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Transformation and Conjugation of Ampicillin-Resistant *Escherichia coli*

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Abstract

There is growing concern regarding the development of antibiotic resistance in clinical and agricultural settings due to the prevalence of antibiotics that exist there. However, antibiotic resistant traits are used extensively in research labs and even in undergraduate classrooms. This study aimed to determine whether undergraduate laboratory transformation experiments could contribute to the spread of antibiotic resistance. Studies have been done on antibiotic resistance in large scale hospital and waste-treatment environments; similar methods were applied to an undergraduate laboratory. First, this study examined whether ampicillin-resistant *E. coli* were left in the laboratory after the General Biology freshmen performed a transformation experiment. In addition, this study tested how efficiently ampicillin-resistant bacteria could transfer its resistance to other bacterial genera. The undergraduate lab was swabbed in five highly trafficked areas; the undersides of work tables, sides of chairs, and doorknob produced no resistant cultures, while swabs of the sink and table tops contained some resistant bacteria. These *E. coli* were plated with various strains of bacteria, including several other *Enterobacteriaceae* as well as gram positive genera with clinical relevance. Then, selective and differential media were used to determine if ampicillin resistance was transferred. The results were assessed by the colony counting plate method. This study's findings could have immediate implications for the safety and cleaning procedures used by undergraduate labs and could provide incentive to test this hypothesis more thoroughly in clinical environments in the future. In addition, the results indicate the possible contamination of sewage water and the release of resistant bacteria into the environment. Further experimentation could better determine the clinical and environmental consequences of the spread of antibiotic resistance in an aquatic environment.

Key Words: ampicillin resistance, transformation, bacteria

1. Introduction

Through the years, resistant bacteria, specifically MRSA, have posed a crucial problem facing the healthcare industry. MRSA infections treated in hospitals have doubled nationwide between the years of 1999 and 2005, growing from an estimated 127,000 reported infections to 278,000 reported infections.[9] Resulting from these bacterial infections, awareness of antibiotic resistance has become a widespread issue. Bacteria are found on all surfaces in our environment, and they possess the potential to transfer resistant genetic information from one strain to another. This resistance can be transferred through mutations in the DNA of the bacteria, or through a process called horizontal gene transfer.[4] Transformation, or the transfer of recombinant DNA between bacteria, is the primary mechanism of horizontal gene transfer in recombinant strains of bacteria,[7] and it consists of releasing DNA into the environment, the induction of the gene of interest into competent host bacteria, interaction of cells and recombinant DNA in the host cell, and the entering of DNA into the cell, which begins expressing the recombinant gene. In conjugation, the donor colony typically uses a plasmid known as an 'R-factor' to transfer the genetic material directly to the target bacterium. Strains with R-factors have been shown to transfer their resistances both in nutrient broth and when found in a supportive natural environment.[8] In order to transfer genetic information,

however, the host bacteria must have competence, or the ability to take foreign DNA into its cell. For this reason, the bacterial strain, *Escherichia coli*, is commonly used due to its competence and ease of use.

In addition to being extremely competent, *E. coli* has shown the ability to transfer resistance to ampicillin to a non-adjacent colony through intercellular signaling,[3] as well as the ability to transfer resistance through direct horizontal transfer, allowing it to both transfer and receive resistance with other strains. Williams demonstrated that *E. coli* is able to transfer a plasmid to other strains of bacteria, such as *Enterococcus faecalis*, *Streptococcus cremoris*, and *Clostridium acetobutylicum.*[15] Due to the possible antibiotic resistance from *E. coli* to other strains of Enterobacteria; it could pose viable clinical issues that could alter the health of those that come into contact with them. The work of Reinthaler revealed that sewage runoff of hospitals can provide a natural environment which encourages the growth and spread of antibiotic resistance, finding multiple resistant bacterial strains in sewers connected to hospitals.[11] For these reasons, the transfer of recombinant DNA across bacterial species is relevant to biology labs across the country. This issue is compounded when general biology students perform bacterial transformation procedures in labs without utilizing proper safety techniques. Sniegowski and Lenski reported that there are high rates of mutation in *E. coli* populations with higher mutators, or bacteria that have mutated to show resistance to a particular characteristic, than wild-type populations, and these populations are more likely to replicate with the mutation.[13] If General Biology laboratories are not safety conscious about preventing *E. coli* transformation of ampicillin resistance to other species of bacteria, it could pose a significant threat to the wellbeing of the community. *Enterococcus faecalis*, for example, has been shown to be competent and capable of receiving resistant genes from affected species.[6] It was the goal of this study to determine whether or not General Biology students' methods lead to an abundance of untreated resistant strains of bacteria, and it aimed to show the importance of minimizing the transformation of recombinant DNA in freshmen-level laboratories. This model could also be applied to hospital settings to determine cleanliness of the facilities and surrounding areas. We expect to find higher bacterial resistance transfer in cases where cleaning procedures are followed less stringently.

2. Materials and Methods

2.1 Obtaining Ampicillin-Resistant *Escherichia Coli*:

The bacteria used in this experiment were *E. coli*, and ampicillin-resistant strains were obtained through the transformation laboratory performed by General Biology students. In order to obtain ampicillin-resistant samples for further experimentation, this experiment was replicated. *E. coli* were mixed with calcium chloride in a micro tube and pGEM plasmid DNA was added to the solution and set on ice for 15 minutes. Next, the competent cells were subjected to a heat shock in a 42°C water bath for exactly two minutes, and then transferred back to the ice. The competent cells then sat for five minutes until Luria (LB) broth was added to the tube. The cells were then left alone in room temperature for 60 minutes and plated, using standard plating methods, onto ampicillin positive agar in a petri dish. This experiment was performed using five different micro tubes, thus five plates of ampicillin-resistant *E. coli* were obtained and allowed to culture overnight in a 37°C incubator.

2.2 Collecting Ampicillin-Resistant *Escherichia Coli* Cultures:

To start, control samples were taken from each chosen location at Xavier University Albers Hall, Room 207, to determine whether or not ampicillin-resistant *E. coli* were present. Control samples were taken from the same location as the experimental samples; the sink, door handle, surface of students' lab benches, underside of students' lab benches, and the underside of students' chairs, but were taken before the students performed the transformation experiment. The control samples consisted of four swabs taken from each location and plated on a single plate split into four quadrants; each swab was sterile and dipped into autoclaved water before sampling. The control samples were then allowed to incubate in a 37°C for 48 hours to allow bacterial growth.

Once the control samples were accounted for, the students began the transformation experiment, and the experimental samples were taken during the time the students were performing their experiments. Again, samples were taken with swabs that were sterile and dipped into autoclaved water before sampling, and four samples were taken from each experimental location and allowed to incubate in a 37°C incubator for 48 hours to allow bacterial growth. Three trials from each experimental location were collected during different periods of the students' experiment.

2.3 Determining Transformation Of Ampicillin Resistance To Surrounding Bacteria:

In order to determine whether or not ampicillin-resistant *E. coli* had the capability to transfer its ampicillin resistance to other surrounding bacteria, resistant *E. coli* colonies were placed on a nutrient agar with various strains of Enterobacteria (*K. pneumoniae*, *E. cloacae*, *S. epidermidis*, *P. vulgaris*, and *P. mirabilis*). The colonies of Enterobacteria were then placed in LB broth and allowed to grow in a 37°C incubator for 48 hours. After 48 hours, a serial dilution was performed and the resultant plates were let to sit overnight in the 37°C incubator. The next morning, the selective plates were observed and the numbers of resistant strains of Enterobacteria were measured. Those plates that showed positive ampicillin-transformation exemplified different colors on the selective media; light green dots were ampicillin-resistant *E. coli* and darker dots on the same petri dish were the resultant strains of Enterobacteria that underwent positive ampicillin-transformation. The data was recorded in the number of colonies that showed ampicillin-resistant transfer.

2.4 Data Analysis:

Swab sample data was counted by hand to determine the existence of bacterial growth and to identify different bacterial strains on the EMB plates. Plates were divided into fourths; a single quadrant was counted and multiplied by four to obtain an estimate for total number when plates exhibited high amounts of growth (greater than 200 colonies). An ANOVA test was performed in order to determine the significance of the results of the antibiotic resistance transfer experiment.

3. Results

During the duration of this research, two different experiments were performed. The first was to determine whether or not there was an abundance of ampicillin-resistant *Escherichia coli* remaining in freshman biology laboratories. We went about this through swabbing various highly trafficked locations in the laboratory and plating the swabs on ampicillin-positive agar to measure growth.

Table 1: Collections of ampicillin resistant *Escherichia coli* in highly trafficked areas in a freshman biology laboratory measured in colonies (col).

Location of Swab	Control	Trial 1	Trial 2	Trial 3
Tabletop	0 col	0 col	0 col	3 col
Table Underside	0 col	0 col	0 col	1 col
Sink	31 col	0 col	TNTC col	0 col
Door Handle	0 col	0 col	0 col	0 col
Chair Underside	0 col	0 col	0 col	0 col
Location of Swab	Control	Trial 1	Trial 2	Trial 3
Tabletop	0 col	0 col	0 col	3 col
Table Underside	0 col	0 col	0 col	1 col
Sink	31 col	0 col	TNTC col	0 col
Door Handle	0 col	0 col	0 col	0 col
Chair Underside	0 col	0 col	0 col	0 col

As seen in Table 1, there was observed growth in three of the five swabbed locations with the sink having the most remaining ampicillin-resistant *E. coli* with measurements TNTC (too numerous to count). Although there was growth in three of five swabbed locations, the results were non-significant ($p=0.43$ by ANOVA).

Table 2: Number of hand-counted colonies on selective ampicillin positive plates created from serial dilutions of broth of *E. coli* and various pathogenic bacterial strains. Three strains of gram negative bacterial showed successful expression of ampicillin resistance. Lack of result from gram positive bacterial strain (*Staphylococcus epidermidis)* supports the occurrence of conjugation over transduction and translation.

Species Name	Number of colonies on 10^6 dilution	Secondary Color Present
Citrobacter freundii	0	No
Klebsiella pneumoniae	TNTC	Yes
Enterobacter cloaceae	42	Yes
Proteus vulgaris	612	Yes
Proteus mirabilis	0	No
Staphylococcus epidermidis (+)	0	No

As seen in Table 2, three of the six experimental dilutions produced ampicillin resistant *E. coli* and ampicillin resistant colonies of the second bacteria. The differential media on those three plates successfully produced color differences for identification of both *E. coli* and non-*E. coli* colonial growth. *E. coli* was the most dominant bacteria on all the plates that exhibited growth. The highest observed rate of resistance transfer occurred on the *Klebsiella pneumonia* experimental plate.

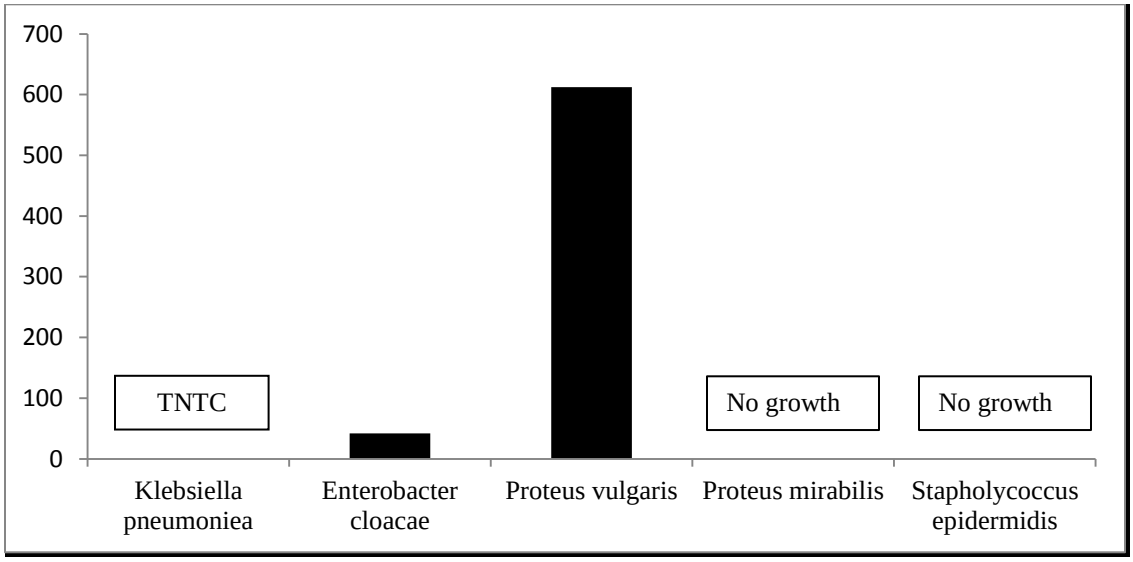

Figure 1: Results of ampicillin resistance transfer from *Escherichia coli* to various strains of gram-negative Enterobacteria measured in colonies).

For the second part of this research, we wanted to observe whether or not ampicillin resistant E.coli transferred its resistance to surrounding Enterobacteria through the process of horizontal gene transfer. As shown in Figure 1, there was successful transformation of the resistant plasmid in *E. cloacae*, *P. vulgaris*, and *K. pneumoniea* with *K. pneumoniea* having the highest rate of transformation). Since the *K. pneumoniea* showed results that were TNTC (too numerous to count), the results were unable to be charted in Figure 1. The results of *E. cloacae* showed 42 ampicillin-resistant colonies, suggesting competence of the resistant plasmid from ampicillin-resistant *E. coli*. In addition, *P. vulgaris* showed 612 ampicillin-resistant colonies, also suggesting competence of the resistant plasmid from ampicillin-resistant *E. coli*. Finally, bacterial species *P. mirabilis* and *S. epidermidis* showed no positive competence of ampicillin resistance from *E. coli*, thus resulting in no growth on the ampicillin-positive agar plates.

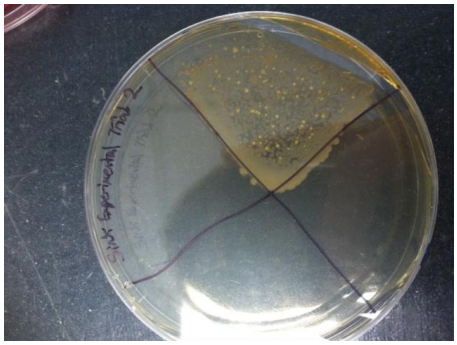

Figure 2, William Gannon, 2014, *E. coli* growth on ampicillin positive LB agar from General Biology laboratory communal sink drain. Colonies were too numerous to count, and growth indicates presence of antibiotic-resistant bacteria.

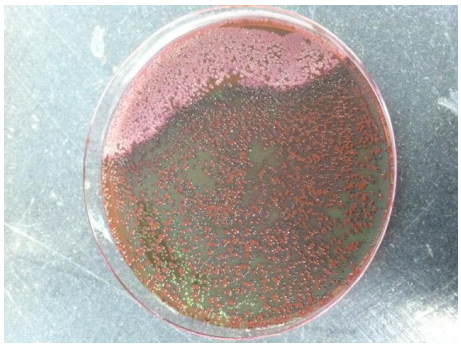

Figure 3. William Gannon, 2014, *E. coli* and K. pneumoniae growth on selective, differential, ampicillin positive EMB agar. EMB agar tests specifically for *E. coli* growth and dyes *E. coli* colonies green due to the lactose fermentation process. All other colonies appear pink-purple in color.

4. Discussion

Initially, we believed that the freshman biology lab would show extremely high levels of antibiotic resistance, specifically ampicillin-resistant *Escherichia coli*, because the students wouldn't follow the cleaning procedures properly. Although experimenters cannot be certain that students did not follow the cleaning procedures carefully, it was determined that there was still some ampicillin-resistant residue remaining after their transformation labs. Ampicillin-resistant *E. coli* was found in the sink drain as well as on the surface and underside of the tables the students were working on. It might be noteworthy to add that the controls of the experiment were taken a day after the transformation labs began, so the resistant strains could be a result of that day's lab, or it could also be a result of the professor enacting the same experiment and leaving traces of resistant strains in the sink due to poor cleaning procedures. This could also indicate that the resistant strains found in the sink have been there for quite some time, indicating that the resistance could have been contaminating the water supply since the last year's experiment. These resistant strains of bacteria could pose a threat to the surrounding environment and agriculture if they get into the water supply because they have the possibility of transferring their resistance to other strains of gram-negative bacteria. Specifically, since there was the highest concentration of ampicillin-resistant *E. coli* centralized in the communal sink, these bacteria could easily seep into the sewage and affect the water supply. Also, because the bacteria have shown the capability to transfer their resistance to other, surrounding species of bacteria, this could become a widespread issue. This was determined through the second experiment, which showed the transfer of ampicillin resistance from *E. coli* to *K. pneumonia, P. vulgaris,* as well as *E. cloacae.*

One implication that the transformation of resistance from *E. coli* to other bacterial species is harmful is that of clinical relevance. For example, the bacteria *Klebsiella pneumoniae* has clinical relevance in that it is believed to be

the major pathogen involved with pyogenic liver abscess as well as one of the leading causes of pneumonia.[5] *Klebsiella pneumoniae* can potentially be pathogenic if it is inhaled, and is a pathogen of concern in hospital environments due to multi-drug resistance phenotypes. Research has found that *K. pneumoniae* is capable of transferring this multi-drug resistance to *E. coli* bacteria.[2] This is relevant because *K. pneumonia* was a bacterial species that tested positive for the transformation of ampicillin resistance from *E. coli*, and if this bacterial species is capable of receiving resistance to treatments and antibacterial medications, then it could pose a significant problem to those suffering from the liver abscess that require specific treatment, or those prevalent to pneumonia.

In addition to *K. pneumoniae*, *P. vulgaris* also may have clinical implications given that it, too, received positive transformation of the ampicillin-resistant gene from *E. coli*. One of the major pathogenic capabilities of *P. vulgaris* is urinary tract infections, specifically bladder and kidney stones.[1] Since *P. vulgaris* was observed to exhibit positive transformation of ampicillin from resistant *E. coli*, it could imply implies that it is competent to receive other resistant genetic material as well. Since this transformation is possible from *E. coli* to *P. vulgaris*, there could be significant health issues if the ampicillin resistant *E. coli* spread in the environment because it could influence resistance into these more virulent strains of bacterial species.

To add, *E. cloacae* also exhibited positive transfer of ampicillin resistance from *E. coli*, and this bacterial species also possesses clinical significance. For example, in neonatal patients, *E. cloaceae* was discovered to cause necrotizing enterocolitis, or the death of intestinal tissue that generally affects premature and sick babies.[10] This is significant because, although not typically treated with ampicillin, the transfer of antibiotic resistance to these pathogenic bacteria could pose a threat to those affected with them.

In addition to the various tested gram-negative bacterial species that showed positive transfer of resistance, it is also important that resistance was not transferred to the gram-positive *S. epidermidis*. Our result for S. epidermidis did not support the idea that *E. coli* is capable of transferring resistance to gram-positive bacterial strains. It did support the occurrence of conjugation within our experiment over transformation or transduction, but further experimentation should be performed to solidify those findings. Gram-positive bacteria have large clinical relevance, especially in hospital settings, because they are the common causes of bloodstream and other infections in hospitalized patients.[12] One of the largest gram-negative infections occurring today is methicillin-resistant *Staphylococcus aureus* (MRSA), and the fact that transformation of resistance from a gram-negative species did not occur means that this infection is influenced in other ways. It is of large concern to minimize the transfer of MRSA and other gram-positive infections because they do not behave the same as our studied gram-negative species.

Finally, although none of these pathogenic bacteria are commonly treated with ampicillin, this research provides a model for the possible transfer of antibiotic resistance to that of virulent, harmful strains. For example, instead of using ampicillin resistance transfer to *K. pneumoniae*, researchers could use more clinically relevant strains of antibiotics, such as Carbapenem.[14] In addition, it would be beneficial to perform further control experiments to determine if the different strains of Enterobacteria could grow without the addition of *E. coli* in order to test for any acquisition of ampicillin resistance. Once this is confirmed, the results would be more definitive if growth were to appear in the presence of ampicillin-resistant *E. coli*. To further confirm the effectiveness of the ampicillin plates, it would also be beneficial to test non-ampicillin resistant bacteria on ampicillin-positive plates to see if the ampicillin effectively kills cells that lack ampicillin resistance. Another way to improve this research modes is to have the experimenter further confirm that the ampicillin-resistant *E. coli* were remaining from students performing the transformation lab as explained earlier. Experimenters should allow students to perform the transformation experiment in a laboratory that has not previously been exposed to this experiment, then swab the different locations in this laboratory and examine the swabs to check for ampicillin-resistant bacterial species.

Antibiotic resistance is of growing concern around the world today, and this research is a model of the different types of virulent strains of bacteria that can be affected if left untreated or if left in areas containing other resistant strains. Furthermore, if transfer of this resistance is not carefully observed and monitored, there could be a resultant outbreak of increasing antibiotic resistant virulent strains of bacteria that our current medication systems could not manipulate and destroy. Further research should be conducted, and a suggestion is to use medically relevant antibiotic-resistant bacteria to determine if virulent strains can positively transfer or conjugate resistant strains of their common antibacterial. Also, our research only performed three trials in five different locations in a General Biology laboratory, which is certainly not enough data to determine whether there is a reoccurring problem with resistant *E. coli* residue. For future studies, researchers could increase the number of trials and locations tested in order to obtain more data. In addition, this research should be carried on through the extent of the year in order to determine if resistance is most prevalent during the transformation lab, or if it is occurring on a more significant basis.

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