Journal of Medicine and Medical Sciences Vol. 10(1) pp 68-76, March, 2019
DOI: http:/dx.doi.org/10.14303/jmms.2019.1
Available online http://www.interesjournals.org/JMMS

Full Length Research Paper

The order in which antibiotics are administered affects the fitness costs of Adherent-Invasive *E. coli* (AIEC) strains

Jordan Gregg*

Department of Biology, College of William and Mary's Integrated Science Centre, Williamsburg, Virginia, USA

*Corresponding author's Email: jbg721@gmail.com

ABSTRACT

AIEC-LF82 is a strain of bacteria that is surmised to have a role in causing IBD and Crohn's disease by activating pro-inflammatory gene expression in organisms. Using antibiotics via combination therapy has been a technique used in clinical settings in an attempt to treat the strains, however, the attempts have not been that effective nor efficient in terms of completely halting the growth and colonization of AIEC to treat IBD and Crohn's disease patients. Research has shown that regarding hindering or preventing the colonization bacterial colonies, sequential therapy tends to be more effective and time-efficient than combination therapy, with fewer adverse effects. To test if this is also the case with the AIEC-LF82 strain of bacteria, I first tested AIEC's response to combination therapy using the Penicillin-Streptomycin, Kanamycin-Chloramphenicol, antimicrobial peptide (AMP), Kanamycin, SPE phase and LB agar plates, all of which were experimental plates other than the LB agar plate that acted as the negative control. I then tested AIEC-LF82's response to sequential therapy were using the LB+Kan+Spe, LB+AMP+Spe, LB+Kan/Cam+Spe, LB+P/S+Spe, LB+P/S+Kan and LB+P/S+AMP and one LB agar plate acting as the negative control. The only differences between Sets A and B were the order in which the antibiotics were administered in the six aforementioned treatment sets. Ultimately, I found that set B of sequential therapy, strong-weak antibiotic treatments, was the most effective treatment but that set A regarding sequential therapy was actually the least effective of all of the treatments. In conclusion, using strong-weak sequential antibiotic therapy treatments appears to be a potentially promising option to treat patients suffering from Crohn's disease and IBD.

Keywords: AIEC, *E. coli*, Crohn's disease, IBD, antibiotics, penicillin, streptomycin, kanamycin, chloramphenicol, resistance.

INTRODUCTION

AIEC is an Adherent-Invasive strain of E. coli bacteria that is highly linked to patients with chronic Crohn's disease and IBD. It is suspected to instigate chronic inflammation in susceptible hosts by altering gut microbiota composition, which would allow it to have a greater chance of activating pro-inflammatory gene expression. AIEC strains tend to colonize the intestinal mucosa by adhering to intestinal epithelial cells, so the important role that is played by the AIEC strain, in Crohn's disease and IBD pathogenicity is due to their ability to invade both intestinal epithelial cells and macrophages (Conte et al. 2014 and Yang et al. 2017). This in turn results in very high levels of secretion of pro-inflammatory cytokines, which ultimately contributes to chronic inflammation. Adherent-Invasive *E. coli* bacteria are also true invasive pathogens because they are able to invade intestinal epithelial cells via a macropinocytosis-like process, allowing them to be able to survive and replicate intracellularly after lysis of the endocytic vacuole (Sevrin G et al. 2018).

Inside macrophages themselves, AIEC strains survive and replicate without inducing host cell death and induce the release of high amounts of TNFα, making

them a very dangerous strain of *E. coli* (Sevrin G et al. 2018). These virulence properties designate AIEC as a pathogen that can potentially induce persistent intestinal inflammation by crossing and breaching the intestinal barrier, moving into deep tissues, and continuously activating macrophages to infect host cells. My research in the IBD lab of the Icahn School of Medicine, at Mount Sinai Hospital in New York City led me to take the stance that the antibiotic treatments would be able to potentially prevent the AIEC bacteria from being able to colonize and thrive. This is since the infection cycle of Adherent-Invasive *E. coli* appears to depend heavily on the ability of these bacteria to first be able to colonize in the gastrointestinal tract of genetically predisposed Crohn's disease and IBD patients (Yang et al. 2017). Another pressing issue is the emergence of mutant strains of bacteria being resistant to one or many antibiotics (Palumbi, 2001).

Although in the past, multi-drug treatments often reduced the prevalence of severe infections, research has shown that excessive use of antibiotics has resulted in the evolution of multidrug resistance in many species of bacteria (Gould IM and van der Meer JWM, 2007). Multi-drug resistance is also extremely frequent in many health care-associated bacterial infections, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* which can tend to make the optimal use of multi-drug therapy more difficult regarding medical treatment. Using antibiotics simultaneously for combination therapy or sequentially for sequential therapy are techniques common used in the healthcare industry, in which two or more different antibiotics are used one after the other. Combination therapy can be used to successfully treat *Helicobacter pylori* (Albert TJ et al. 2005), which is an agent of peptic ulcers or in this case the AIEC variants of *E. coli*. However, one of the flaws is that combination therapy can often be associated with uncomfortable side effects if the drugs used in combination create an adverse reaction in the organism's body.

However, unlike the previously mentioned therapy sequential therapy within a single host exposes bacterial infections to a rapid change in antibiotics. The cycling process of antibiotics via combination therapy within a hospital system can take months to years to implement, but with sequential therapy, it is possible to switch antibiotics within a single host over a matter of days (Perron GG et al. 2012). It is also important to note assuming that the antibiotics chosen for sequential therapy don't elicit cross-resistance, the mutants that are resistant against one antibiotic are unlikely to reach high frequencies within the host before a second antibiotic is applied. Though combination therapy can prove to be very effective if the correct antibiotics are used, sequential therapy is generally a more reliable technique overall. This is because a rapid switch in antibiotic use has the potential to minimize multi-drug resistance while greatly minimizing any potential negative clinical consequences of combination therapy.

MATERIALS AND METHODS

The first step that I took in order to test the response of the AIEC LF82 strain of bacteria to combination therapy that I used, was to pipette 50 µl of AIEC competent cells into an eppendorf tube and place it in a tray of ice for half an hour. Afterwards, I heat shocked the cells in the eppendorf tube in a 42°C water bath for 60 seconds to ensure that the plasmid would enter the bateria. Next, I left the eppendorf tube with the AIEC on ice again for 3 minutes. I then added 900 ul of LB at room temperature to the eppendorf tube and left the cells in the lab's incubator for an hour, at 37°C to ensure proper growth. Afterwards, I plated 100 µl of the AIEC bacteria onto several lb agar plates with Penicillin-Streptomycin, Kanamycin-Chloramphenicol, antimicrobial peptide (AMP), Kanamycin, SPE phase tetracycline antibiotic solutions depending on the plate and left them to incubate at 37°C overnight for approximately 24 hours. Plate #1 was made with lb agar and Penicillin-Streptomycin antibiotic solutions, plate #2 was made with lb agar and a Kanamycin-Chloramphenicol antibiotic solution, plate #3 was made with lb agar mixed with an antimicrobial peptide (AMP) antibiotic solution, plate #4 was made with an lb agar and a Kanamycin antibiotic solution, plate #5 was made with lb agar and a SPE phase tetracycline antibiotic solution and plate #6 with only LB broth.

The experimental groups were plate #1, which had both Penicillin and Streptomycin antibiotics used simultaneously to treat the AIEC bacterial samples, and plate #2 both Kanamycin and Chloramphenicol antibiotics used simultaneously to treat the AIEC bacterial samples. However, there were several controls for the experiment with the positive control groups for this set being the LB+AMP, LB+Kan and LB +Spe with no second antibiotic added. The negative control group was the LB broth agar plate with no antibiotics added whatsoever. A day after observing the results of the first set of plates, sealing the original agar plates with parafilm and preserving them in the laboratory freezer, I then made one set of replicates to make sure that the trends observed from the plates were accurate. I once again pipetted 50 µl of AIEC competent cells into an eppendorf tube and placed it in a tray of ice for half an hour. Afterwards, I once again heat shocked the cells in the eppendorf tube in a 42°C water bath for 60 seconds to ensure that the plasmid (Albert TJ et al. 2005) would enter the bacteria. Next, I left the eppendorf tube with the AIEC on ice again for 3 minutes once more. I then added 900 µl of LB at room

temperature to the eppendorf tube and incubated the cells for an hour at 37°C again.

I then plated 100 µl of the AIEC bacteria onto several lb agar plates with Penicillin-Streptomycin, Kanamycin-Chloramphenicol, antimicrobial peptide (AMP), Kanamycin, SPE phase tetracycline antibiotic solutions depending on the plates, and left them to incubate at 37°C overnight again, with plates 1-6 corresponding to the same types of antibiotics used in the original setup. The next day, after observing the results, I then sealed the replicates with parafilm and placed the replicate plates in the laboratory fridge. Afterwards, I made a second set of replicate plates, via the aforementioned steps and preserved those plates as well to compare against the first replicate and the original plate to test for accuracy. Regarding testing the effectiveness and response of the of the AIEC LF82 strain of bacteria to sequential therapy, the first step that I took in order to test the response of the AIEC LF82 strain of bacteria to sequential therapy that I used was to pipette 50 µl of AIEC competent cells into an eppendorf tube and place it in a tray of ice for half an hour. Afterwards, I heat shocked the cells in the eppendorf tube in a 42°C water bath for 60 seconds to ensure that the plasmid (Albert TJ et al. 2005) would enter the bacteria. Next, I left the eppendorf tube with the AIEC on ice again for 3 minutes to ensure that the samples would not be overheated and to preserve the samples. I then added 900 µl of LB at room temperature to the eppendorf tube and incubated the cells for an hour at 37°C.

I then made two different sets of treatment groups with 7 different plates, Set A and B. For Set A, first the bacterial colonies were grown on LB broth plates mixed with weak antibiotics that after three days were then transferred to LB broth plates mixed with strong antibiotics. I then spread and plated 150 µl of the AIEC bacteria colonies that were separated by quadrants onto several different lb agar plates with LB+Spe then Kan, LB+Spe then AMP, LB+Spe then Kan/Cam, LB +Spe then P/S, LB+Kan then P/S, LB+AMP and P/S and then lastly the control group with no added antibiotics whatsoever. Plates 1-4 were made with lb agar and a SPE phase tetracycline antibiotic solution. Plate #5 was made with a lb agar and a Kanamycin antibiotic solution. Then plate #6 was made with a lb agar and an antimicrobial peptide antibiotic solution that later had Penicillin-Streptomycin added to the bacteria colonies. Lastly, plate #7 served as a control respectively. Two replicate sets of the original set A sample, using the previously aforementioned methods to test for consistency were made. The replicates, as well as the original plates, were sealed with parafilm and put into the laboratory freezer.

Afterwards, the colonies grown on plates 1-6 were transferred to new LB plates with strong antibiotics incorporated into the LB agar, rather than weak antibiotics. The AIEC colonies from plate #1 were also then picked and spread to an LB plate with Kanamycin mixed in the agar and were separated by quadrants once again. LB agar plate, with antimicrobial peptide (AMP) solution, was used to regrow the colonies picked from plate #2 and was spread and separated by quadrants. The colonies from plate #3 were picked and spread on a new plate with Kanamycin-Chloramphenicol mixed in the agar in the solution and were spread and separated by quadrants once again. The colonies from plates 4-6 were spread and picked on new LB agar plates with Penicillin-Streptomycin mixed in the solution and were also spread and separated by quadrants once again. The colonies were incubated for 24 hours to stimulate growth and afterwards, were sealed with parafilm and put into our laboratory freezer.

For Set B, a strong, where a strong antibiotic was first used and mixed with the LB broth of the plates, then after a 3 day period, they were plated on LB broth plates that contained weak antibiotics. Directly following, I plated 150 µl of the AIEC bacteria colonies that were separated by quadrants onto several different lb agar plates with LB+Kan then Spe, LB+AMP the Spe, LB+Kan/Cam then Spe, LB+P/S then Spe, LB +P/S+Kan, LB+P/S+AMP and then lastly the control with no added antibiotics whatsoever. Plate #1 was made with lb agar and a Kanamycin antibiotic solution. Afterwards, plate #2 was made with lb agar and an antimicrobial peptide (AMP) antibiotic solution. Plate #3 was made with lb agar and a Kanamycin-Chloramphenicol antibiotic solution. Then plate #4 was made with lb agar and a Penicillin-Streptomycin antibiotic solution. Next, plate #5 was made with a lb agar and a Penicillin-Streptomycin antibiotic solution. Plate #6 was made with a lb agar and a Penicillin-Streptomycin antibiotic solution. Lastly, plate #7 served as a control respectively. Two replicate sets of the original set B sample, using the previously aforementioned methods to test for consistency, were made. Once again, the replicates, as well as the original plates, were sealed with parafilm and put into the laboratory freezer.

Afterwards, the colonies grown on plates 1-6 were transferred to new LB plates with weak antibiotics incorporated into the LB agar, rather than strong antibiotics. The AIEC colonies from plates 1-4 were then picked and spread to an LB plate with SPE phase tetracycline antibiotic solution mixed in the agar and were separated by quadrants. AIEC colonies from plate #5 were also then picked and spread to an LB plate with Kanamycin mixed in the agar and were separated by quadrants once again. In addition, the AIEC colonies from plate #6 were also then picked and spread to a LB plate with a antimicrobial peptide (AMP), added agar, and were separated by quadrants. The colonies were

incubated for 24 hours to stimulate growth (Gould IM and van der Meer JWM, 2007) and afterwards were sealed with parafilm and put into the laboratory freezer. For all of the aforementioned treatments, regarding each of the plates, for every 25 ml of lb agar, 1 ml of antibiotic solution was used in conjunction with the agar so that each plate had an antibiotic concentration that was proportional to the amount of AIEC bacteria plated.

RESULTS AND DISCUSSION

I grew the AIEC LF82 bacteria on six agar plates (as previously mentioned) with Penicillin-Streptomycin, Kanamycin-Chloramphenicol, antimicrobial peptide (AMP), Kanamycin, SPE phase tetracycline antibiotics to test their resistance and noticed that all of the antibiotics alone, proved to be quite infective since the untransformed AIEC bacteria were still able to grow very similarly to how they would on a regular lb agar plate.

Antibiotic and probiotic therapies appear to be very poor potential choices for therapeutic treatment of ileal Crohn's disease and IBD, at least at first glance. However, I noticed that plates #1 and #2 were able to completely halt the colonization and therefore the growth of AIEC LF82 bacteria.

The effects of combination therapy using antibiotics ended up being more potent than initially predicted. When two strong antibiotics, used via combination therapy, tended to actually be more successful than the weak-strong sequential therapy treatment used in set A, the effects of combination therapy on the AIEC bacterial colonies also affected the colonies quicker than expected in only a 24 hour period.

The significance of this is that clinical combination therapy using antibiotics can tend not to be the most time effective; however the P/S and Kan/Cam antibiotic solutions were able to act and work quickly on eradicating the AIEC colonies when used in conjunction. When two strong antibiotics were used in conjunction, they tended to interfere with AIEC bacteria reproduction and halted the growth and colonization of AIEC colonies (Tables 1-5).

The data from replicate sets 1 and 2, represented by Tables 2 and 3 respectively, has slight numerical deviations from the original set. The replicate sets also retain the general trend of having fewer colonies when strong antibiotics are used singularly and having no AIEC colonies when two strong antibiotics are simultaneously used.

Table 1. AIEC bacteria response to combination therapy.

AIEC Colonies	LB+Kan/Cam (Plate #1)	LB+P/S (Plate #2)	LB+AMP (Plate #3)	LB+Kan (Plate #4)	LB+Spe (Plate #5)	LB (Plate #6)
	0	0	16	22	25	30

Table 2. AIEC combination therapy replicates plate #1.

AIEC Colonies	LB+P/S (Plate #1)	LB+Kan/Cam (Plate #2)	LB+AMP (Plate #3)	LB+Kan (Plate #4)	LB+Spe (Plate #5)	LB (Plate #6)
	0	0	15	20	23	27

Table 3. AIEC combination therapy replicates plate #2.

AIEC Colonies	LB+P/S (Plate#1)	LB+Kan/Cam (Plate#2)	LB+AMP (Plate #3)	LB+Kan (Plate #4)	LB+Spe (Plate #5)	LB (Plate #6)
	0	0	14	21	24	29

The data regarding sequential therapy set A showed that ultimately the data was less effective than combination therapy. This is ultimately because it affects DNA. The DNA of AIEC bacteria ultimately evolves after being exposed to weak antibiotics, mutates, and becomes resistant against several of the properties of the previous antibiotics.

This process will in turn make it less susceptible to treatment from a following antibiotic. In the original set and the two replicate sets, there were lower amounts of surviving AIEC colonies when P/S was used, than in any of the other treatments in set A. However, the treatment set had a higher amount of surviving AIEC colonies, than the combination therapy.

The AIEC bacteria that were treated via sequential therapy in Set B were not able to replicate nearly as well as the bacteria that were treated via combination therapy. This is because during sequential therapy,

specifically in Set B, select antibiotics in sequential order were able to destroy DNA of AIEC bacteria and prevent them from multiplying (Perron GG, 2012). Set B was also found to be more effective than set A because since the strong antibiotics were applied first, this eliminated most bacterial colonies before they have a chance to evolve. This was a much more effective method than the weak-strong treatment and yielded a much lower number of surviving.

Table 4. Average amount of AIEC colonies that survived under sequential therapy (Set A).

	LB+Spe then Kan (Plate #1)	LB+Spe then AMP (Plate #2)	LB+Spe then Kan/Cam (Plate #3)	LB+Spe then P/S (Plate #4)	LB+Kan then P/S (Plate #5)	LB+AP and P/S (Plate #6)	LB Agar (Control) (Plate #7)
AIEC Colonies (Original Set A Plates)	24	19	7	6	4	2	32
AIEC Colonies (Replicate #l of Set A Plates)	22	17	6	6	3	2	29
AIEC Colonies (Replicate #2 of Set A Plates)	24	19	7	6	4	2	31

Table 5. Average amount of AIEC colonies that survived under sequential therapy (Set B).

	LB+Kan then Spe (Plate #1)	LB+AMP then Spe (Plate #2)	LB+Kan/Cam then Spe (Plate #3)	LB+P/S then Spe (Plate #4)	LB+P/S then Kan (Plate #5)	LB+P/S then AMP (Plate #6)	LB Agar (Control) (Plate #7)
AIEC Colonies (Original Set B Plates)	19	14	0	0	0	0	34
AIEC Colonies (Replicate #l of Set B Plates)	17	13	0	0	0	0	31
AIEC Colonies (Replicate #2 of Set B Plates)	19	12	0	0	0	0	33

Plates that consisted of LB+AMP, LB+Kan and LB+Spe treatments only had one antibiotic mixed in solution and served as positive controls to test whether or not singular antibiotic therapy was more or less effective than combination therapy. The LB+P/S and LB +Kan/Cam plates however had two antibiotics mixed in agar plates, which were the plates that happened to have no surviving colonies when combination therapy was initiated. The standard error of the mean (SEM) calculated is 5.043 (Figure 1). The X axis presents all of the types of antibiotics that the AIEC colonies were exposed to and the Y axis represents the average number of surviving AIEC colonies after treatment. For sets A and B, all 7 plates listed were exposed to the same antibiotics but in different order. Set A represented the weak-strong antibiotic therapy and set B represented the strong-weak antibiotic therapy.

The plates with LB+Kan+Spe tended to yield the most AIEC colonies of any sequential antibiotic treatments no matter what order antibiotics were given in. However, the AIEC colonies treated with LB+P/S+AMP tended to yield the lowest number of colonies no matter what order the treatments were given. The standard error of the mean (SEM) calculated for set A is 4.196 and for Set B is 4.87 (Figure 2).

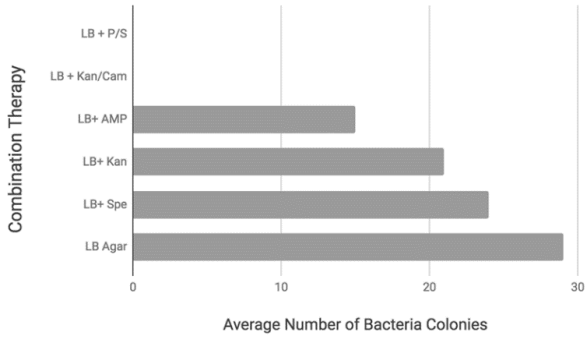

Figure 1. The average number of AIEC colonies that survived antibiotic treatments under combination therapy during the original and replicate tests are represented by the figure above.

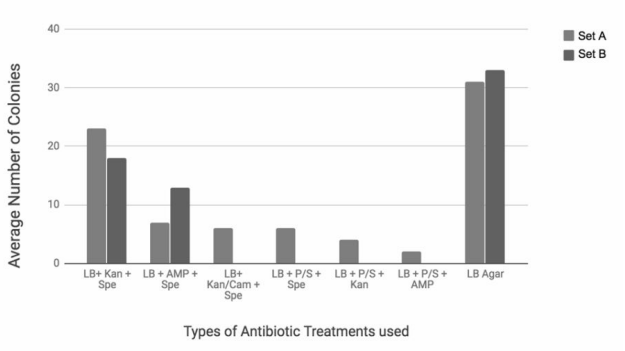

Figure 2. The results of sequential therapy on AIEC bacterial colonies are illustrated. The averages of the original and replicate experimental test results for both Sets A and B were taken to visualize the results in the figure above.

Regarding measuring the effect of antibiotic treatments on AIEC under sequential therapy and the evolution of MDR in the strains, a population dynamics model regarding the evolution of MDR in the AIEC bacterial population was made.

The model was made with the assumption that bacteria acquire resistance mutations against the first and the second antibiotics with rates α1 and α2 and that an antibiotic-resistant allele confers a cost of resistance in the absence of that antibiotic (Perron GG, 2012). Other assumptions that are made is that mutations that confer resistance against the first antibiotic are deleterious with selection coefficient c1 in the absence of the following antibiotic and that the fitness cost of mutation will confer resistance to the second antibiotic, which corresponds to variable c2. Other variables used in the population dynamics model include ω which is the number of MDR cells in AIEC, r1 which is the intrinsic growth rate, B which is the antibiotic value for each antibiotic and is a value dependent on whether a strong or weak antibiotic is used, ω refers to the colony population number, and f which is the specific value associated with each following antibiotic.

In the first phase of the experiment, it was assumed that the AIEC bacterial population grew and by the end of this phase, it reached the mutation-selection balance (Perron GG, 2012), α1/c1, and that there were no MDR mutants. During this specific portion of the phase, the population then grew for time tin the presence of the first antibiotic, and then grew for time t again after the second antibiotic was applied on a new plate with the bacterial samples (MacLean RC et al. 2010 and Andes D et al. 2004).

An assumption regarding my experiment, is that MDR tended to evolve best if on average there was at least one cell harboring resistance to both antibiotics at the time that the second antibiotic was applied. Therefore if $\omega_{15}(t)$ is the expected number of MDR cells at time t during the second growth phase, the equation for MDR evolution is ω_{15} (T) $\geq$ 1(Ochman H et al. 2000 and Perron GG et al. 2012). Relating the aforementioned equation to variables α_1, α_2, c_1, and c_2 in terms of MDR evolution, an assumption that must be made is that the population stays below its carrying capacity and grows exponentially for time T under the presence of the first antibiotic. Therefore the term $\omega_1(t)$ which refers to the expected number of bacteria resistant to the first antibiotic only at time t, can be described and defined by these equations:

$\omega_1=r_1\omega_1-\alpha_2\omega_1$,

$\omega_{12}=(r_1-c_2)\omega_{12}+\alpha_2\omega$

Which then changes to this equation assuming that conditions stay the same at the beginning of the second growth phase:

$\omega_1(0)=\omega_0\alpha_1/c_1$,

$\omega_{15}(0)=0$

Relating the previously mentioned equations to the intrinsic growth rate of single-drug-resistant bacteria in the presence of the first antibiotic then expands the equation for:

$\omega_1(t)=(\omega_0\alpha_1/c_1)*e^(r_1-\alpha_2)t$,

$\omega_{15}(t)=((\omega_0\alpha_1/c_1)\mu_2/(\alpha_2-c_2))*(e^(r_1-c_2)t-e^(r_1-\alpha_2)t)$

which then becomes: $((\omega_0\alpha_1\alpha_2*e^(r_1-\alpha_2)t/(c_1))*(1-e^-(c_2-\alpha_2)t/(c_2-\alpha_2)))/\geq 1$ which can re-evaluated as:

$c_1 \leq Bf(c_2)$ where $B=((\omega_0\alpha_1\alpha_2)*e^(r_1-\alpha_2)t*(t))$

and $f(c)=(1-(e^-(c-\alpha_2)t))/((c-\alpha_2)t)$

In the experiment, it is possible to relate the one strong and one weak antibiotic used per LB agar plate as, X and Y, in which resistance incurs the costs CX and CY, respectively. Without loss of generality then CX>CY. Relating the aforementioned equation into a term that relates to set A where the weak antibiotic is applied before the strong antibiotic would yield the equation CY $\leq$ Bf (CX) when simplified.

As for the case relating to Set B where the strong antibiotic is applied before the weak antibiotic, the equation that would be yielded is the equation CX $\leq$ Bf (CY) when simplified. For the mathematical model below, only the equations Cy $\leq$ Bf (CX) and Cx $\leq$ Bf (CY) are relevant since the aforementioned equations are just the unsimplified versions of Cy $\leq$ Bf (CX) and Cx $\leq$ Bf (CY) (Figure 3).

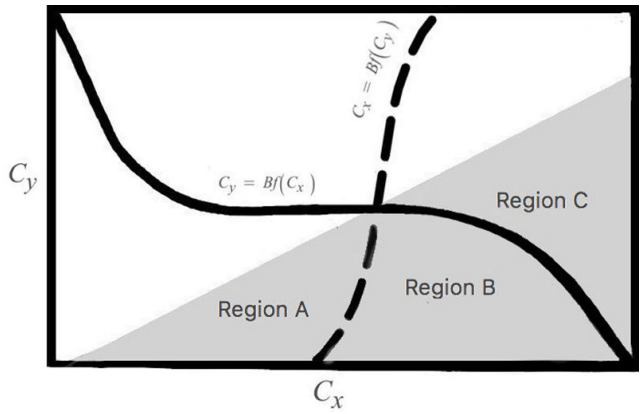

Figure 3. The illustration above is an experimental model that shows the regions where MDR is expected to evolve under different antibiotic treatments.

The region to the right of the dashed line is the region where MDR is expected to be hindered when a strong antibiotic is administered first. In contrast, the region on the left-side, below the solid line, is the region where MDR is expected to evolve when a weak antibiotic is administered first. The Cx and Cy referring to the axes in the planes relate to CY ≤ Bf (CX) and CX ≤ Bf (CY) and divide to plane to have separate regions. Regarding the two formulas, variables Cx and Cy refer to the fitness costs of the treated bacteria and the Bf variable refers to the strength of the antibiotic (Bell G, 2008).

This aforementioned diagram illustrates the differences between the formulas CY ≤ Bf(CX) and CX ≤ Bf(CY) and divides the grey plane into three separate regions. Note that only the area under the grey plane is relevant regarding the data where CX>CY. In region A, both equations are satisfied, which implies that MDR is tends to evolve independent of the order in which antibiotic treatments are applied to AIEC. However, in region B, CY ≤ Bf (CX) is satisfied but not CY ≤ Bf(CX), which means that MDR tends to evolve when a weak antibiotic treatment is administered before a strong antibiotic. Lastly, region C shows that both equations are violated, meaning that MDR tends not to evolve under either treatment. In summation, the model shows that MDR is less likely to evolve under sequential therapy when a strong antibiotic is applied before a strong antibiotic; meaning that the cost of fitness resistance is ultimately represented by region B (Levy SB and Marshall B, 2004).

CONCLUSION

In an attempt to determine whether or not antibiotics would be a useful method of therapy for disrupting the growth of AIEC-LF82 bacteria and treating Crohn's disease and IBD, I observed that the lb agar plates that had two combined types of antibiotics were able to completely stop the colonization and growth of AIEC bacteria. I found that each of the aforementioned antibiotics would have a much weaker negative effect on the growth and colonization of AIEC bacteria when used singularly rather than when used in conjunction. When used singularly, most of the antibiotics only hindered the growth of AIEC bacteria to a relatively small degree, with AMPs, seeming to be the most effective antibiotics. It also appeared that using two different antibiotics combined, may be a viable future treatment for patients suffering from Crohn's disease, since they appeared to completely prevent the growth of AIEC bacteria and destroy any plasmids that the bacteria may have obtained in the process. Thus, Penicillin-Streptomycin and Kanamycin-Chloramphenicol antibiotic solutions appear to be a substantially potent treatment for patients suffering from Crohn's disease and IBD regarding combination therapy.

The results of the experiment also showed that strong-weak sequential therapy is more effective overall than weak-strong sequential therapy and that the former type of sequential therapy significantly hinders MDR evolution in AIEC-LF82 bacteria. The population dynamics model in the experiment showed that the order of when the antibiotic is given affects the fitness cost and that strong-weak sequential therapy is also more effective than combination therapy but not weak-strong sequential therapy. In addition, it was shown that MDR evolution was reduced by first using the antibiotic for which resistant mutation confers the highest fitness cost and vice versa. The results from the experiment show that MDR evolution overall tends not be that affected when antibiotics are given simultaneous as opposed to being given at different times as seen in the model. Given that results from the experiment, it also appears that specific antibiotic combinations tend to be more effective at lowering MDR evolution and affect the fitness costs of AIEC bacteria than other combinations.

The time scale between the use of the two antibiotics tends to determine the frequency of resistance mutations found at each phase in the experiment. In the pre-antibiotic phase the population size post-treatment must remain low relative to carrying capacity; and it must be long enough for the population to reach the mutation-selection balance regarding resistance in order for the assumptions in the model to be true, which was the case. The experiment was ultimately successful because of the ease and effectiveness of switching antibiotics in a short period of time, despite the potential difficulty of controlling the exact concentration of antibiotics that reach the site of infection. When the antibiotics were used in conjunction, I also made sure we that there was no cross-resistance, no recombination between the

resistance mutations, no possibility of compensatory mutations, and no epistasis between resistance mutations, hence the antibiotic combinations were chosen carefully. If cross-resistance between the two antibiotics was present in the experiment then the order effect would be cancelled since the evolution of resistance to one antibiotic would ultimately lead to the resistance to the following antibiotic. Hence, for the future of combination therapy and especially sequential therapy, it would seem that medical institutions should avoid the use of two drugs that are known to lead to cross-resistance. Horizontal gene transfer is also another factor that can lead to the assembly resistance genes in bacterial lineages and ultimately can lead to MDR evolution.

In summation, the order in which antibiotics are given can affect bacterial populations is because there is a large amount of competition in AIEC-LF82 bacterial combinations and because of that the frequency of resistant mutants is limited and lowered by the rate at which they form. Ultimately, resistance mutations will arise at a low rate and resistance mutations that incur a large cost on fitness will be less frequent in a population that is not treated with antibiotics, which is a process known as mutation-selection balance. In strong-weak sequential therapy, the first antibiotic greatly reduces population density and the competition between bacterial cells is lowered as a result. With extremely small competition, the rate of resistance evolution against the second antibiotic is limited primarily by the rate at which resistance mutations arise. Resistance mutations against the two antibiotics incur different costs, therefore the order of antibiotics treatment will greatly determine and effect MDR evolution.

Since multi-drug therapy is a clinical practice that is growing in popularity to treat bacterial infection, it is crucial to understand the full evolutionary consequences associated with drug deployments. The results from my experiment not only demonstrate the influence cost of resistance has on the evolution of MDR, but also mentions approaches that will ultimately improve multidrug therapy, particularly regarding AIEC bacteria. For example, even though combination therapy is overall less effective than sequential therapy due to the fact that combination therapy tends to be more timely and can cause adverse and/or effects in the human body depending on the combination of antibiotics used, it is actually more effective at eliminating AIEC bacterial colonies in a short period of time if antibiotic solutions composed of two antibiotics such as Penicillin-Streptomycin and Kanamycin-Chloramphenicol solutions are used simultaneously. Regarding sequential therapy, even though strong-weak sequential therapy was shown by the results to be the most successful therapy and weak-strong sequential therapy was shown by the results to be the least effective, the most effective combinations in both sequential therapy types were LB+P/S+AMP and the least effective combinations were LB+Kan+Spe. Since combination therapy was also shown to be the most effective, only when Penicillin-Streptomycin and Kanamycin-Chloramphenicol antibiotics solutions were used and these specific combinations were not known to cause uncomfortable nor adverse effects in the human body, using these antibiotic solutions via combination therapy for the future treatment of AIEC holds promising potential. Even though weak-strong antibiotic sequential therapy was demonstrated to be much more effective than initially hypothesized, strong-weak sequential antibiotic therapy particularly treated has significant potential for treating Crohn's disease and IBD given the fact that it has the potential to severely hinder and/or completely halt the colonization of AIEC-LF82 bacterial colonies.

REFERENCES

Conte MP, et al. (2014). Adherent-Invasive Escherichia coli (AIEC) in pediatric crohn’s disease patients: Phenotypic and genetic pathogenic features. BMC Research Notes. 7(1)-748.

Yang Y, et al. (2017). The role of major virulence factors of AIEC involved in inflammatory bowel disease: A mini-review. Appl. Microbiol. Biotechnol. 101(21): 7781-7787.

Sevrin G, et al. (2018). Adaptation of adherent-invasive E. coli to gut environment: Impact on flagellum expression and bacterial colonization ability. Gut Microbes. pp: 1-17.

Palumbi SR. (2001). Humans as the world's greatest evolutionary force. Science. 293(5536): 1786-1790.

Gould IM and van der Meer JWM (2007). Antibiotic policies: Fighting resistance, 1st ed. Springer Science Business Media, LLC, New York, NY.

Albert TJ, et al. (2005). Mutation discovery in bacterial genomes: metronidazole resistance in Helicobacter pylori. Nat. Methods. 2(12): 951–953.

Perron GG, et al. (2012). Bacterial recombination promotes the evolution of multidrug-resistance in functionally diverse populations. Proc. Biol. Sci. 279(1733): 1477-1484.

MacLean RC et al. (2010). The population genetics of antibiotic resistance: Integrating molecular mechanisms and treatment contexts. Nat. Rev. Genet. 11(6): 405-414.

Andes D, et al. (2004). Application of pharmacokinetics and pharmacodynamics to antimicrobial therapy of respiratory tract infections. Clin. Lab. Med. 24(2): 477-502.

Ochman H, et al. (2000). Lateral gene transfer and the nature of bacterial innovation. Nature. 405(6784): 299-304.

Bell G. (2008). Selection: The mechanism of evolution. Oxford University Press, Oxford, United Kingdom.

Levy SB and Marshall B. (2004). Antibacterial resistance worldwide: causes, challenges and responses. Nat. Med. 10 (Suppl 12): S122-S129.