Plasmid-Mediated Transfer of Antibiotic Resistance Genes within Biofilms

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Abstract

Antibiotic-resistant bacteria pose one of the most consequential medical threats to human health. Antibiotic resistance is most often encoded in the DNA of bacterial plasmids, which allows the resistance to be horizontally transferred through conjugation to other bacteria. The objective of this study was to determine the transfer frequency of an environmentally extracted, multidrug resistance plasmid, pTRE-131, under three different mating conditions: liquid cultures, batch biofilms, and drip-flow biofilms. All three mating methods were conducted for 1 h and 24 h using *Escherichia coli* JM109 (pTRE-131) as plasmid donor and *Escherichia coli* ATCC 25922 as the recipient. Mated bacteria were serially diluted and plated on selective agar to estimate cell density of total recipients and transconjugants. Generalized linear modeling revealed no significant difference in conjugation efficiency at the different exposure times; however, the drip-flow biofilms showed a significantly greater efficiency than the other two conditions tested. These findings suggest that a complete understanding of the evolution of antibiotic-resistant bacteria requires the investigation of genetic exchange between planktonic and biofilm phases.

Introduction

Antibiotic-resistant microbes are selected for by the presence of antimicrobial agents in the environment and clinical setting. It is a common misconception that the presence of antibiotics creates resistant bacteria; the use of antibiotics only selects for the bacteria already carrying the resistance. By eliminating all susceptible bacteria, resistant bacteria are given the space and nutrients to flourish due to compromised microbial antagonism, resulting in them becoming the dominating bacterial strain in that specific environment. Bacteria carry antibiotic resistance genes (ARGs) on their plasmids, which are defined as small circular strands of DNA that replicate independently of chromosomal DNA and are able to be horizontally transferred through conjugation. Conjugative plasmids are the greatest contributor to the spread of ARGs (1). Once a recipient bacterial cell receives the new plasmid, the plasmid can be replicated and kept at a high copy number in the new cell depending on the selective pressures put on that bacterium from their environment. One of the greatest selective pressures that bacteria face are antibiotics. The increasing presence of antibiotics directly increases the abundance of plasmids in the environment that contain genes encoding for antibiotic resistance.

Bacterial resistance to antibiotics is spread by horizontal gene transfer, specifically bacterial conjugation. Bacterial conjugation is when genetic material is passed from one bacterium to another via direct cell-to-cell contact through a sex pilus. When two bacteria are connected by a sex pilus, the plasmid in one bacterium can be replicated and transferred to the new bacterium where it is replicated and maintained (2). The result of conjugation is that all of the bacteria involved now have the plasmid, which contains antibiotic resistance genes and oftentimes other virulence factors. Conjugation in a liquid culture mating, a mixture of cultured donor and recipient bacterial strains, is based on a random chance that the two bacterial cells will "run into" each other and attach by the donor's sex pilus (Figure 1). Batch biofilms, a recipient strain biofilm grown on a microscope slide and exposed to a cultured donor strain, allow for a greater chance of bacterial contact; however, this method still relies on chance (Figure 2). Conjugation in a drip-flow biofilm, biofilm grown on a microscope slide exposed to a constant flow of media containing donor with plasmid, insures direct cell-to-cell contact of the donor's plasmid with the recipient biofilm (Figure 3).

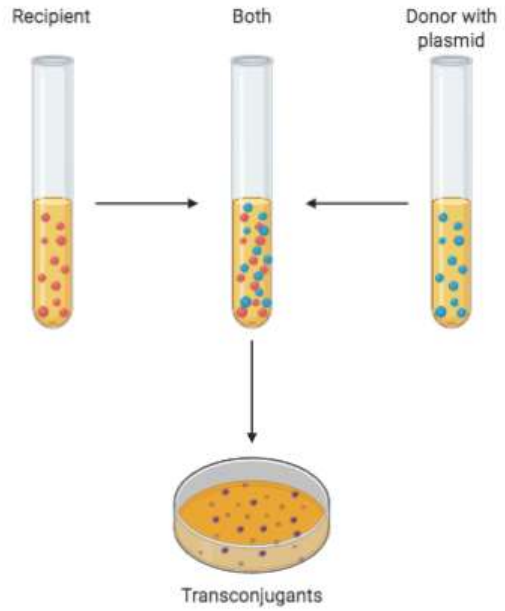


Figure 1: Liquid Culture Mating. Donor with plasmid and recipient are combined for a liquid culture mating. Bacterial growth on selective agar results from a successful conjugation.

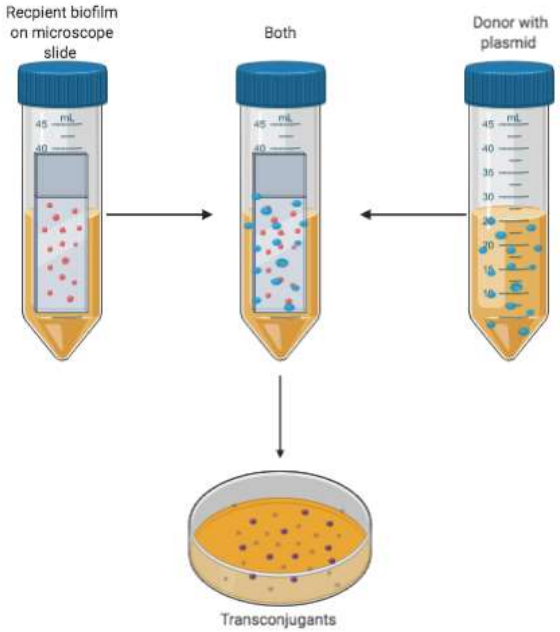


Figure 2: Batch Biofilm Mating. Recipient biofilm is exposed to cultured donor with plasmid and allowed time for conjugation. Bacterial growth on selective agar results from a successful conjugation.

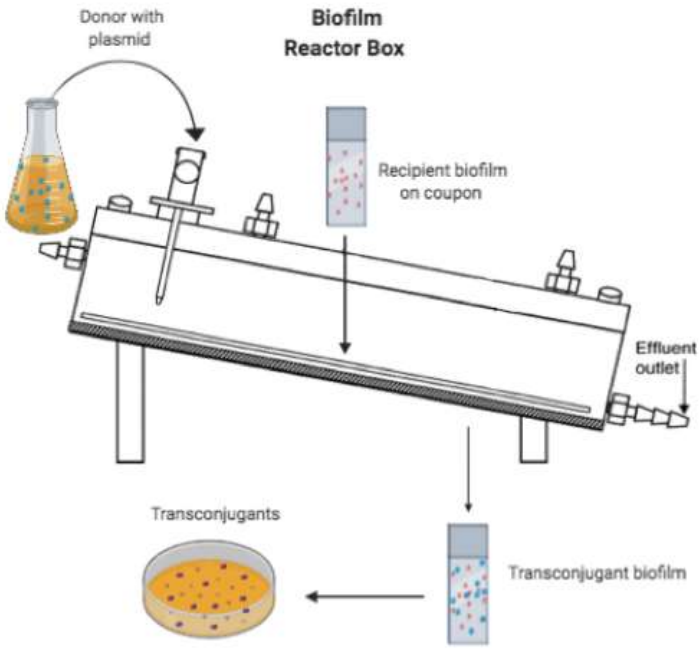


Figure 3: Drip-Flow Biofilm Mating. Recipient biofilm grown with a continuous flow of fresh media are exposed to a continuous flow of donor with plasmid for conjugation. Bacterial growth on selective agar results from a successful conjugation.

Biofilms, a thick, slimy layer of multiple bacterial cells, adhered to a solid surface and enclosed in their own extracellular polymeric substance (EPS), can grow in any moist and nutrient-rich environment. Common clinical environments that tend to be colonized by biofilms include catheters, transplants, ear canals and dental plaque (3). Up to 80% of all lethal bacterial infections are a caused by biofilm forming bacteria (4). In addition, studies suggest that antibiotic resistance can increase up to 1000-fold in a biofilm as compared to planktonic, free-floating, bacteria, yet there is still no consensus in the literature on multidrug resistance plasmids transmissibility within biofilms (5). The most important difference between planktonic as compared to bacteria incorporated in a biofilm, is that in a planktonic state, bacteria are more susceptible to antimicrobial agents. One of the many reasons why biofilm-based infections are more likely to persist than infections colonized by planktonic bacteria is because of a biofilm's increased ability to resist antimicrobial agents such as antibiotics (6). Part of what aids in a biofilms ability to resist antimicrobials is the secretion of EPS. EPS is composed of proteins, polysaccharides and extracellular DNA (eDNA). The highly hydrated biopolymers of EP form a matrix which interacts with the environment resulting in the attachment of a biofilms to a surface and keeping the biofilm together (7).

Our recipient bacteria, *Escherichia coli* ATCC 25922, is commonly used in biofilm experiments as a control because it is a proficient biofilm producer. Our donor bacteria, *Escherichia coli* JM109 is a lab strain of *E. coli* that was altered to contain the multidrug-resistant plasmid pTRE-131. This plasmid was extracted from the Tijuana River Estuary and fully sequenced for its chromosomal and plasmid DNA (8). The plasmid, pTRE-131 encodes for multiple ARGs, including ticarcillin, ciprofloxacin and chloramphenicol (8) (Figure 4). This 48kb plasmid is an IncN-group plasmid, meaning it is found in high copy number and has a broad host-range replicon (8). The key aspect of this specific plasmid is that it is a self-transmissible multidrug resistance plasmid, and for the purpose of this experiment it contains the bla_{OXA-1} gene (Figure 4), encoding resistance to the antibiotic ampicillin (8). Given its high copy number,

broad host range, and its multidrug resistance, pTRE-131 is an ideal plasmid to study ARG transmissibility between bacteria.

While there is minimal research on multidrug resistance plasmid conjugation in biofilms, there is also no standardized conjugation exposure time frame. Current publications suggest that exposing recipient and donor bacteria to each other for one-hour is sufficient to study conjugation patterns; however, there are other studies that suggest twenty-four hours is optimal to study conjugation. This project aims to identify the difference in transfer frequencies of antibiotic resistance genes between conjugation of planktonic bacteria and bacteria incorporated in biofilms.

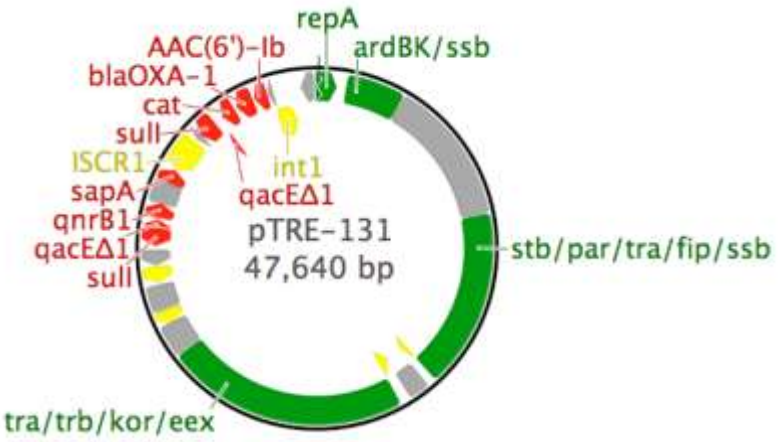


Figure 4: pTRE-131. The annotated plasmid categorizes the genes carried on this particular plasmid. The undefined genes are represented in grey, backbone genes in green, mobile genetic elements in yellow, and virulence factors in red. The *bla*$_{OXA-1}$ gene encodes for ampicillin resistance and is the selectable marker for this plasmid throughout the experiment (6).

Materials and Methods

Bacterial Strains and Antibiotics

The recipient bacterial strain was *E. coli* ATCC 25922 and *E. coli* JM109 pTRE-131 was the donor strain. *E. coli* ATCC 25922 is resistant to rifampicin (RIF), whereas *E. coli* JM109 (pTRE-131) is multidrug resistant, but for the purpose of this experiment it is important to note that it is resistant to ampicillin (AM). A cocktail (AM-RIF) of both antibiotics is selective for recipient bacteria who have been successfully transferred the donor plasmid (pTRE-131), which is a transconjugant. All bacteria were grown up in Luria-Bertani (LB) broth in a shaking incubator set at 37°C and 200 rpm. To keep the selective pressure for the pTRE-131 plasmid, *E. coli* JM109 (pTRE-131) was cultured in the presence of AM (10 ug/mL). All agar plates were made with LB agar and were enriched with either RIF or a cocktail of AM and RIF. AM was used at a 20 ug/mL and RIF was used at a 5 ug/mL concentration.

Antibiotic MIC Determination

The recipient *E. coli* ATCC 25922 shows chromosomal resistance to the antibiotic rifampicin (RIF), while the donor *E. coli* JM109 (pTRE-131) contains plasmid-mediated resistance to ampicillin (AM). In order to determine the minimum inhibitory concentrations (MIC) for both recipient and donor for AM and RIF, recipient and donor strains were plated on a series of agar plates enriched with a concentration gradient of AM and RIF. Final MIC values were determined for optimal concentrations for mating selections. Liquid stocks of RIF and AM were prepared for a final stock concentration of 50 ug/mL.

Liquid Mating

Cultures of *E. coli* ATCC 25922 and *E. coli* JM109 (pTRE-131) were grown in 5 mL of LB for 24 h in a shaking incubator. After 24 h of growth, *E. coli* JM109 (pTRE-131) was centrifuged at 14,000 rpm for 5 minutes, supernatant containing any residual antibiotic (AM) was discarded and the cell pellet was resuspended in fresh LB. For the mating, 1 mL each of donor and recipient were combined and incubated at 1 h and 24 h. After the respective mating times, the cultures were serial diluted following the procedure described above.

Batch Biofilm Mating

Cultures of *E. coli* ATCC 25922 and *E. coli* JM109 (pTRE-131) were grown in 25 mL of LB in a conical vial for 24 h in a shaking incubator. A sterile microscope slide was placed in the conical vial containing *E. coli* ATCC 25922, for biofilm attachment. After 24 h of growth, *E. coli* JM109 (pTRE-131) was centrifuged at 14,000 rpm for 5 minutes, supernatant containing any residual antibiotic (AM) was discarded, and the cell pellet was resuspended in 25 mL of fresh LB. For mating, the microscope slide containing the recipient biofilms was aseptically transferred to the fresh conical vial containing the donor and plasmid. Mated bacteria were incubated for 1 h and 24 h. After the specific amount of incubation time, microscope slides were aseptically removed and rinsed three times with sterile saline. Using a sterile cotton swab, biofilm mass was scraped from the microscope slide and resuspended in 900 *u*L of sterile saline and serially diluted. The viable plate counts were performed as described above.

Drip-Flow Biofilm Reactor Mating

Recipient bacteria were cultured after a 24 h incubation period and 1 mL was added to 15 mL of fresh LB in each of the biofilm wells. Each biofilm well contained a sterile microscope slide for biofilm attachment, located in the reactor box (Figure 6). Biofilm wells were left for 6 h to allow for the attachment phase of biofilm formation. After 6 h, the reactor box was tilted to a 45° angle and the continuous flow of fresh media began to drip at a rate of 0.8mL/min, controlled through the peristaltic pump (Figure 5). Media was dripped at the same constant rate for 24 h. After 24 h, 1 L of cultured donor bacteria (centrifuged and resuspended in fresh media) was added to the fresh media carboy. The donor dripped at a constant rate over the recipient biofilms for 1 h and 24 h. After the specific amount of exposure time, microscope slides were aseptically removed and rinsed three times with sterile saline. Using a sterile cotton swab, biofilm mass was scraped from the microscope slide and resuspended in 900 *u*L of sterile saline and serially diluted. The viable plate counts were performed as described above. The Drip-Flow Biofilm Reactor was purchased from Biosurface Technologies Corporation (9). All autoclave and sterilization of the biofilm reactor was done according to the set-up videos produce by the Center for Biofilm Engineering (CBE) at Montana State University. Inoculation procedures and methods were also done according to the CBE protocols (10).

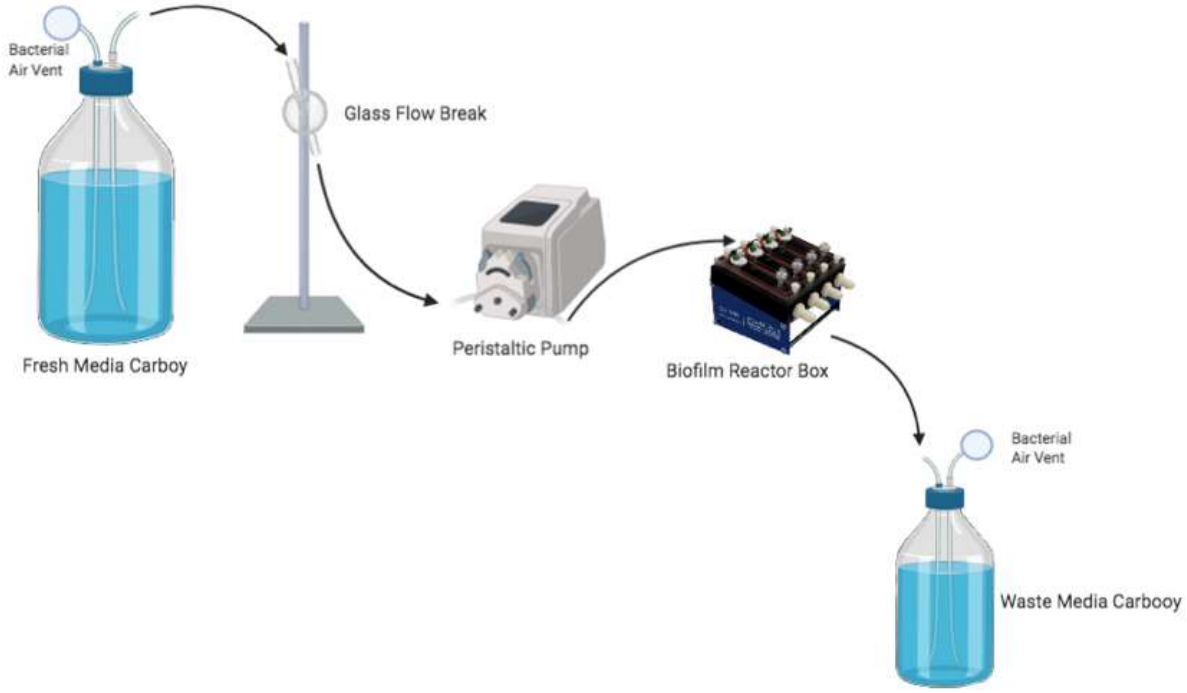


Figure 5: Drip-Flow Biofilm Reactor Setup. LB broth flows from the fresh media carboy through the glass flow break, and peristaltic pump through a serious of tubing until it drips into the biofilm reactor box where the recipient biofilm is forming. For conjugation, cultured donor with plasmid is added to the fresh media carboy and follows the same drip procedure. Waste flows from the biofilm reactor box into the waste carboy for sterilization.

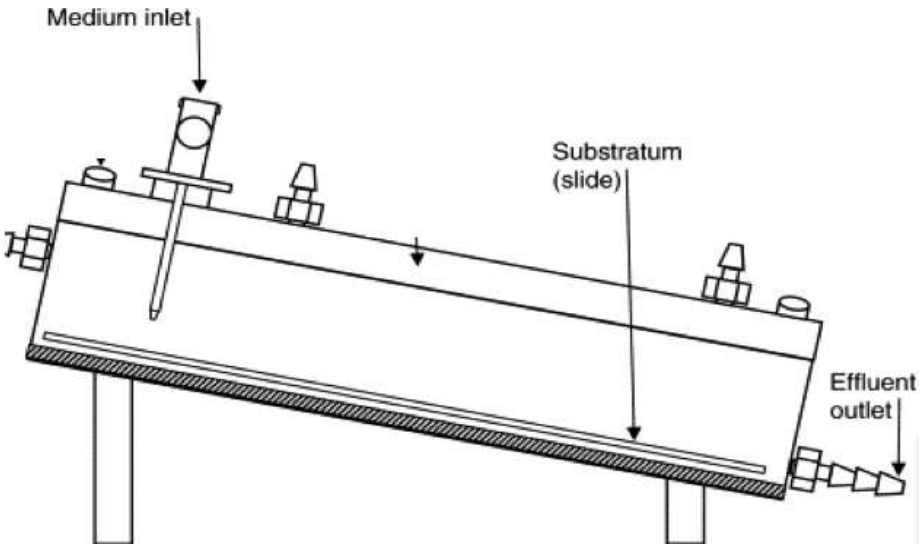


Figure 6: Close up of Biofilm Reactor Box. Biofilms grown on the microscope (substratum) slide while the media (with and without donor bacteria drips over the slide through a needle in the medium inlet. Excess media, waste, and unattached bacteria flow out of the biofilm box through the effluent outlet into the waste carboy.

Viable Plate Counts

A 10-fold serial dilution of the resuspended mated bacteria was spread onto LB agar plates (AM-RIF), selecting for transconjugants, and on a series of plates with RIF selecting for total recipients.

After 24 h incubation at 37°C, twelve colonies from the AM-RIF plates were selected, prepared as glycerol stocks, and stored at -80°C for further analysis. All mating experiments were done in replicates of 12.

Transconjugant Confirmation

Individual colonies of putative transconjugants were selected from cocktail plates, resuspended in 5 mL of LB and incubated for 24 h. After the 24 h incubation, glycerol stocks were prepared, and samples were stored at -80° C for further analysis. The first step of confirmation was genomic DNA preps (gDNA). Using the Sigma Aldrich Kit, the full genomes from transconjugants were extracted and later prepped for RAPD PCR (11). After running a random amplification of polymorphic DNA (RAPD) polymerase chain reactions (PCR), samples were subjected to gel electrophoresis for recipient genome confirmation (PCR products, 1.5% agarose, 45 min at 100 V). *E. coli* ATCC 25922 DNA was used as a positive control and that of *E. coli* JM109 (pTRE-131) was used as a negative control. Transconjugants whose gDNA matched the recipients where then prepared for plasmid extraction via the QIAprep Spin Miniprep Kit (12). Plasmid preps were subjected to gel electrophoresis (Plasmid, 1.0% agarose, 1.5 h at 100 V) against *E. coli* JM109 (pTRE-131) as a positive control; and *E. coli* ATCC 25922 as the negative control. Transconjugants were confirmed only if the sample matched both the recipient gDNA by RAPD PCR and the donor's plasmid profile.

Data Analysis

Colony counts from the viable plate counts were collected and used to calculate the cell density (CFU/mL) of both recipients and transconjugants. The ratios of transconjugants to possible recipients were calculated and subjected to further analysis. Significant differences between the three mating conditions and two exposure times were determined through a generalized linear model using R.

Results

Minimum Inhibitory Concentrations (MIC)

E. coli ATCC 25922 is susceptible to AM at 10 ug/mL and RIF at 25 ug/mL. *E. coli* JM109 (pTRE-131) is resistant to AM up to 50 ug/mL but is susceptible to RIF at 5 ug/mL. Concentrations of AM at 20 ug/mL and RIF at 5 ug/mL were chosen to conduct the following conjugation experiments because a cocktail (AM-RIF) will select for only bacterial cells that have successfully undergone conjugation. Concentrations were decreased by two-fold for inoculations (Table 1a and 1b).

Liquid Culture Mating

Mean CFU/mL ratio for 1 h is 1.20E-02 with a standard deviation of 1.20E-03 (Table 5). Based on the mean ratio for 1 h, for every 10 recipients, 0.83 received donor plasmid. Means CFU/mL ratio for 24 h is 1.62E-04 with a standard deviation of 1.90E-04 (Table 5). For 24 h, for every 10 recipients, 0.617 out of them received donor plasmid. Conjugation from 1 h produced more transconjugants than conjugation from 24 h (Table 2).

Batch Biofilm Mating

Mean CFU/mL ratio for 1 h is 7.03E-02 with a standard deviation of 1.77E-0 (Table 5). Based on the mean ratio for 1 h, for every 10 recipients, 1.44 received donor plasmid. Means CFU/mL ratio for 24 h is 0 with a standard deviation of 0 (Table 5). For 24 h there were no successful transconjugants (Table 3).

Drip-Flow Biofilm Mating

Mean CFU/mL ratio for 1 h is 4.40E-01 with a standard deviation of 4.01E-01 (Table 5). Based on the mean ratio for 1 h, for every 10 recipients, 2.27 received donor plasmid. Means CFU/mL ratio for 24 h is 2.91E-01 with a standard deviation of 3.05E-01 (Table 5). For 24 h, for every 10 recipients, 3.45 out of them received donor plasmid. Drip-flow biofilm 24 h mating produced the highest average of transconjugants between all growth conditions and exposure times (Table 4).

Transconjugant Confirmation

Genomic and plasmid DNA extraction gel electrophoresis confirmed successful transfer of pTRE-131 from *E. coli* JM109 to *E. coli* ATCC 25922. Throughout all samples tested, 55.6% were confirmed transconjugants.

Exposure Time and Growth Condition Analysis

Means and standard deviations were obtained from the ratio of successful transconjugants that were produced from the number of total recipients available for conjugation. Means and standard deviation were collected from all 12 recipients except the case for Batch Biofilm at 24 h. One outlier was excluded from that specific data analysis.

A generalized linear model identifies the effects of growth condition and exposure time, finding that collectively they had a significant effect on growth ($F(5,66) = 7.09$, $p<.001$, $R^2+ 0.30$). Post-Hoc tests revealed significant differences between growth conditions. Liquid-Batch (95% CI (-0.18, 0.08), p=0.61). Liquid-Drip (95% CI (-0.43, -0.17) p=0.00002). Batch-Drip (95% CI (0.12, 0.38), p=0.00006). Additionally, there was no overall significant difference between the exposure times (95% CI (-0.08, 0.09) p=0.94), however, there are significant differences between the time effects for the different treatment groups. Drip and Batch 1 h (95% CI (-0.01, 0.44) p=0.078). Liquid and Batch 1 h (95% CI (-0.28, 0.17), p=0.97). Liquid and Drip 1 h (95% CI (-0.49, -0.044), p=0.01). Drip and Batch 24 h (95% CI (0.06, 0.51), p=0.005). Liquid and Batch 24 h (95% CI (-0.27, 0.18), p=0.99). Liquid and Drip 24 h (95% CI (-0.55, -0.104), p=0.0008).

Table 1a: *E. coli's* MIC of Ampicillin

AM	0 abx	10 ug/mL	20 ug/mL[a]	30 ug/mL	40 ug/mL	50 ug/mL
E. coli ATCC 25922	+	-	-	-	-	-
E. coli JM109 (pTRE-131)	+	+	+	+	+	+

Table 1b: *E. coli's* MIC of Rifampicin

RIF	0 abx	5 ug/mL[b]	10 ug/mL	15 ug/mL	20 ug/mL	25 ug/mL	50 ug/mL	100 ug/mL	150 ug/mL	200 ug/mL
E. coli ATCC 25922	+	+	+	+	+	-	-	-	-	-
E. coli JM109 (pTRE-131)	+	-	-	-	-	-	-	-	-	-

[a] Concentration of AM used to conduct the following conjugation experiments. [b] Concentration of RIF used to conduct the following conjugation experiments.

Table 2: Liquid Culture Mating Ratios of Transconjugants per Total Recipients

Ratios from liquid culture 1 h mating (CFU/mL)	Ratios from liquid culture 24 h mating (CFU/mL)
4.46E-02	6.13E-04
6.71E-03	3.16E-04
8.00E-03	1.78E-04
2.86E-02	3.54E-04
6.98E-03	5.21E-05
1.57E-02	2.43E-04
5.82E-04	1.83E-04
3.81E-03	6.37E-07
1.63E-02	1.82E-08
1.02E-02	1.29E-08
2.94E-03	2.39E-08
1.95E-04	4.29E-08

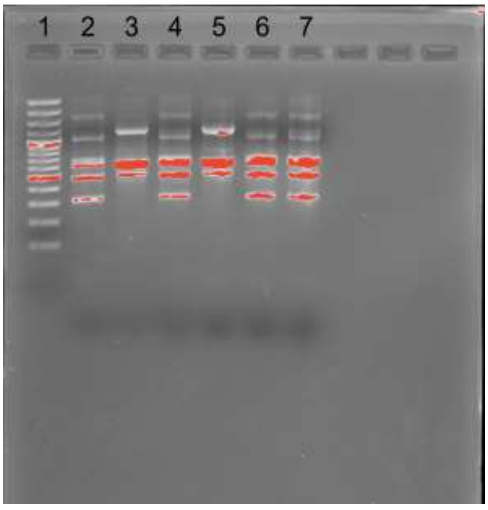
Table 3: Batch Biofilm Mating Ratios of Transconjugants per Total Recipients

Ratios from batch biofilm 1 h mating (CFU/mL)	Ratios from batch biofilm 24 h mating (CFU/mL)
1.08E-02	0
3.40E-02	0
6.17E-04	0
7.43E-02	5.32E-01[a]
2.88E-02	0
4.64E-02	0
7.69E-03	0
2.31E-04	0
5.36E-04	0
1.37E-03	0
6.27E-01	0
1.09E-02	0

[a] Outlier was not included in data analysis.

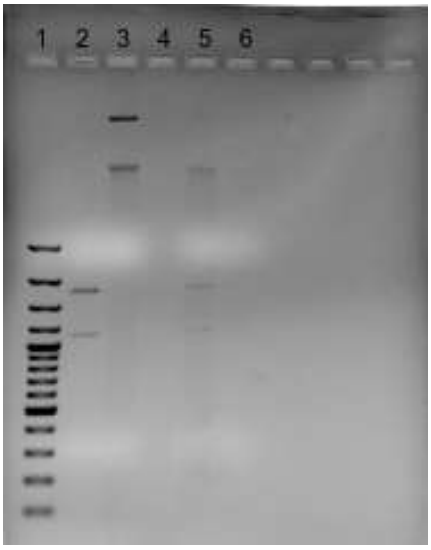
Table 4: Drip-Flow Biofilm Mating Ratios of Transconjugants per Total Recipients

Ratios from drip-flow 1 h mating (CFU/mL)	Ratios from drip-flow 24 h mating (CFU/mL)
1.68E-01	3.87E-01
3.86E-01	1.75E-01
8.39E-03	8.45E-01
4.95E-03	5.35E-02
5.69E-01	5.00E-01
1.61E-03	7.11E-02
1.24E+00	5.91E-03
6.11E-01	2.80E-01
6.60E-01	6.11E-01
7.09E-01	6.60E-01
1.24E-01	2.90E-01
9.00E-03	2.20E-02



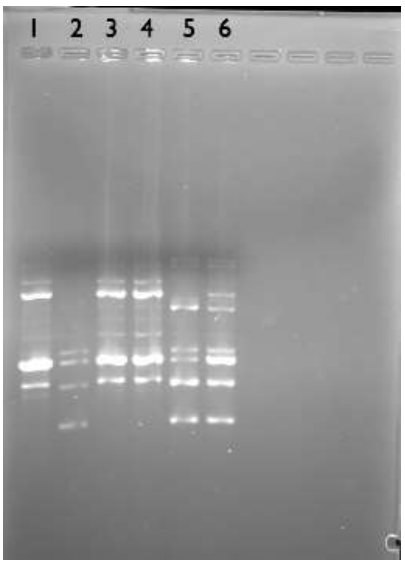
1. Ladder
2. *E. coli* ATCC 25922
3. Sample 1
4. Sample 2
5. Sample 3
6. Sample 4
7. Sample 5

Figure 7a: Liquid Culture Transconjugant RAPD PCR Gel. Samples 2, 4 and 5 show similar binding pattern to the recipient strain, *E. coli* ATCC 25922.



1. Ladder
2. *E. coli* ATCC 25922
3. *E. coli* JM109 (pTRE- 131)
4. Sample 2
5. Sample 4
6. Sample 5

Figure 7b: Liquid Culture Transconjugant Plasmid Mini Prep Gel. Sample 4 shows the strongest plasmid band, while samples 2 and 5 show very faint plasmid bands. All plasmid bands match the donor plasmid in well 3.



1. *E. coli* ATCC 25922
2. *E. coli* JM109 (pTRE- 131)
3. Sample 1*
4. Sample 2*
5. Sample 3**
6. Sample 4**

* 1 h mating, ** 24 h mating

Figure 8a: Drip-Flow Biofilm Transconjugant RAPD PCR Gel. Samples 1 and 2 show similar banding pattern to the recipient strain *E. coli* ATCC 25922, while samples 3 and 4 show similar banding pattern to both recipient and donor *E. coli* JM109 (pTRE- 131).

1. Ladder
2. *E. coli* JM109 (pTRE- 131)
3. Sample 2

Figure 8b: Drip-Flow Biofilm Transconjugant Mini Plasmid Prep Gel. Sample 2 show the same plasmid band as the plasmid donor shown in well 2.

Table 5: Mean and standard deviation of exposure times and conjugation conditions of transconjugant to total recipient ratios.

	1 h		24 h	
Condition	m	**sd**	**m**	**sd**
Liquid Culture	1.20E-02	1.20E-03	1.62E-04	1.90E-04
Batch Biofilm	7.03E-02	1.77E-01	0	0
Drip-Flow Biofilm	2.85E-01	2.81E-01	2.74E-01	3.29E-01

Discussion

For all three mating conditions, there was no significant difference between the one-hour and twenty-four-hour mating exposure. Based on that information, it can be concluded that subjecting donor and recipient strains to conjugation for one hour is sufficient to study the transmissibility of antibiotic resistance genes. One-hour mating is optimal for conserving resources and producing the least amount of waste possible. Additionally, in the case of batch biofilms, one-hour mating is ideal because the twenty-four-hour protocol produced no viable transconjugants. This may be due to the anerobic conditions of the batch biofilm system, which is more prevalent after twenty-four-hours than after one-hour. The batch biofilm is a closed system; there is no supply of oxygen available to the donor and recipient strains, which results in lower survival rate; therefore, resulting in a lower rate of transconjugant production. Overall, the of batch biofilms produced the lowest mean for both one and twenty-four hours which can be seen in Table 5 (7.03E-02 and 0, respectively). One-hour batch biofilm

also produced the highest standard deviation (1.77E-01) when compared to all other growth conditions and times.

Statistical analysis revealed that drip-flow biofilms produced a significantly higher ratio of transconjugants to total recipients for both one-hour and twenty-four-hour mattings ($p=0.00118$). While liquid culture had the overall lowest standard deviation for both exposure times (1.20E-03 and 1.90E-04), drip-flow biofilms had the greatest average for ratio of transconjugants (4.40E-01 and 2.91E-01). Liquid culture conditions are subjected to the potential of anerobic conditions whereas drip-flow biofilms are not. Additionally, mated liquid cultures were prepared in test tubes, which does not allow for the removal of waste products or the addition of fresh nutrients. This is mostly a concern after twenty-four-hours. Because drip-flow biofilms are grown in the Drip-Flow Biofilm Reactor they are subjected to a constant supply of fresh media, which contains all the nutrients and oxygen needed for them to flourish. In addition to a fresh supply of nutrients, the reactor was tilted at a 45° angle, allowing for waste such as dead cells and toxins to be removed. This is especially important in comparison to liquid cultures and batch biofilms because in those conditions, waste products build up and might inhibit growth and the ability of the donor and recipient to do conjugation.

Confirming transconjugant production become much more complex in the drip-flow biofilms when compared to liquid cultures. As seen in Figures 7a, samples either show the same banding patter as the recipient or the donor. Figure 8a depicts a much more complicated banding pattern in the drip-flow biofilm transconjugants. Samples 1 and 2, which come from the one-hour mating, have an identical banding pattern to the recipient, while samples 3 and 4, from the twenty-four-hour mattings, have a banding pattern that is a mixture of recipient and donor. Because samples 3 and 4 can grow in the presence of ampicillin and rifampicin, we know antibiotic resistance genes are being transferred from our donor and recipient strains; however, because the banding pattern matched the donor more closely, this process is more complicated than bacterial conjugation. The recipient contains chromosomal resistance to rifampicin while the donor contains plasmid-mediated resistance to ampicillin. If the RIF resistance gene is located near a transposon region in the recipient, the gene may be able to be passed on to the donor. The complexity in the banding pattern of transconjugants obtained from the twenty-four-hour drip-flow biofilm system implies there is more going on than conjugation. These findings contribute to the importance behind the study of antibiotic resistance gene transfer within biofilms.

While there is much knowledge regarding biofilm formation, there are few studies that are concerned with the ability of plasmid-mediated ARGs to be conjugated between planktonic bacteria and biofilms (13). Because biofilms are the leading cause of nosocomial infections, those obtained in a hospital, it is imperative that the ability of ARGs to persist and spread within biofilms is understood. These findings suggest that the transfer for ARGs is the highest when conjugation takes place between planktonic and biofilms as compared to between planktonic bacteria. The system of drip-flow biofilms aims to mimic the constant supply of nutrients that biofilms in infections persist on, which is why it is the best model to study how antibiotic resistance is transferred in biofilms. In order to discover ways to combat multidrug resistance in the clinic, there needs to be a complete understanding of the evolution of antibiotic-resistant bacteria, which requires the investigation of genetic exchange between planktonic and biofilm phases.

References

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