Ampicillin and kanamycin resistant bacteria comparison

Antibiotic use throughout the world has increased tremendously over the decades. In the past, antibiotic resistance was most prevalent in areas of frequent antibiotic use, such as in medical or laboratory settings. However, the increasing use of antibiotics and antibacterial products outside of hospitals, such as in homes and schools, echoes the expansion of antibiotic resistant bacteria (LBC Biology Staff, 2010). One major source of the growing problem is that antibiotics are being over prescribed by doctors to millions of people around the world. It is currently believed that about only half of the antibiotics prescribed to patients are administered properly (Levy, 1998). In addition to over prescription by doctors, many patients misuse the antibiotics and further increase the spread of resistance. For example, some patients discontinue use of antibiotics upon feeling symptom relief, not at the end of their antibiotic schedule prescribed by the doctor. In actuality, patients are killing off the weakest bacteria, causing temporary relief, and allowing the stronger and more resistant bacteria to multiply at a faster rate (Levy, 1998). This and other types of antibiotic misuse have promoted the growth of strains of bacteria with resistance to antibiotic attack. This can be seen through studies that have shown Tetracycline resistance by normal human intestinal flora that exploded from 2% in the 1950s to 80% in the 1990s (Criswell, 2004). Other studies have shown Kanamycin, an antibiotic from the 1950s, has become clinically useless as a result of the prevalence of Kanamycin-resistant bacteria (Criswell, 2004). It has become visible that the development of resistance to any antibiotic, new or old, will happen in a matter of time (LBC Biology Staff, 2010). Due to the inevitability of mutation, natural selection, time and environmental conditions, resistance will be seen in more common areas like work and home.

As a consequence of the every growing expansion of antiobiotic resistance, places previously thought to be uncontaminated like schools and homes have become overwhelmed with antibiotic resistant bacteria. In one household study, it was discovered that kitchen sinks contained many different types of resistant bacteria, primarily from food waste and human hands (Rusin et al., 1998). Only the application of strong bleaches and specific cleaning products on a regimented cleaning schedule led to a decreased amount of bacteria in kitchen sinks (Rusin et al., 1998). The cleaning products used in this study did not contain antibacterial ingredients, which helped reduce the spread of resistance by killing all bacteria instead of the most susceptible strains. Antibacterial products and cleaning supplies are less effective and in turn can lead to reproduction of stronger antibiotic resistant bacteria. The large amount of antibacterial cleaning products, food and waste combined with the constant water supply in sink drains allows for a greater chance of survival of antibiotic-resistant bacteria (Levy, 1998). Optimal conditions for bacterial growth with a wet environment cause a higher frequency of bacterial transmission of resistance (Perryman and Flournoy, 1980). In scientific laboratories, regulations are in place to monitor the disposal of solid and liquid wastes. Some regulations include specific waste baskets for toxic or contaminated substances and use of certain sinks only when dealing with harmful liquids in laboratory settings. This ensures that unnecessary amounts of harmful substances that could lead to resistance are not continually poured down laboratory sink drains. However, no such regulations are in effect in household environments.

In a study performed in Oklahoma City the extent of growing antibiotic resistance was seen in multiple environments. Bacterial samples were gathered from sink drains in the Veterans Administration Medical Center, libraries, private homes, shopping centers, and other similar environments for comparison (Perryman and Flournoy, 1980). The goal of the experiment was to determine the types of resistant bacteria that were most prevalent in sink drains, the abundance of bacteria in sink drains, and the life span of bacteria in dry and wet environments (Perryman and Flournoy, 1980). Through testing, bacteria were found to have longer life spans in wet environments than in dry environments, and many bacteria survived for over 180 days in wet environments (Perryman and Flournoy, 1980). The high survival rate of bacteria in areas with constant water supply, such as in laboratory and kitchen sinks, supports the prediction that sinks are ideal environments for ample bacterial growth. In the aforementioned study, bacterial growth occurred on plates containing the antibiotics gentamicin and amikacin, and it was determined that the sink drains from the medical hospital contained the highest amount of antibiotic resistant organisms. Overall, 88% of the sink drains sampled from the Veterans Administration Medical Center contained some type of antibiotic resistant bacteria (Perryman and Flournoy, 1980). While bacteria could come from other sources such as the patients and tap water, the great quantity of antibiotic resistant bacteria in all environments illustrates the need for a reduction in the overuse of antibiotics and the essential awareness of the consequences.

Places with high levels of exposure to antibiotics and antibacterial products provide ideal environments for bacteria to develop resistance through

replicated mutations or transmissions between bacteria. Some factors that severely add to the growing problem of antibiotic resistant bacteria include increased applications of antibacterial soaps and cleaning products, over prescription of antibiotics by doctors, misuse of antibiotics by patients, and improper care of waste products (Levy, 1998). Bacteria can become resistant to antibiotics through genetic mutation, transfer of the mutation between bacteria, or transmission of the mutated DNA on a plasmid between bacteria when the resistant gene is carried on the plasmid DNA. A plasmid is a relatively small piece of circular DNA that is self replicating and independent of the chromosomal DNA of the cell. Resistant chromosomal DNA and plasmid DNA can be transmitted to the next generation through cell replication. Plasmids can be passed through bacterial conjugation, which involves a bacterium copying the plasmid with resistant DNA and inserting the copied plasmid into a second bacterium. Plasmid DNA can also be transferred through bacterial transformation when plasmid DNA invades another bacterium and is incorporated into the bacterium's DNA (Cognato, 2010). Understanding these problems and the mechanisms of resistance transmission is the first step in preventing further development of resistant strains of bacteria.

The focus of the experiment at hand is to determine whether the bacteria located in a laboratory sink or in an apartment garbage disposal contains more antibiotic resistant strains. It was hypothesized that the apartment garbage disposal would contain more antibiotic resistant bacteria than the laboratory sink. This is due to the abundance of contaminated materials that pass through garbage disposals in comparison to the regulated materials

that pass through laboratory sinks. The null hypothesis is that the amounts of antibiotic resistant bacteria that exist in the garbage disposal sink and laboratory sink will be equal.

Many steps were needed to accomplish this research and obtain the sample bacteria to determine the resistance. Samples from the laboratory sink and the apartment garbage disposal were swabbed on agar plates to obtain a culture of bacteria. Colonies were selected based on growth and seclusion from the bacterial " lawn". Individual bacteria were then streaked on master patch plates for each environment. After the bacteria had grown, individual colonies were selected to be streaked on antibiotic plates containing Ampicillin, Kanamycin, and Tetracycline. Antibiotic resistant bacteria were chosen from the antibiotic plates, separated and characterized. Next, plasmids from the antibiotic resistant bacteria were isolated and spliced using restriction endonucleases to determine band length of resistant plasmid DNA to help identify the type of bacteria. Competent E. coli cells were transformed with the control plasmid DNA to convey antibiotic resistance and support bacteria identification. Finally, the bacterial DNA was replicated by polymerase chain reaction to amplify the 16S rRNA gene in hopes to obtain sequencing information of a known bacterium. It was predicted that resistant bacteria, for all antibiotics, will be Gram negative due to easier entry of resistant plasmid DNA into the cell. Bacteria with a thin cell wall layer and an outer membrane surrounding the peptidoglycan layer are Gram negative. Bacteria with a thick wall layer that do not have the peptidoglycan layer surrounding are Gram positive. Gram identity was

verified through Gram staining, a KOH test, and observing growth on a MacConkey agar and Eosin Methylene Blue Agar plate.

Methods

Swab Plates

A sterile cotton swab saturated in sterile phosphate-buffered saline was used to gather samples from the laboratory sink and an apartment garbage disposal. Bacterial samples from the disposal and lab sinks were collected from the underside of the drain. Bacteria were then swabbed onto Lysogeny broth agar plates (three per environment). Plates were placed into an incubator for 24 hours at 37°C. Following the incubation period, plates were removed, parafilmed, and refrigerated at 4°C until needed.

Master Patch Plates

Master plates were made by placing sixteen individual colonies onto a 4×4 grid on Lysogeny broth (LB) only plates. An inoculation loop was used to transfer the 16 individual colonies from the sample plate onto a grid of the master plate. Plates were labeled with D for the apartment garbage disposal and L for the laboratory sink along with a number (1, 2, or 3) to distinguish between swabbed samples. Plates were incubated at 37°C for 24 hours, removed, sealed with parafilm, and refrigerated at 4°C until needed.

Antibiotic Patch Plates

Antibiotic agar plates were made by mixing 8. 4g agar with 12g LB powder and 600mL of distilled water (dH2O), and then autoclaved. After cooling, 2. 4μL of Ampicillin, 1. 2μL of Kanamycin, or 2. 4μL of Tetracycline were added

appropriately and plates were poured. One colony per grid of the master patch plate was obtained with an inoculation loop, and the bacteria were transferred in a line onto a corresponding grid on the antibiotic plates. The number of squares that contained bacterial growth was observed and recorded. One colony of the bacteria grown on the antibiotic patch plates was then streaked onto a new antibiotic plate to obtain individual colonies of bacteria for further study.

Miniprep

A liquid culture was performed in preparation for the Promega Wizard Plus SV Miniprep DNA Purification System, which was used to isolate plasmid DNA from antibiotic resistant bacteria. First, 5µL of antibiotic was added to a 5mL tube filled with a liquid medium made of LB. A single colony of bacteria was added to the medium and placed in a shaker at 37°C for 24 hours. The liquid culture was then transferred into an Eppendorf tube and centrifuged for 5 minutes at 4, 400rpm. Liquid media waste was disposed of and the pellet was thoroughly re-suspended in 250µL of Cell Resuspension Solution. If the bacteria were Gram positive, 63µL of lysozyme would be added to the solution. Since the bacteria studied was Gram negative, the process continued with the addition of 250µL of Cell Lysis Solution was added to the Eppendorf tube containing the resuspended bacterial solution and the sample was mixed. Subsequently, 10µL Alkaline Protease Solution was added, mixed, and incubated for 5 minutes at room temperature. Then, 350µL Neutralization Solution was added, mixed, and centrifuged for 10 minutes at 13, 500rpm. A Spin Column was inserted in a Collection Tube and the clear lysate was decanted into the Spin Column. This was centrifuged for

1 minute at 13, 500rpm and the flowthrough was discarded. The Spin Column was replaced, 750µL of wash solution was added, and the solution was centrifuged for 1 minute at 13, 500rpm. The flowthrough was discarded, and this process was repeated with a 250µL wash. The solution was centrifuged for 2 minutes at 13, 500rpm. The Spin Column was transferred to a 1. 5mL Eppendorf tube. Finally, 50µL of Nuclease-Free Water was added and then the solution was centrifuged for 1 minute at 13, 500rpm. The column was discarded and the DNA was stored at -20ËšC.

Gel Electrophoresis

DNA electrophoresis was used to determine the length of the plasmid DNA of the environmental samples and Blue plasmid control (pKAN). First, 0. 7g of agarose powder was added to 70mL of 1X TBE. The solution was heated in a microwave for 1 minute so the agarose powder was completely dissolved. After the mixture cooled, 3µL of Ethidium bromide was added and the gel was taken out of the mold and put on the rig. The gel was submerged in a 1X TBE buffer. The wells of the gel were filled with 10µL of a mixture containing 8µL of plasmid DNA and 2µL of plasmid dye, and the gel ran for 60 minutes on 80 volts. The 1% agarose gel was viewed under an ultraviolet light to compare lengths of DNA with the 1KB ladder.

Gram Staining

Gram staining was used to determine the Gram identity of bacteria. Bacteria that are Gram negative stained red and bacteria that are Gram positive stained violet. A colony of bacteria was added to an Eppendorf tube with 400µL of dH2O. After vortexing, 5µL of the solution was pipetted onto a slide.

Once dry, the slide was passed over a flame to affix the bacteria to the glass, preventing the removal of bacteria. The slide was flooded drop-wise with crystal violet and iodine, and rinsed with dH2O for 5 seconds after the addition of each reactant. Ethanol was added until the color was no longer emitted, then rinsed with dH2O for 5 seconds. Safranin was added drop-wise for 1 minute and then rinsed with dH2O for 5 seconds. The slide was observed under a microscope to determine Gram identity.

KOH Test

The KOH test for Gram positive and negative bacteria was begun by pipetting 20µL of 3% KOH on a slide. After adding one clump of bacteria to the KOH, the consistency of the solution was observed. If the solution was thick, viscous and adhered to the inoculation loop, the bacteria were Gram negative. If the solution was thin and not viscous, the bacteria were Gram positive.

MacConkey Agar Plate

A MacConkey agar plate was streaked with antibiotic resistant bacteria from the garbage disposal and laboratory sink. After incubation at 37ËšC for 24 hours, the plates were observed for growth to indicate Gram negative bacteria. The MacConkey agar plate also signaled lactose fermentation with the appearance of pink colonies.

Eosin Methylene Blue Agar Plate (EMB)

An EMB plate was streaked with antibiotic resistant bacteria from the apartment garbage disposal and the laboratory sink as well as a positive E.

coli control. After incubation at 37ËšC for 24 hours, the plates were observed for growth to indicate Gram negative bacteria. The EMB agar plate indicated strong lactose fermentation through the appearance of dark green metallic colonies and a lesser degree of lactose fermentation through the appearance of purple or pink colonies.

Restriction Digest

Restriction enzymes cut the control pKAN DNA at specific restriction sites identified by the NEBcutter V2. 0. The enzymes used in restriction digest were BamHI and EcoRI in Buffer II, and PvuI and PstI in Buffer III. The reaction solution used in restriction digest consists of 10μL of DNA, 1μL of each enzyme, 2μL of NEBuffer, and 7μL of de-ionized distilled water (ddH2O) added together in an Eppendorf tube. The solution was centrifuged at 14, 500rpm for 30 seconds and then incubated for 24 hours at 37ËšC. A plasmid map created from the NEBcutter V2. 0 was compared to a gel electrophoresis run on a 1% aragose gel with plasmid DNA. The gel electrophoresis compared Blue plasmid (pKAN) DNA that was uncut with the Blue control plasmid (pKAN) that was cut with restriction enzymes.

Transformation

After plasmid DNA preparation, 22μL of E. coli competent cells were added to three separate Eppendorf tubes. In one tube, 5μL of control DNA, pKAN, was added and stirred with the pipette tip. In the second tube, a negative control was made with the addition of 5μL of dH2O that was then stirred with a pipette tip. In the third tube, a positive control was made with the addition of 1μL of known pKAN, and the solution was stirred with a pipette tip. The tubes

were then incubated in ice 30 minutes. The cells were heat shocked for 45 seconds at 42ĚšC and then placed on ice for 2 minutes. 250µL of pre-warmed (37ĚšC) SOC medium was added to all three of the Eppendorf tubes, and the tubes were then incubated in a shaker at 37ĚšC for 1 hour at 2, 250rpm. Upon removal from the incubator, 75µL of each transformation were spread onto plates with a sterilized " hockey stick". The transformed control DNA, pKAN, cells and the negative control dH2O transformed cells were spread onto LB only plates, ampicillin antibiotic plates, and kanamycin antibiotic plates to determine if resistance to antibiotics was transferred in the transformation. The transformed positive control, known pKAN, cells was spread onto a LB only plate and a kanamycin plate since pKAN is known to be resistant to kanamycin. Plates were incubated for 24 hours at 37ĚšC and numbers of resistant bacterial colonies were observed. Bacterial growth on the control DNA, pKAN, transformation antibiotic plates would signal resistance to the antibiotic in the plate, and growth on the LB only plate would signal the existence of bacterial cells from the transformation. No growth on the dH2O negative control plates containing ampicillin and kanamycin antibiotics would signal a correct transformation as long as there was bacterial growth on the LB only plate. Growth on the positive control, known pKAN, transformation plate signaled the correct transfer of kanamycin resistant plasmid DNA into the competent E. coli cells.

Polymerase Chain Reaction

The Polymerase Chain Reaction (PCR) involved mixing a reaction cocktail that included 80µL of Nuclease-free water, 10µL of 10X Thermopol buffer, 3µL of 10mM dNTPs, 2µL of 11F @ 10µM, 2µL of 1492R @ 10µM, and 1µL of

Taq polymerase @ 5000U/mL. The solution was then mixed through vortexing. Subsequently, 22μL of the cocktail was transferred to each of the 4 PCR tubes. A small portion of each bacterial colony was added to SOC medium and mixed. Then 5μL of SOC medium with bacteria was added to each tube. Tube 1 had environmental bacteria, tube 2 had different environmental bacteria, tube 3 had the control E. coli and 5μL of H_2O was added to tube 4. The reactions were placed in the thermocycler in C4. The PCR cycling program consists of five steps. The first step is pre-denaturation in which the PCR mixing reaction cocktail is heated at 95°C for 5 minutes. The second step is denaturation, which involves heating the reaction cocktail at 95°C for 30 seconds to unwind and separate the DNA. The third step is annealing, which is run at 50°C for 30 seconds to allow the 11F and 1492R primers to attach to the DNA template strands. The fourth step is elongation, which is run at 72°C for 45 seconds to allow the DNA polymerase (Taq polymerase) to add dNTPs and replicate the 16S gene. The fifth step is the final elongation, which is run at 72°C for 7 minutes. The hold between cycles is run at 4°C, and the PCR is run for 35 cycles. Gel electrophoresis was run to determine if a successful PCR reaction took place. 10μL of the PCR solution from each tube was mixed with 2μL of plasmid dye, and 10μL of the mixtures were loaded into the wells of the 1% agarose gel.

Chi Squared Test of Independence

A Chi Squared Test of Independence was run to determine if a statistically significant difference exists between the numbers of antibiotic resistant bacteria from the two environments. The number of grids on the antibiotic plates was recorded only if the bacteria grew on both the antibiotic plate and

the LB only plate. The test was run on Vassar Stats and gave a p-value to correspond to the data and indicate if there was a significant difference.

Results

Swab and Master Patch Plates

After the incubation period of 24 hours at 37 C, the swab plates, labeled L for laboratory sink samples (L1-L3) and D for garbage disposal sink samples (D1-D3), were observed and found that 100% of the environmental bacteria grew (Figure 1). Bacteria growth in both environments was indicated by white colored spots or streaks within the plate's grid. Master plates were observed from both experimental environments and found to have growth on all of the 16 grids on each plate (Figure 2).

Antibiotic Patch Plates

From the garbage disposal sink, the three samples all had some level of growth (Figure 3). The following percentages were calculated by dividing the number of grids with bacterial development on the antibiotic plates by the number of grids with growth from the LB plates (Table 1). Plate D1 showed 100%, 62. 5%, 0%, and 100% growth on the Ampicillin, Kanamycin, Tetracycline, and LB only plates respectively. Plate D2 demonstrated 93. 75%, 93. 75%, 0%, and 100% growth on the Ampicillin, Kanamycin, Tetracycline, and LB only plates respectively. Plate D3 showed 93. 75%, 75%, 0%, and 100% growth on the Ampicillin, Kanamycin, Tetracycline, and LB only plates respectively. From the laboratory sink, all samples had bacteria development (Figure 4). Plate L1 demonstrated 100%, 93. 75%, 12. 5%, and 100% growth on the Ampicillin, Kanamycin, Tetracycline, and LB

only plates respectively. Plate L2 showed 100%, 73. 33%, 6. 67%, and 93. 75% growth on the Ampicillin, Kanamycin, Tetracycline, and LB only plates respectively. Plate L3 demonstrated 57. 14%, 42. 86%, 7. 14%, and 87. 5% growth on the Ampicillin, Kanamycin, Tetracycline, and LB only plates respectively.

Chi Squared Test of Independence

Data obtained from the number of antibiotic resistant colonies on the antibiotic patch plates was used to run the Chi-squared Test of Independence for Ampicillin and Kanamycin resistant bacteria. For Ampicillin resistant bacteria, the p-value obtained was 0. 74. With one degree of freedom, the Chi-squared critical value of 3. 84 obtained from a Chi-squared Distribution Table in comparison to the Chi-squared statistical value denoted no statistically significant difference. For Kanamycin resistant bacteris, the calculated p-value was 0. 81. With one degree of freedom, comparison of the Chi-squared critical value of 3. 84 found in a Chi-squared Distribution Table and the Chi-squared statistical value demonstrated no statistically significant difference (Table 1).

Gram Staining, KOH, MacConkey Agar and Eosin Methylene Blue Agar Plates

Four tests were used to determine the gram identity of bacteria from the experimental environments. The results showed that the three environmental bacteria slides were stained pink indicating gram negative bacteria (Figure 5, Table 2). For the KOH test, all three samples from both environments appeared viscous and thick, indicating gram negative bacteria (Table 2). The MacConkey Agar Plate was divided into three sections for the

different antibiotic resistant bacteria. The environmental bacterial sample in Section 1 was obtained from the Ampicillin antibiotic plate L2 grid #3. The bacterial sample in Section 2 was obtained from the Kanamycin antibiotic plate L2 grid #14. The bacterial sample in Section 3 was obtained from the Kanamycin antibiotic plate D2 grid #16. All three samples in the three sections grew bacteria that were stained pink, indicating Gram negative bacteria that ferment lactose (Figure 6, Table 2). The Eosin Methylene Blue Agar Plate was sectioned off into four parts and bacteria from three environmental samples and one E. coli positive control were plated. The bacterial sample in Section 1 was taken from the Ampicillin antibiotic plate L2 grid #3. The bacterial sample in Section 2 was obtained from the Kanamycin antibiotic plate L2 grid #14. The bacterial sample in Section 3 was gathered from the Kanamycin antibiotic plate D2 grid #16. The bacterial sample in Section 4 was obtained from an E. coli plate that was known to be Gram negative. Pink colonies formed in all four sections, signaling Gram negative identity of the bacteria and lactose fermentation (Figure 6, Table 2).

Mini Prep and Gel Electrophoresis

Promega Wizard Plus SV Miniprep DNA Purification System was run to isolate plasmid DNA. This plasmid DNA was run on a 1% agarose gel. The lengths of bands in Trial A could not be determined because the DNA in the wells did not run with the ladder. The Blue control plasmid, which was pKAN, was located in lane 3 in Trial A and Trial B and was used to indicate a successful Miniprep. The band length of the pKAN control DNA in Trial B was about 4, 200 base pairs. An environmental plasmid found on Ampicillin streak plate L2, grid #3 was used in lane 7 in Trial A and lane 5 in Trial B. In Trial B, the

base pair length of the environmental bacteria plasmid used in lane 5 could not be determined due to the appearance of many bands of varying length. An environmental plasmid from Kanamycin streak plate L2, grid #14 was used in lane 5 in Trial A and lane 7 in Trial B. The band length of this environmental plasmid in Trial B could not be determined due to the faint appearance of a band greater than 10, 000bp. Another environmental plasmid from Kanamycin streak plate D2, grid#16 was used in lane 6 in both Trial A and Trial B. The band length of this environmental plasmid in Trial B also could not be determined from the faint appearance of a band greater than 10, 000bp (Figure 7).

Restriction Digest

In Trial A, restriction digest was used to cut the Blue control pKAN DNA with the enzymes BamHI, EcoRI, PstI, and PvuI. Lane 3 displays pKAN cut with PstI and PvuI. Lane 4 displays pKAN cut with BamHI and EcoRI. The lengths of the bands shown are about 4, 000bp, 3, 000bp, 2, 500bp, 1, 500bp, and 1, 200bp. The lengths of the bands shown are about 1, 700bp, 1, 100bp, 750bp, 600bp, and 500bp. Lanes 5-8 contained environmental bacterial DNA that was cut with BamHI, EcoRI, PstI, and PvuI as well, but no bands were observed (Figure 8).

In Trial B, restriction digest was used to cut pKAN DNA with only the enzymes BamHI and EcoRI. Lane 3 displays pKAN that was cut with BamHI, showing a band length that is about 4, 200bp. Lane 4 shows pKAN that was cut with EcoRI, and the band lengths shown are about 8, 000bp, 5, 000bp, and 4, 000bp. Lane 5 displays pKAN that was cut with BamHI and EcoRI, and the

band lengths shown are about 4, 100bp, 3, 100bp, and 2, 000bp. Lane 6 shows pKAN that remained uncut with a band length of about 4, 200bp (Figure 9).

Transformation

Transformation was performed to convey resistance carried on plasmid DNA into competent E. coli cells. Blue plasmid control DNA (pKAN) was used for the transformation, which was successful. This was indicated by the growth of transformed bacteria on Kanamycin antibacterial plates (Figure 10).

Polymerase Chain Reaction

A Polymerase Chain Reaction (PCR) was used to amplify and prepare the 16S gene of rRNA. Gel electrophoresis was run on the PCR product to determine if a successful PCR reaction had taken place. Lane 3 contains PCR product from the Kanamycin plate L1 grid #14 and lane 4 contains PCR product from the master patch plate D3 grid #16. Bands were not seen in these lanes containing environmental bacteria, signaling an unsuccessful PCR. Lane 5 displays the negative water control without bands. Lane 6 shows the positive E. coli control PCR product with a band length of about 2, 000bp (Figure 11).

Discussion

The study showed that no statistically significant difference existed between the amount of antibiotic resistant bacteria in the garbage disposal and laboratory sink and it also characterized all of the environmental bacteria as Gram negative. To determine the amount of bacteria located in the experimental areas, many tests were utilized to analyze the bacterium.

Patch plates containing Tetracycline, Ampicillin, Kanamycin and LB were

made in order to verify antibiotic resistant bacteria and growth. The plates with bacterial growth that was resistant to Ampicillin and Kanamycin were used in a statistical analysis to determine a correlation between the amounts of growth and the two environments. Our prediction that the amount of bacterial growth from the garbage disposal sink in Capitol Villa would be greater than the Lyman Briggs lab sink in C5 was refuted due to the Chi-squared Test for Independence that showed no statistically significant difference. We failed to reject the null hypothesis that no difference existed between the amounts of antibiotic resistant bacteria found in each environment.

A Chi-squared Test for Independence was run to compare the amounts of antibiotic resistant bacteria on the Ampicillin and Kanamycin plates. Tetracycline was not used because no data indicated resistance. The existence of Ampicillin and Kanamycin resistant bacteria in both the garbage disposal and the laboratory sink is unsurprising due to the widespread clinical use of both antibiotics over the past decades (Criswell, 2004). For Ampicillin, a total of 178 bacterial streaks grew between the two environments and a p-value of 0. 74 was calculated. With one degree of freedom, the Chi-squared critical value of 3. 84 obtained from a Chi-squared Distribution Table in comparison to the Chi-squared statistical value denoted no statistically significant difference. For Kanamycin, 162 streaks grew between the two environments and a p-value of 0. 81 was calculated. With one degree of freedom, the a comparison of the Chi-squared critical value of 3. 84 found from a Chi-squared Distribution Table to the Chi-squared statistical value denoted no statistically significant difference as well.

Therefore, the prediction that the garbage disposal sink would contain more antibiotic resistant bacteria than the laboratory sink was rejected.

To further understand why bacteria were resistant, four tests were run to categorize the Gram identity of the environmental samples. The structure of the bacteria plays a large role in determining resistance. Importantly, it is easier for the plasmid DNA to penetrate a Gram negative bacterium due to the lack of an outer membrane around the peptidoglycan layer. The Gram staining process showed pink rod shaped bacterium, demonstrating that the bacteria was Gram negative. The KOH tests resulted in a viscous substance, indicating that all the environmental bacteria obtained from the garbage disposal and the laboratory sink were Gram negative. The MacConkey agar plates identified the bacteria to be Gram negative through growth on the plate. The growth on the plate was a pink color, signifying lactose fermentation from the bacteria. The environmental bacteria developed pink colonies on the EMB agar plates, further supporting the Gram negative identity and a low production of lactose fermentation of the environmental bacteria gathered from the garbage disposal and laboratory sink.

Gel electrophoresis was used in determining the existence and length of environmental plasmid DNA. The Miniprep isolated the plasmid DNA from the bacteria, but upon running the gel, it was discovered that no environmental plasmid DNA was present. The absence of bands