differences in the resistance of coliform assemblages

were less prominent. Increases in antibiotic concentration rarely yielded significant decreases in the amount of resistant coliform isolates. Since coliforms are more antibiotic resistant, test concentrations of antibiotics should be raised accordingly to expose significant differences.

There were no significant temporal or spatial differences in the percentage of fecal coliform in the microbial community. The percentage of total coliforms in the community was approximately 0.7 percent. Total coliform enumeration data is incomplete. The ratio of fecal to total coliforms, 1:7, was similar to those of the rivers Indus and Gomat in India (Pathak et al., 1993). The Indus is considered to be less chemically and microbially polluted than the Gomati (Pathak et al., 1993). Also interesting is that the percentage of fecal and total coliforms in the James River, 0.1% and 0.7%, was closely related to the percentage in the polluted Gomati, 0.07 and 0.5%, respectively. Another thing the James has in common with the Gomati River is both have areas of urbanization, industrialization, agriculture and are heavily populated. These pollution causing factors have been shown to cause imbalances in the microbial ecology and population of an aquatic system (Pathak et al., 1993).

These imbalances are part of the problem of comparing temporal and spatial bacterial assemblages. These assemblages may not be comprised of statistically similar amounts of microbes and some bacteria are inherently more resistant than others. If one assemblage is dominated by a strain resistant to an

antibiotic this may result in a statistically significant difference in resistance. It has been shown that sewage treatment and urbanization increase the incidence of antibiotic resistant and MAR bacteria (Bell et al., 1983). The James has an abundance of both these factors and they may be contributing to microbial variation and antibiotic resistance. However, this contribution does not appear to be significant.

There were five major discoveries in this study. 1)Coliform bacteria in the James River are significantly more resistant to antibiotics than non-coliforms. 2) Coliform bacteria comprise less than 1% of the microbial community. 3) Antibiotics used at the highest test concentrations most often exposed spatial differences in the mean number of resistant bacteria. 4) There appeared to be a decrease in bacterial density and antibiotic resistance with increased salinity. 5) No relationship between municipal discharges, non-point source pollution or urbanization and the density of antibiotic resistant bacteria could be determined.

Literature Cited

Alam, M.N., Haq, S.A., Das, K.K., Baral, P.K., Mazid. M.N., Siddique, R.U., Rahman, K.M., Hasan, Z., Khan, M.A.S. and Dutta. P. (1995) Efficacy of Ciproflaxacin in Enteric Fever: Comparison of Treatment Duration in Sensitive and Multidrug Resistant Salmonella. Amer. J. of Trop. Med. and Hygiene. 53(3) 306-311

Al-Jebouri, M.M. (1985) A Note on Antibiotic Resistance in the Bacterial Flora of Raw Sewage and Sewage -Polluted River Tigris in Mosul, Iraq. J. of Appl. Bacteriol. 58 401-405

Baldry, P. (1976) The Battle Against Bacteria: A Fresh Start. Cambridge University Press

Bell, J., Elliott, G.E. and Smith, D.W. (1983) Influence of Sewage Treatment and Urbanization on Selection of Multiple Resistance in Fecal Coliform Populations. Appl. Environ. Microbiol. 46(1) 227-232

Bell, J., Macrae, W. and Elliot G.E. (1981) R Factors in Coliform-Fecal Coliform Sewage Flora of the Prairies and Northwest Territories of Canada. 42(2) 204-210

Bell, R. (1978) Antibiotic Resistance Patterns of Fecal Coliforms Isolated from Domestic Sewage Before and After Treatment in an Aerobic Lagoon. Cam. J. Microbiol. 24 886-888

Davies, J. (1994) Inactivation of Antibiotics and the Dissemination of Resistance Genes. Science 264 375-381

De Vicente, M. Aviles, J.C. Codina, J.J. Borrego and Romero, P. (1989) Resistance to Antibiotics and Heavy Metals of

Pseudomonas aeruginosa Isolated From Natural Waters. J. of Appl. Bacteriol. 68 625-632

Flint K.P (1987) The Long Term Survival of Escherichia coli in River Water. J. of Appl. Bacteriol. 63 261-270

French G.L., Ling J., Chow K.L. & Mark K.K. (1987) Occurrence of Multiple Antibiotic Resistance and R-plasmids in Gram-negative Bacteria Isolated from Faecally contaminated Fresh-water Streams in Hong Kong. Epidem. Info. 98 285-299

Gedebou M. & Tassew A. (1980) Neisseria gonorrhoeae Isolates from Ethiopia* 2. Pair Correlations Between Minimal Inhibitory Concentration Values of five Antibiotics and Frequency of Multiple Antibiotic Resistance. Bulletin of the World H. Org. 58(1) 73-79

Gregory J., Kennedy J., Snead L., Sykes M., Ayers R., Cosby G., La Vern C., Hunter m., McEachern R., Martin C., Torbeck S., Wilson S., Sale C., Morgan C., McMillan L. and Reid M. (1994) Virginia Water Quality Assesment for 1994. Virginia Department of Environmental Quality

Hof, H., (1991) Bases of the Antibacterial Effect of Beta-lactam Antibiotics. Immun. Infect. 19(6) 175-178

Iseman M. D. (1993) Treatment of Multidrug-Resistant Tuberculosis. The New England J. of Med. 329(11) 784-787

Mach, P.A. & Grimes, J.D. (1982) R-plasmid transfer in a Waste Water Treatment Plant. Appl. and Environ. Microbiol. 44(6) 1395-1403

Maniatis, T., Fritsch, E. and Sambrook, J. (1982) Molecular

Cloning a Laboratory Manual. Cold Spring Harbor Laboratory 121

Martinez, M., Mondaca, M.A. and Zemelman R. (1994) Antibiotic-Resistant Gram-Negative Bacilli in the Sewage of the City of Concepcion, Chile. Rev. Latinoam. Microbiol. 36(1) 39-46

Meckes, M. (1982) Effect of UV Light Disinfection on Antibiotic Resistant Coliforms in Wastewater Effluents. 43(2) 371-377

Monfort, P. and Baleux, B. (1991) Distribution and survival of Motile Aeromonas spp. in Brackish Water Receiving Sewage treatment Effluent. Appl. and Environ. Microbiol. 57(9) 2459-2467

Murray, G.E., Tobin, R.S., Junkins, B. and Kushner, D.J. (1984) Effect of Chlorination on Antibiotic Resistance Profiles of Sewage-Related Bacteria. Appl. and Environ. Microbiol. 48(1) 73-77

Nikaido, H. (1994) Prevention of Drug Access to Bacterial Targets: Permeability Barriers and Active Efflux. Science 264

Nowak, R. (1994) Hungary Sees an Improvement in Penicillin Resistance. Science 264(15) 364

Ogan, M.T. & Nwiika, D.E. (1993) Studies on the Ecology of Aquatic Bacteria of the Lower Niger Delta: Multiple Antibiotic Resistance Among the Standard Plate Count Organisms. J. of Appl. Bacteriol. 74 595-602

Painchaud, J., Therriault, J.C. and Legendre, L. ((1995) Assessment of Salinity Related Mortality of freshwater Bacteria in the Saint Lawrence river Estuary. Appl. and

Environ. Microbiol. 61(1) 205-208

Papapetropoulou, M. (1996) Spread of Bacterial Resistance to Antibiotics Through the Aquatic Environment. Environ. Microbiol. 40(6) 473-479

Pathak, S. P., Gaur, A. and Bhattacherjee, J. W. (1993) Distribution of Antibiotic Resistance Among Aerobic Heterotrophic Bacteria from Rivers in Relation to Pollution. J. of Environ. Sci. Health A28(1) 73-87

Qureshi, A.A. & Qureshi, M.A. (1992) Multiple Antibiotic Resistant Fecal Coliforms in Raw Sewqage. Water Air and Soil Pollut. 61 47-56

Rheinheimer, G. (1992) Aquatic Microbiology 4th edition. 1992 John Wiley & Sons Ltd.

Roberts, M.C. (1992) Antibiotic Resistance ed. Maniloff, J. Mycoplasmas Molecular Biology and Pathogenesis. American Society for Microbiology. 513-521

Rump, H. & Krist, H. (1988) Laboratory Manual for the Examination of Water, Waste Water and Soil. VCH Weinheim Germany

Sevebeck K., Kahn H. and Chapman N. (1989) Virginia's Waters. Virginia Water Resources Research Center, VPI and State University

Summers, A.O. (1993) Mercury Released from Dental "Silver" Provokes an Increase in Mercury and Antibiotic resistant Bacteria in Oral and Intestinal Floras of Primates. Antimocrob. Agents and Chemotherapy 37(4) 825-834

Travis J. (1994) Reviving the Antimicrobial Miracle. Science 264 (15) 360-362

Walter, M.V. & Vennes, V.W. (1985) Occurrence of Multiple-Antibiotic-Resistant Enteric bacteria in Domestic Sewage and Oxidation Lagoons. Appl. and Environ. Microbiol. 50 930-933

Wnorowski, A.U. (1993) Resistance to Antibiotics of Heavy Metal-Tolerant and Heavy Metal-Sensitive Bacterial Strains. J. of Environ. Sci. Health A28(1) 203-215

Zelibor, Jr. J.L., Doughten, W.M., Grimes, D.J. and Colwell, R.R. (1987) Testing for Bacterial Resistance to Arsenic in Monitoring Well Water by the Direct Viable Counting Method. Appl. and Environ. Microbiol. 53(12) 2929-2934

Tables

Table 1. Mean salinity levels measured in parts per thousand (ppt).

Site	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Mean salinity per site
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0.9	0.9	0.4	1.0	0.7	0.7	0.7
6	8.0	8.0	9.0	12.0	10.0	10.0	9.5*
7	16.0	16.0	19.0	21.0	18.0	18.0	18**

*=Mean salinity level significantly greater $P<0.05$ than sites 1-5.
**=Mean salinity level significantly greater $P<0.05$ than sites 1-6.
No significant difference in salinity levels between trials.
*** All salinity testing was done by the Virginia Commonwealth DEQ

Table 2. Bactericidal effects of antibiotic concentrations on isolated bacteria. N=84

Antibiotic	Conc. ug/ml	*Mean # resistant colonies	ANOVA Table	**Tukey's p<0.05 Mean resistant colonies
Ampicillin	10	32.6 (5.3)	F(2, 249)=40.8	32.6>28.8> 23.4
Ampicillin	100	28.8 (6.3)		
Ampicillin	500	23.4 (8.0)		
Chloramphenicol	10	7.0 (5.6)	F(2, 249)=18.6	7.0>4.8> 3.2
Chloramphenicol	25	4.8 (3.7)		
Chloramphenicol	50	3.2 (2.9)		
Streptomycin	10	20.0 (7.8)	F(2, 249)=454	20.0>2.3, 1.4
Streptomycin	75	2.3 (2.8)		
Streptomycin	100	1.4 (1.6)		
Tetracycline	5	9.5 (6.2)	F(2, 249)=125	9.5>1.9, 0.6
Tetracycline	20	1.9 (2.5)		
Tetracycline	50	0.6 (1.0)		

*=Mean antibiotic resistant colonies per TSA plate, 38 colonies tested per plate, 84 plates. (standard deviation)
**=Comparison of the mean number of resistant colonies per antibiotic concentration. Significantly different means separated by '>'.

Table 3. Mean number of antibiotic resistant bacteria isolated at each sampling site. N=12

Antibiotic	Conc. ug/ml	*Mean antibiotic resistant colonies per site							ANOVA Table	**Tukey's p<0.05
		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7		
Ampicillin	10	35.0	36.0	33.8	33.1	33.8	29.6	26.8	F (6, 77)=6.13	6<2; 7<1, 2, 3, 4, 5
Ampicillin	100	31.1	33.3	30.7	29.7	31.9	23.5	20.7	F (6, 77)=11.95	6<1, 2, 3, 4, 5; 7<1, 2, 3, 4, 5
Ampicillin	500	26.0	27.8	26.0	26.2	28.5	15.0	14.0	F (6, 77)=12.78	6<1, 2, 3, 4, 5; 7<1, 2, 3, 4, 5
Chloramphenicol	10	9.8	11.4	8.3	7.7	8.0	0.9	2.5	F (6, 77)=8.8	6<1, 2, 3, 4, 5; 7<1, 2, 3
Chloramphenicol	25	7.0	7.4	4.6	5.8	6.6	0.6	1.4	F (6, 77)=11.43	6<1, 2, 3, 4, 5; 7< 1, 2, 4, 5
Chloramphenicol	50	4.8	4.6	3.3	4.1	4.4	0.5	0.5	F (6, 77)=7.85	6<1, 2, 4, 5; 7<1, 2, 4, 5
Streptomycin	10	26.5	24.6	25.0	20.4	19.9	18.2	14.4	F (6, 77)=4.67	7<1, 2, 3
Streptomycin	75	3.1	5.0	2.5	3.4	1.1	0.3	0.1	F (6, 77)=6.65	5<2; 6<2, 4; 7<1 ,2, 4
Streptomycin	100	1.5	2.5	2.1	2.1	0.9	0.1	0.0	F (6, 77)=7.04	5<2; 6<2, 3, 4; 7<2, 3, 4
Tetracycline	5	12.9	12.9	9.5	11.2	11.5	2.0	6.0	F (6, 77)=7.36	6<1, 2, 3, 4, 5; 7<1, 2
Tetracycline	20	2.9	3.1	3.1	2.0	1.4	0.1	0.5	F (6, 77)=3.43	6<2, 3
Tetracycline	50	0.4	1.3	1.6	0.4	0.0	0.0	0.3	F (6, 77)=6.4	1<3; 4<3; 5<2, 3; 6<2, 3; 7<3

*=Mean antibiotic resistant colonies, 38 colonies tested per replica, 12 replicates per site.

**= Comparison of the mean number of antibiotic resistant bacteria isolated per site. Significantly different means separated by '<'.

Table 4. Mean number of of antibiotic resistant bacteria isolated per trial date. N=14

Antibiotic	Conc. ug/ml	*Mean resistant colonies per trial						ANOVA Table	**Tukey's p<0.05
		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6		
Ampicillin	10	29.9	29.2	31.5	34.7	34.0	36.2	F (5, 78)=4.96	1<6; 2<4, 6
Ampicillin	100	27.9	26.4	26.1	30.4	29.2	32.2	F (5, 78)=2.14	No difference
Ampicillin	500	21.9	21.0	20.3	27.2	24.3	25.4	F (5, 78)=1.68	No difference
Chloramphenicol	10	4.8	8.2	8.2	5.2	8.7	6.5	F(5,78)=1.26	No difference
Chloramphenicol	25	3.9	6.5	5.7	3.7	5.0	4.0	F (5,78)=1.31	No difference
Chloramphenicol	50	2.7	3.0	3.3	3.0	4.2	2.8	F (5, 78)=0.52	No difference
Streptomycin	10	18.6	13.4	19.0	22.9	26.3	27.6	F (5, 78)=10.12	1<5, 6; 2<4, 5, 6; 3<5, 6
Streptomycin	75	1.8	2.2	1.7	1.4	4.3	1.9	F (5, 78)=2.06	No difference
Streptomycin	100	1.3	1.6	1.4	0.9	1.5	1.3	F (5, 78)=0.31	No difference
Tetracycline	5	8.0	7.8	15.2	11.5	7.8	6.2	F (5, 78)=5.03	1, 2, 5,6<3
Tetracycline	20	1.2	1.5	4.2	1.7	1.4	1.2	F (5, 78)=3.45	1, 2 ,5, 6<3
Tetracycline	50	0.7	0.7	0.8	0.3	0.2	0.6	F (5, 78)=0.71	No difference

*=Mean antibiotic resistant colonies, 38 colonies tested per replica, 14 replicates.

**= Comparison of the mean number of antibiotic resistant bacteria isolated per trial. Significantly different means separated by '<'.

Table 5. Mean number of MAR bacteria isolated between sites. N=6

*Conc. Bracket	**Mean MAR bacteria per site							ANOVA Table	***Tukey's p<0.05
	SITE								
	1	2	3	4	5	6	7		
Low	60	58	55	49	48	34	31	F(6, 56)=4.19	6<1; 7<1,2
Med	15	22	17	13	14	6	2	F(6, 56)=6.42	6<2 7<1, 2, 3, 5
High	11	14	9	8	8	1	1	F(6, 56)=6.35	6<1; 7<1, 2

*=Antibiotics grouped by relative concentration level.
**=Mean number of MAR bacteria per site 76 colonies tested per replicate, 6 replicates per site.
***=Significantly different means separated by '<'.
Low=(colonies resistant to two or more of) ampicillin at 10 ug/ml, chloramphenicol at 10 ug/ml, streptomycin at 10 ug/ml and tetracycline at 5 ug/ml.
Med=(colonies resistant to two or more of) ampicillin at 100 ug/ml, chloramphenicol at 25 ug/ml, streptomycin at 75 ug/ml and tetracycline at 20 ug/ml.
High=(colonies resistant to two or more of) ampicillin at 500 ug/ml, chloramphenicol at 50 ug/ml, streptomycin at 100 ug/ml and tetracycline at 50 ug/ml

Table 6A. Mean colony forming units per milliliter (CFU's/ml).

	Mean CFU's/ml					
Site	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5***	Trial 6
1	615	615	685	480	4000	1017
2**	2285	4460	905	967	5000	3062
3*	3732	3300	2080	2215	3380	6075
4	555	650	625	1105	3330	2700
5	1350	1130	695	1770	2270	870
6	575	513	865	385	3400	920
7	172	1300	930	940	1500	925

*=Mean CFU's/ml significantly greater $P<0.05$ than sites 1 and 4-7.
**=Mean CFU's/ml significantly greater $P<0.05$ than sites 6 and 7.
***=Mean significantly greater $P<0.05$ than trials 1-4 and 6.

Table 6B. Fecal and total coliform colonies per 100ml.

	*Fecal coliforms/ 100ml (**Mean total coliforms/100ml)					
Site	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
1	330	330	18	18	2200	18
1	(nd)	(nd)	(nd)	(900)	(nd)	(2100)
2	110	110	68	20	9200	68
2	(nd)	(nd)	(nd)	(700)	(nd)	(nd)
3	490	490	1400	130	78	78
3	(nd)	(nd)	(nd)	(2000)	(nd)	(nd)
4	68	68	130	18	20	20
4	(nd)	(nd)	(nd)	(600)	(nd)	(1900)
5	70	70	170	8	2	2
5	(nd)	(nd)	(nd)	(900)	(nd)	(300)
6	5	5	13	2	2	2
6	(nd)	(nd)	(nd)	(100)	(nd)	(100)
7	2	2	2	8	2	2
7	(nd)	(nd)	(nd)	(nd)	(nd)	(200)

nd=No data
*=Fecal coliform datum was supplied by the Virginia Department of Environmental Quality.
**=Total coliform colonies were determined by the researcher via the membrane filter technique and represent the mean number of number of countable colonies.

Table 7. Antibiotic resistance of coliform bacteria. N=42

Antibiotic	Conc. ug/ml	*Mean # of resistant colonies	ANOVA Table	**Tukey's p<0.05
Ampicillin	10	9.6 (1.5)	F(2, 123)=2.33	No diff. between means
Ampicillin	100	9.2 (2.0)		
Ampicillin	500	8.6 (2.6)		
Chloramphenicol	10	5.2 (3.0)	F(2, 123)=12.5	5.2, 4.5>2.2
Chloramphenicol	25	4.5 (2.9)		
Chloramphenicol	50	2.2 (2.4)		
Streptomycin	10	5.4 (2.9)	F(2, 123)=47	5.4>1.6, 1.0
Streptomycin	75	1.6 (2.0)		
Streptomycin	100	1.0 (1.4)		
Tetracycline	5	4.2 (2.9)	F(2, 123)=40.8	4.2>1.3, 0.5
Tetracycline	20	1.3 (1.6)		
Tetracycline	50	0.5 (0.7)		

*=Mean number of antibiotic resistant colonies, 10 colonies tested per replicate, 42 replicates representing trials 4-6 (standard deviation).
**=Comparison of the mean number of resistant coliform colonies per antibiotic concentration. Significantly different means separated by '>'.

Table 8. Comparison of mean antibiotic resistant coliform isolates and mean resistant isolates from the microbial community. N=42

Antibiotic	Conc. ug/ml	*Mean # of resistant coliform isolates	*Mean # of resistant isolates from the microbial community	**Tukey's p<0.05
Ampicillin	10	9.6	8.7	9.6>8.7
Ampicillin	100	9.2	7.6	9.2>7.6
Ampicillin	500	8.6	6.4	8.6>6.4
Chloramphenicol	10	5.2	1.5	5.2>1.5
Chloramphenicol	25	4.5	0.9	4.5>0.9
Chloramphenicol	50	2.2	0.8	2.2>0.8
Streptomycin	10	5.4	6.4	No difference
Streptomycin	75	1.6	0.4	1.6>0.4
Streptomycin	100	1.0	0.1	1.0>0.1
Tetracycline	5	4.3	2.1	4.3>2.1
Tetracycline	20	1.2	0.2	1.2>0.2
Tetracycline	50	0.5	0.0	0.5>0.0

*=Mean antibiotic resistant colonies, 42 replicates (representing trials 4-6) 10 colonies tested per replicate.
**=Comparison of mean resistant isolates. Significantly different means separated by `>'.

*** Table 8 is a comparison of antibiotic resistance between the ten coliform isolated per sample in trials 4-6 and a subset of ten bacteria, from the corresponding water sample representing the microbial community. Bacteria from the microbial community were numbered 1-40 and chosen via a random number table.

Table 9. Significant differences in the amounts of antibiotic resistant coliform bacteria isolated between sites. N=6

Antibiotic	Conc. ug/ml	**Mean resistant coliform colonies per site							ANOVA Table	***Tukey's p<0.05
		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7		
Ampicillin	10	10.0	9.0	10.0	10.0	10.0	10.0	7.5	F (6, 35)=2.86	7<1, 3, 4, 5, 6
Ampicillin	100	10.0	9.8	9.5	10.0	10.0	8.5	8.6	F (6, 35)=2.93	7<1, 4, 5
Ampicillin	500	9.3	9.1	9.0	10.0	10.0	6.5	6.5	F (6, 35)=2.57	No difference
Chloramphenicol	10	7.3	7.0	7.1	5.1	4.5	3.1	2.0	F (6, 35)=4.39	7<1, 2, 3
Chloramphenicol	25	6.1	5.6	6.1	2.5	4.6	3.5	3.1	F (6, 35)=1.68	No difference
Chloramphenicol	50	2.3	3.1	4.6	1.6	2.6	0.3	1.0	F (6, 35)=2.63	6<3
Streptomycin	10	5.5	7.1	7.6	7.3	3.6	4.3	2.3	F (6, 35)=4.16	7<2, 3, 4
Streptomycin	75	2.6	1.3	4.3	1.6	0.1	0.1	1.0	F (6, 35)=4.75	5, 6, 7<3
Streptomycin	100	1.3	0.5	2.5	1.6	0.1	0.1	0.8	F (6, 35)=2.78	5, 6<3
Tetracycline	5	7.0	5.0	5.8	4.3	3.1	2.6	2.0	F (6, 35)=2.69	7<1
Tetracycline	20	1.0	1.0	2.1	1.3	1.8	0.5	1.0	F (6, 35)=0.67	No difference
Tetracycline	50	0.3	0.5	0.5	1.0	0.1	0.1	1.0	F (6, 35)=1.45	No difference

*=Mean number of antibiotc resistant coliform colonies, 10 colonies tested per replica, 6 replicates.
**=Comparison of the mean number of antibiotic resistant coliform bacteria isolated per site. Significantly different means separated by '<'.

Figures

Figure 1. DEQ Water Monitoring Stations/ Project water sampling sites.

x=Wastewater discharge

Figure 1

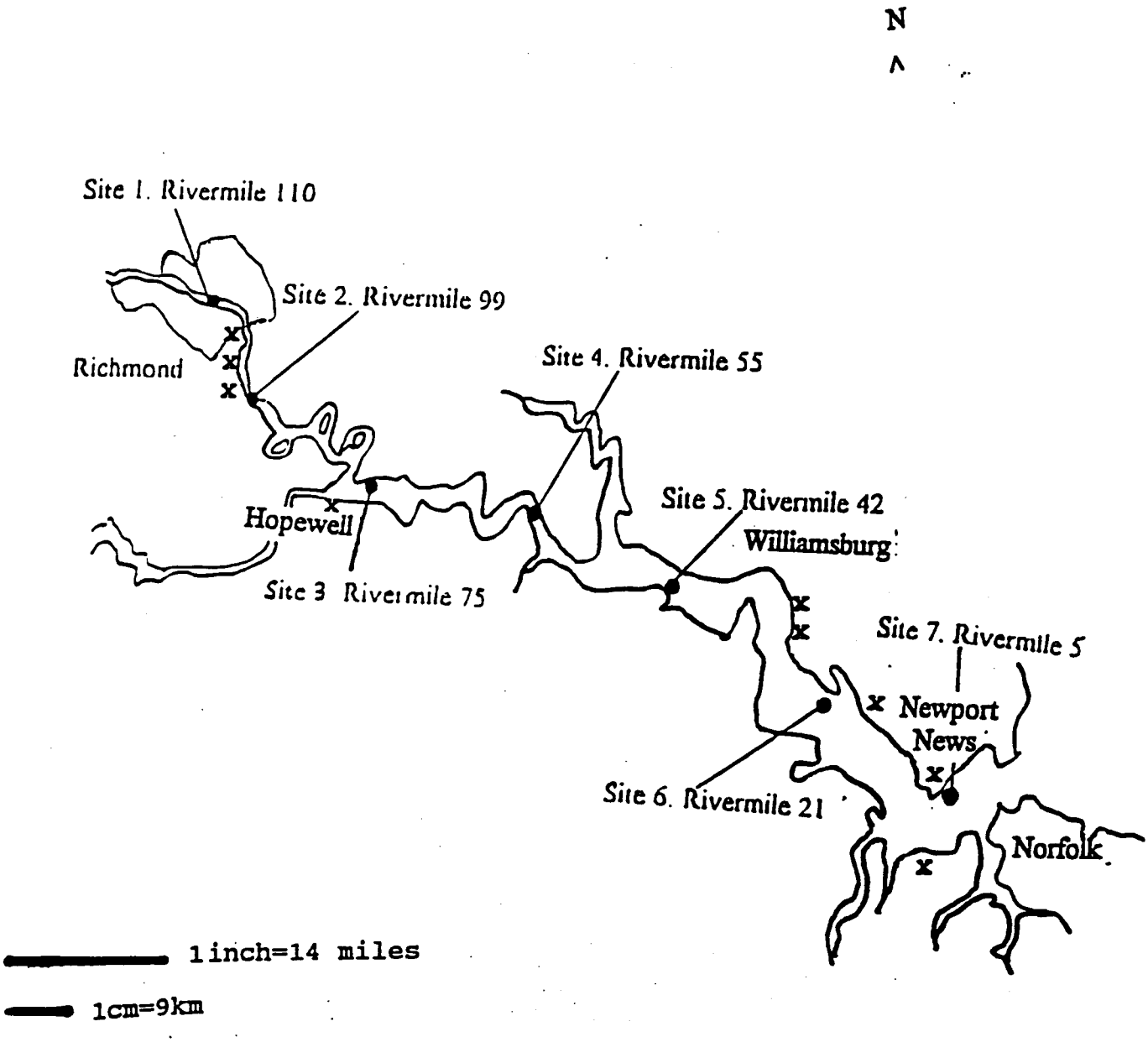

N
Site 1. Rivermile 110
Site 2. Rivermile 99
Richmond
Site 4. Rivermile 55
Hopewell
Site 5. Rivermile 42
Williamsburg
Site 3 Rivermile 75
Site 7. Rivermile 5
Newport News
Site 6. Rivermile 21
Norfolk
1inch=14 miles
1cm=9km

Figure 2. Effect of chloramphenicol on bacteria isolated per site, all trials inclusive.

Error bars= Absolute error, $P<0.05$

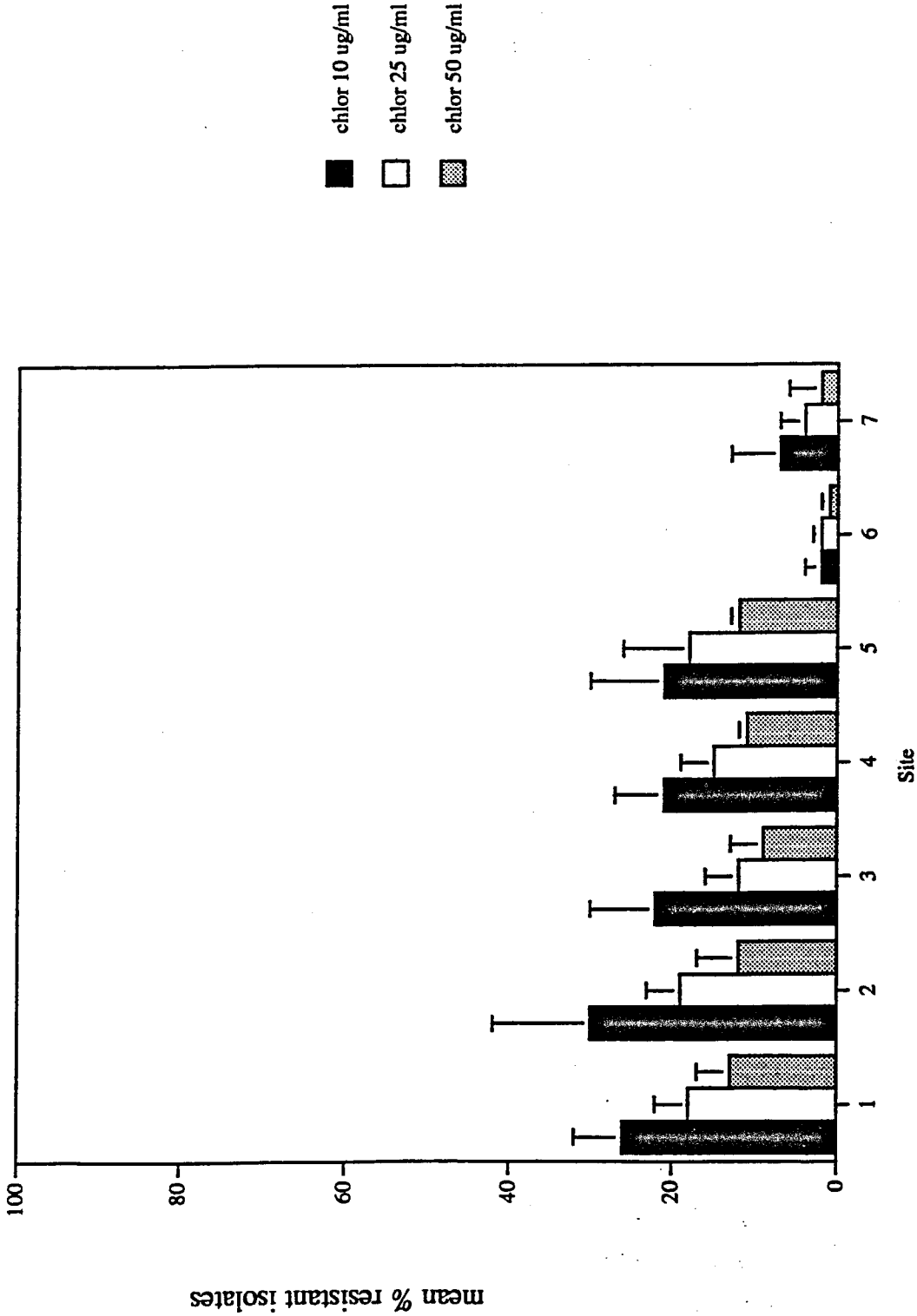
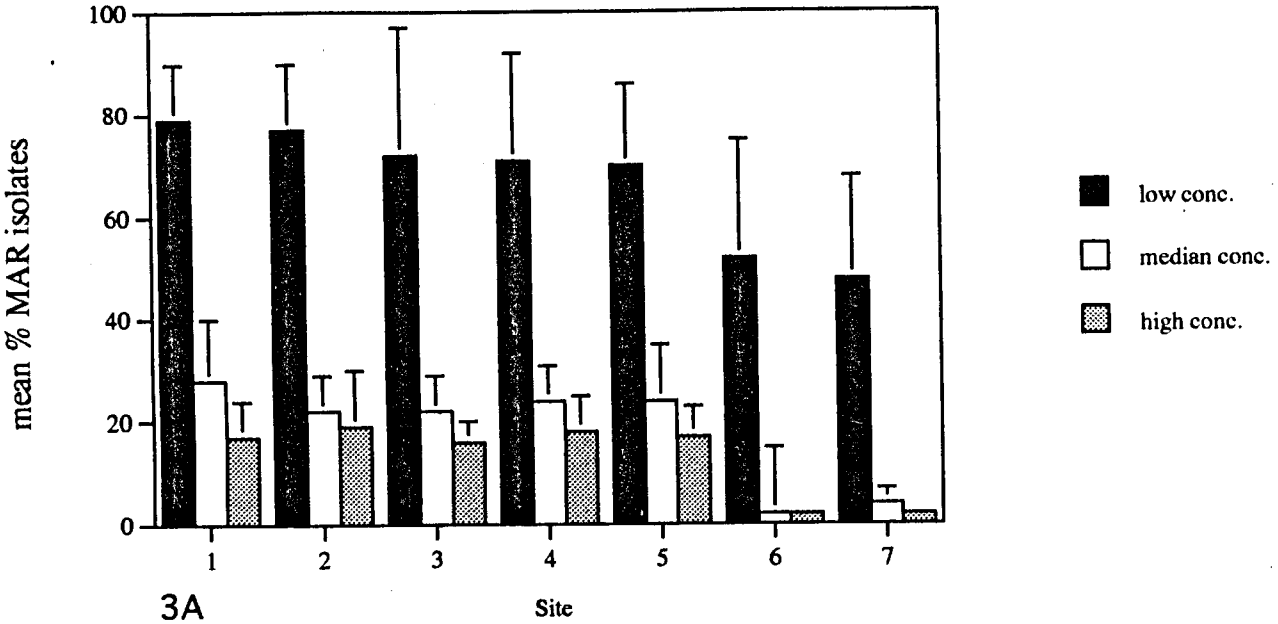

chlor 10 ug/ml
chlor 25 ug/ml
chlor 50 ug/ml
mean % resistant isolates
100
80
60
40
20
0
1
2
3
4
5
6
7
Site

Figure 2

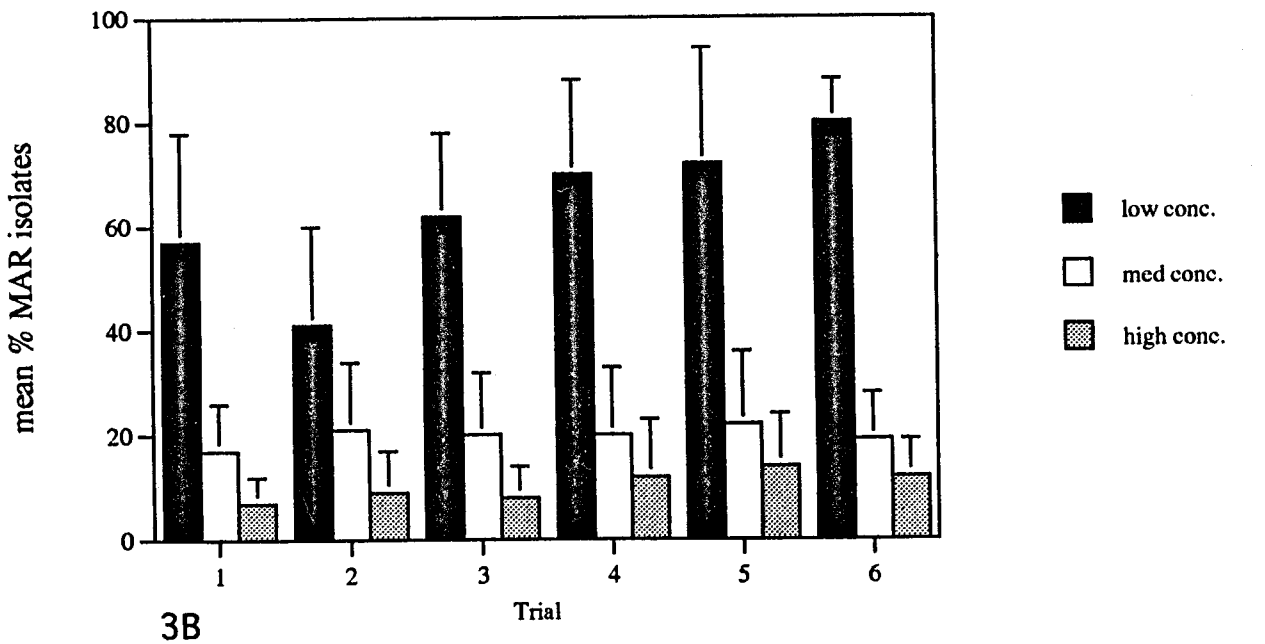
Figure 3A. Mean percentage of MAR bacteria isolated per site, all trials inclusive.

Figure 3B. Mean percentage of MAR bacteria isolated per trial, all sites incliusive.

Error bars=Absolute error, $p<0.05$


mean % MAR isolates
100
80
60
40
20
0
1
2
3
4
5
6
7
Site
low conc.
median conc.
high conc.

3A

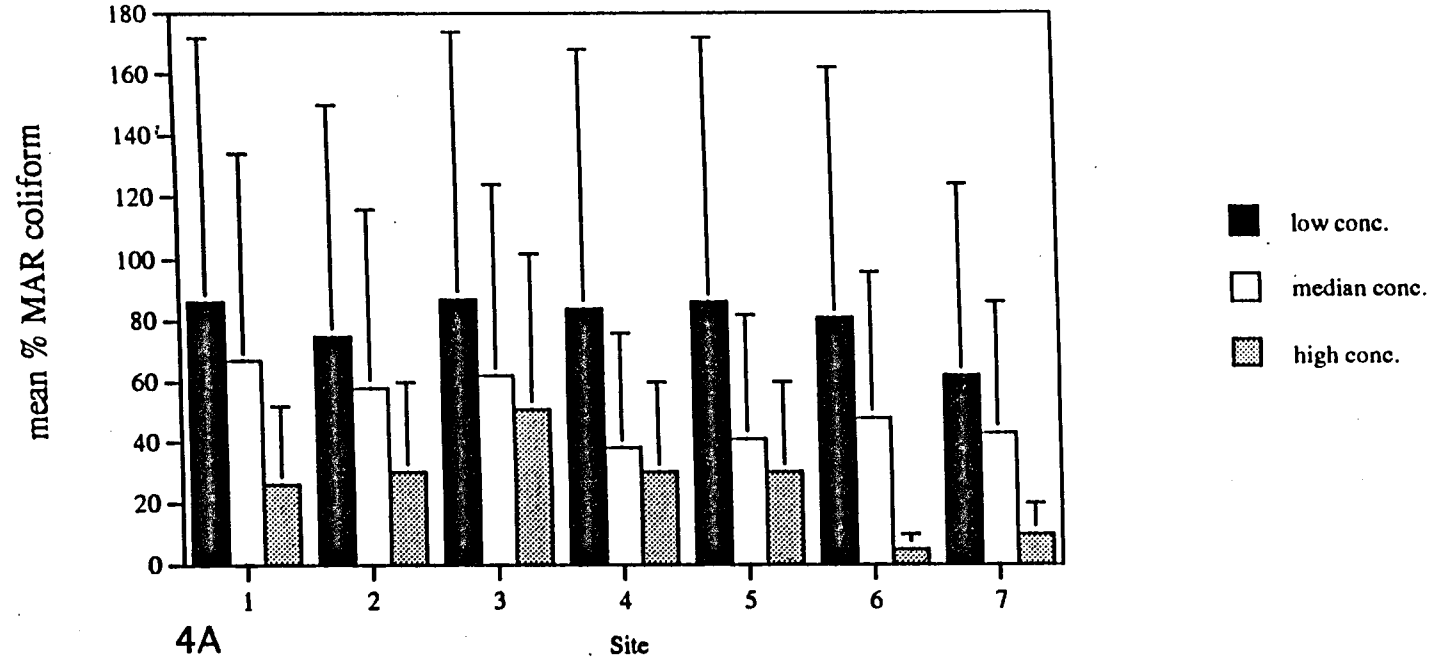

mean % MAR isolates
100
80
60
40
20
0
1
2
3
4
5
6
Trial
low conc.
med conc.
high conc.

3B

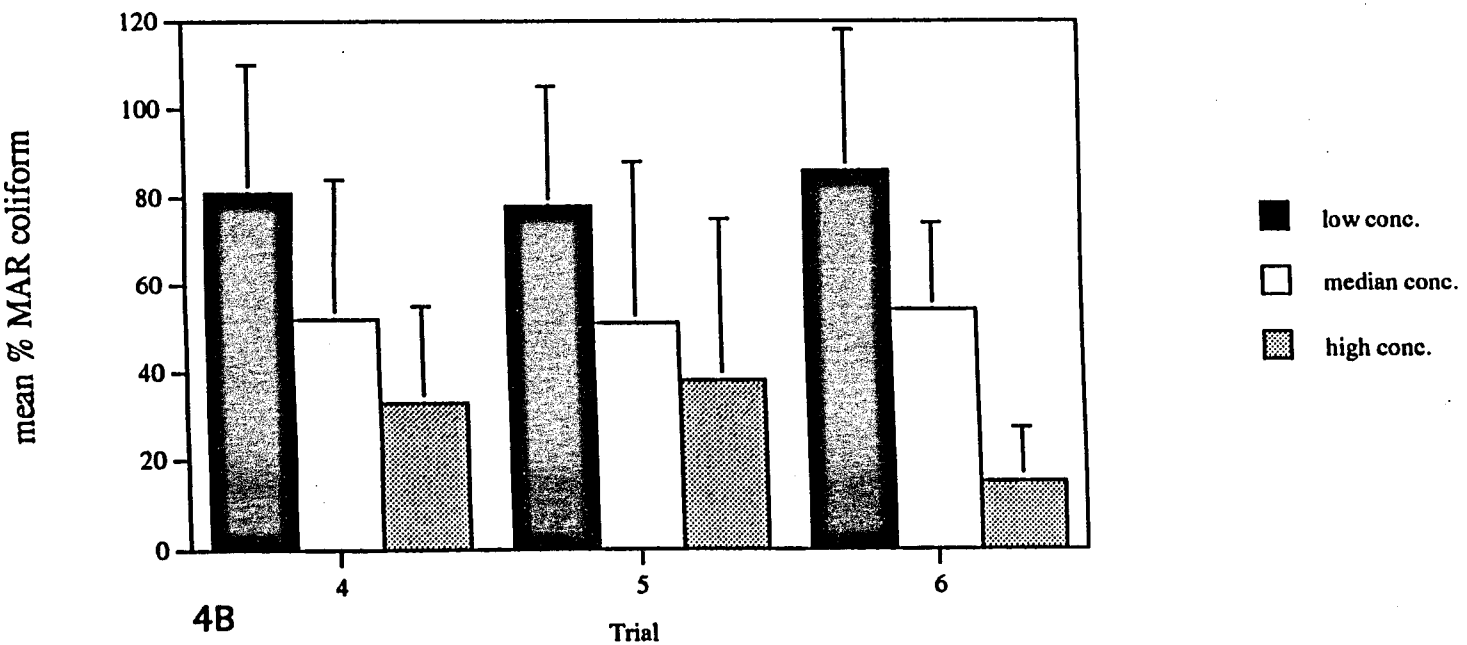
Figure 4A. Mean percentage of Mar coliforms isolated per site, trials 4-6 inclusive.

Figure 4B. Mean percentage of MAR coliforms isolated per trial, all sites inclusive.

Error bars= Absolute error, $P<0.05$


mean % MAR coliform
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
Site
low conc.
median conc.
high conc.

4A


mean % MAR coliform
120
100
80
60
40
20
0
4
5
6
Trial
low conc.
median conc.
high conc.

4B

Vita

Keith Allen Boisvert was born in Worcester, Massachusetts on August 15, 1968. He was raised in Cherry Valley, MA and graduated with honors from Leicester High School in 1986. He received a B.S. in Natural Science from Worcester State College in 1991. He completed an M.S. in Biology at the University of Richmond in May of 1997.