

Examining the Effects of Common Pharmaceuticals on the Growth of Freshwater Bacteria Isolated from Streams in LaGrange, GA

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Abstract

This study examined how different pharmaceuticals would affect different strains of bacteria found in freshwater streams. The bacteria were obtained from two different fresh water streams located in West Central Georgia. The pharmaceuticals tested in this study include: ibuprofen, acetaminophen, and Zyrtec (cetirizine). These pharmaceuticals were diluted to create multiple concentrations known to be found in freshwater streams in order to see if differing concentrations had any effect on the growth of these bacterial isolates. To determine the total effect that these pharmaceuticals had on the growth of the bacterial isolates, optical densities were recorded by using a plate reader to read absorbances at 600 nm. From these readings, Zyrtec (cetirizine) produced the most significant effect on the growth of all bacterial samples among higher concentrations; whereas acetaminophen and ibuprofen produced little significant effects among all samples collected and concentrations tested. These results suggest that certain pharmaceuticals produce a significant effect in inhibiting the growth of certain freshwater stream bacteria, but do not have an overbearing effect on the entirety of bacteria in freshwater streams at the concentrations frequently measured in streams.

Introduction

Bacteria play a vital role in keeping freshwater streams healthy. The effects of bacterial life on overall ecosystem health can be seen in two ways: either by the bacteria providing resources for other forms of life to grow or by getting rid of harmful toxins that could be in the water. For example, bacteria can produce carbon dioxide which allows for the growth of plants in these streams (Haack, 2017). Another way in which bacteria can aid in the health of streams is by breaking down any chemicals that may be found in the water ways. These chemicals, especially if they are bioactive, have negative effects on the different life forms found in the water (Suja et al., 2009). Another form of chemical that proves to be toxic to water life forms are pesticides. However, bacteria are able to break these down, so toxins are not released that could permanently harm an ecosystem (Haack, 2017).

Pharmaceuticals can also be classified as toxins if they reach the water ways. Some pharmaceuticals that reach these water ways may still have bioactive parts that can actively, and perhaps negatively, affect the bacteria that can be found in the water depending on the concentration of pharmaceutical that is found in the ecosystem (Corcoran, Winter, and Tyler, 2010). For the pharmaceuticals tested in this study the ranges include: acetaminophen from 25 ng/L (Ebele, Abdallah, and Harrad, 2017) to 10,000 ng/L (Kolpin et al., 2002), ibuprofen from 1 ng/L (Ebele, Abdallah, and Harrad, 2017) to 1,000 ng/L (Kolpin et al., 2002), and Zyrtec 97,000 to 530,000 ng/L (Fick et al., 2009). If these pharmaceuticals are found to have negative effects on bacteria, then the other organisms living in the

water may also be in danger if the bacteria are no longer able to function in providing carbon dioxide or no longer have the ability to break down toxins that reach the water.

From 2000 to 2010, global consumption of pharmaceuticals increased by about 36% (Van Boeckel et al., 2014), and some believe that this number is still rising today. As this use of pharmaceuticals rises, more may be able to seep into freshwater streams and affect the wildlife more than what is currently known (Corcoran, Winter, and Tyler, 2010). Therefore, the overall goal of this study is to determine if different strains of bacteria behave similarly if exposed to the same over the counter pharmaceuticals, and to provide a better understanding of how these pharmaceuticals directly affect the bacteria living in freshwater streams. We hypothesize that for each pharmaceutical as the concentration increases there will be a decrease in the amount of bacterial growth.

Materials and Methods

Water Sample and Bacteria Collection. Water samples were collected from two different streams: Park Creek and Granger Park Creek located in West Central Georgia. Samples were placed on ice and returned to the lab for processing. Once in the lab, 100 μ l of each sample was spread on a TSA plate, and the plates were incubated for 24 hours at room temperature in a method similar to that of Mulamattathil et al. (2014). Five different bacterial isolates were chosen based on diversity of colony morphology for this study. Three isolates were from Granger Park Creek (T3, T4, T5), and two isolates were from Park Creek (P3, P4). Each of the 5 bacterial isolates were further isolated from their original plates by streaking onto individual TSA plates in order to obtain pure cultures.

Pharmaceutical Dilutions. Three pharmaceuticals were chosen for this study based on those commonly used and found within freshwater streams. The pharmaceuticals chosen included: acetaminophen, ibuprofen, and Zyrtec. In order to dissolve the pharmaceuticals easier, the liquid versions of each pharmaceutical were used, which involved using the children's versions of these specific medicines. Each of the three pharmaceuticals tested were diluted to produce concentrations that could be found in freshwater streams as described earlier. As a guide, the highest concentration was set to be half of the concentration of the obtained medicine and each subsequent concentration was a 1:10 dilution of the pharmaceutical into LB broth. A total of 8 dilutions were used for each pharmaceutical to test against each bacterial sample ranging from zero pharmaceutical (positive controls) to half the concentration on each bottle.

Bacterial Growth Measurements. A GloMax plate reader was used to quantify bacterial growth in the presence of the various pharmaceuticals. To prepare for the plate reader, a pure colony of each bacterial sample was placed in 5 mL of LB broth and incubated overnight at room temperature. The next day, 10 μ L of bacterial culture was placed in a microcentrifuge tube with 1 mL of the corresponding pharmaceutical concentration, a 1:100 dilution. These were mixed thoroughly to ensure that the bacteria were exposed to the entire pharmaceutical. Then, 200 μ L from each set of pharmaceutical and bacteria was dispensed into a well on a 96-well plate with 3 replications. Also, there was only pharmaceutical placed in a well to act as a negative control for each concentration. Controls were performed in multiples of three. After the plates were filled with each sample, they were placed in a shaking incubator for 24 hours to allow the bacteria to grow. After 24 hours, the plates were taken to a plate reader and absorbances were read for each well at 600 nm, and the results were recorded. This process was completed for each pharmaceutical for a total of three trials.

Results

In order to determine the overall effectiveness of the pharmaceuticals on the growth of bacteria, optical densities were measured at 600nm. With this data over three different trials, averages were calculated and displayed in a graph to signify overall effectiveness of the specific pharmaceutical. For each trial, each specific pharmaceutical had two plates that were read that contained the 5 different bacterial isolates. The data and graphs were used to determine general trends in the effectiveness of higher concentrations on bacterial growth. This information is displayed in Figures 1-3.

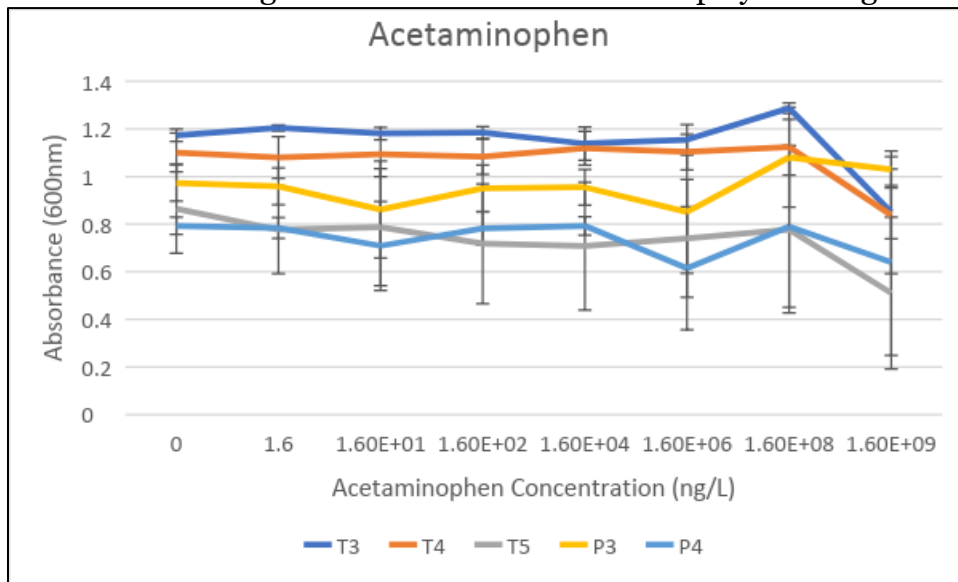


Figure 1. Bacterial growth measured as absorbance (600 nm) in the presence of various concentrations of acetaminophen.

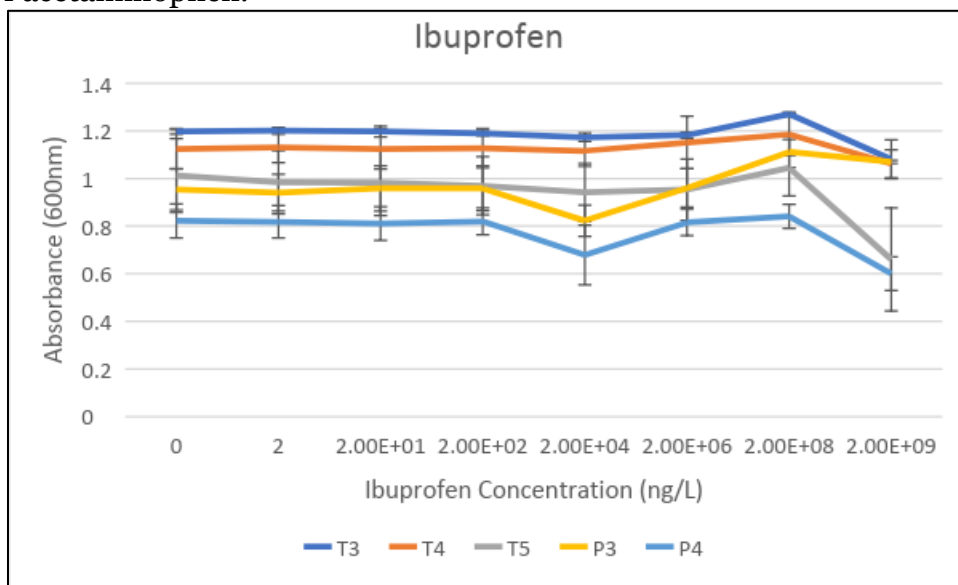


Figure 2. Bacterial growth measured as absorbance (600 nm) in the presence of various concentrations of ibuprofen.

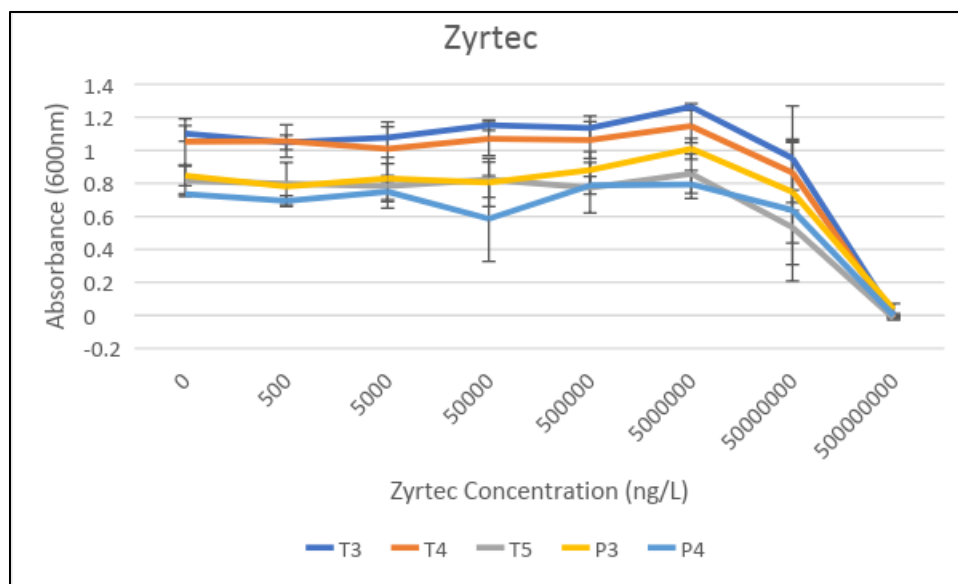


Figure 3. Bacterial growth measured as absorbance (600 nm) in the presence of various concentrations of Zyrtec.

Also, in order to determine the true effects of these pharmaceuticals on bacterial growth, a t-test was performed based off the averages that were calculated for each trial. A t-test was run for each pharmaceutical and concentration to see if there was any significant loss of bacterial growth relative to the positive controls. This data could also show if pharmaceuticals had specific effects against any single bacterial isolate. The p-values of these t-tests can be seen in Tables 1-3.

Concentration (ng/L)	Isolates				
	T3	T4	T5	P3	P4
1.6	0.1303	0.4225	0.357	0.444	0.420
1.60E+01	0.4155	0.473	0.3871	0.2816	0.302
1.60E+02	0.3665	0.424	0.295	0.4178	0.4421
1.60E+04	0.290	0.4241	0.292	0.429	0.4975
1.60E+06	0.3772	0.485	0.3237	0.3051	0.220
1.60E+08	0.0077	0.4265	0.400	0.2926	0.498
1.60E+09	0.0129	0.1400	0.1528	0.2786	0.328

Table 1. P-values obtained from t-tests comparing growth at each concentration of Acetaminophen to the corresponding positive control. Highlighted p-values indicate a significant decrease in bacterial growth ($p < 0.05$).

Concentration (ng/L)	Isolates				
	T3	T4	T5	P3	P4
2	0.447	0.4661	0.4349	0.4441	0.4821
2.00E+01	0.478	0.4962	0.4304	0.4871	0.448

2.00E+02	0.262	0.486	0.3999	0.4856	0.4891
2.00E+04	0.1117	0.4701	0.3396	0.1055	0.1453
2.00E+06	0.1520	0.409	0.3661	0.4792	0.4677
2.00E+08	0.009	0.2834	0.4254	0.0750	0.3994
2.00E+09	0.0771	0.2525	0.090	0.086	0.0274

Table 2. P-values obtained from t-tests comparing growth at each concentration of ibuprofen to the corresponding positive control. Highlighted p-values indicate a significant decrease in bacterial growth ($p < 0.05$).

Concentration (ng/L)	Isolates				
	T3	T4	T5	P3	P4
5.00E+02	0.1832	0.4890	0.4527	0.1271	0.1021
5.00E+03	0.3598	0.4111	0.4140	0.448	0.3895
5.00E+04	0.1732	0.4555	0.4774	0.3827	0.2617
5.00E+05	0.3334	0.4719	0.3962	0.3761	0.1438
5.00E+06	0.000	0.2684	0.3688	0.0410	0.2188
5.00E+07	0.2982	0.1875	0.1166	0.3614	0.3984
5.00E+08	0.000	0.004	0.002	0.001	0.000

Table 3. P-values obtained from t-tests comparing growth at each concentration of Zyrtec to the corresponding positive control. Highlighted p-values indicate a significant decrease in bacterial growth ($p < 0.05$).

Discussion

This study shows that pharmaceuticals can have detrimental effects on bacterial growth, especially at higher concentrations. The pharmaceutical which was deemed most effective at halting bacterial growth was Zyrtec, or cetirizine. While looking at the results, it is obvious that Zyrtec at higher concentrations very negatively affects the growth of bacteria, but also, upon completing the t-tests, it shows that Zyrtec most significantly stops the growth of all bacterial isolates. Acetaminophen, on the other hand, does not produce as much significance in halting bacterial growth, but some significance is seen. Also, ibuprofen does not have a large effect on bacterial growth, but still some significance is noticed.

We are not sure why Zyrtec was so effective at blocking bacterial growth at high concentrations, but we believe that it may have to do with an active agent that was found in the medicine. Some reasons that we believe the other pharmaceuticals did not produce a significant effect is because the children's versions of these pharmaceuticals contain a fair amount of sugar that the bacteria may have been able

to use and grow. However, the fact that all pharmaceuticals tested did display a significant effect on at least one bacterial isolate reiterates that the increase of pharmaceutical consumption may lead to a weaker freshwater ecosystem (Corcoran, Winter, and Tyler, 2010). Also, the observation that the different pharmaceuticals had different effects on the various bacterial isolates shows that when these pharmaceuticals are found in water ways, differing effects may be observed as one pharmaceutical may affect bacterial strains that are responsible for varying functions. Overall, the exposure of bacteria to pharmaceuticals can lead to a changing ecosystem that may not be beneficial for the life forms in the water.

As mentioned, the presence of the pharmaceuticals may lead to a weaker water ecosystem (Corcoran, Winter, and Tyler, 2010). This can happen in a variety of ways. For instance, bacteria are responsible for breaking down pesticides (Haack, 2017), and if these bacteria are killed and no longer able to break down these pesticides, then the fish or plants living in the ecosystem may also be affected. This could create a cascade of events where the food web is destroyed, and the ecosystem may no longer be able to come back as bacteria can serve as the basis for freshwater food webs (Haack, 2017). The findings of this study, if repeated, may require pharmaceutical companies to assess how their products are discarded and if necessary make changes so that these products do not reach the water ways and cause problems that can affect entire ecosystems.

Possible future experiments based on this data might be to test how the presence of these pharmaceuticals at troubling concentrations fully impact a growing ecosystem over time. Another possible experiment may be to test how a mixture of pharmaceuticals impact bacterial growth.

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