Computational Model To Quantify the Growth of Antibiotic-Resistant Bacteria in Wastewater

Abstract

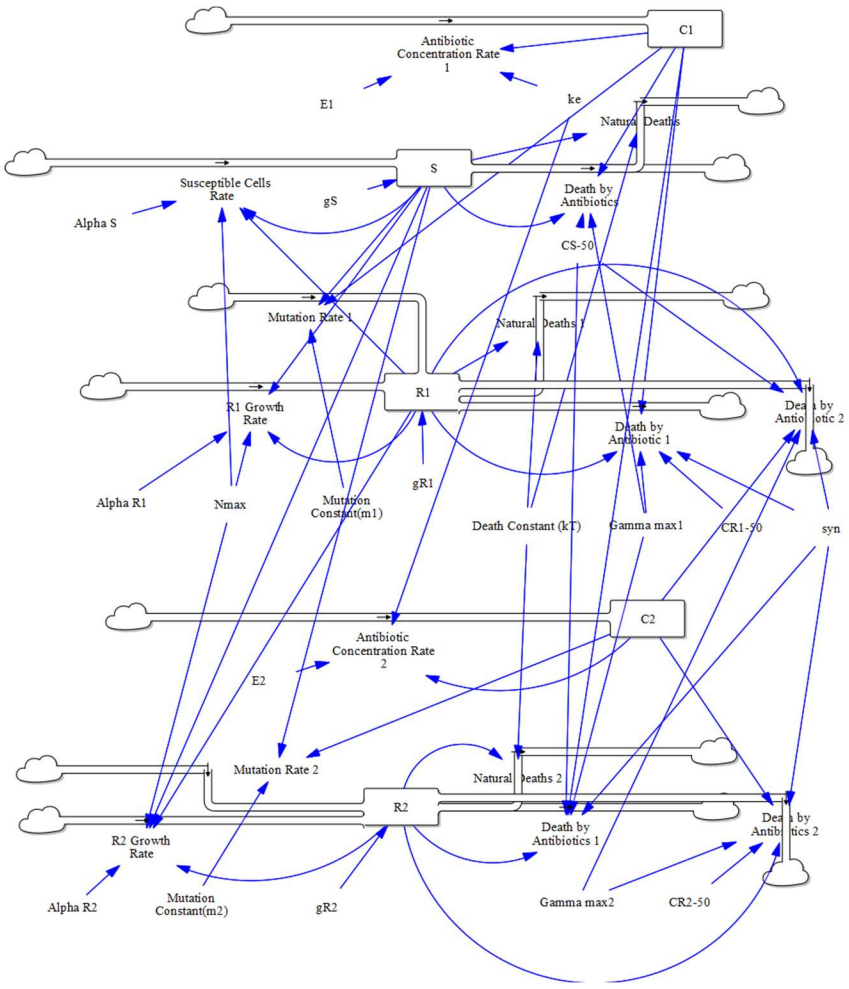
Antibiotics end up in wastewater treatment plants due to improper disposal. As a result, bacteria present in the water mutate and grow resistant to the antibiotics. This pollutes the water and causes diseases. We model this interaction between the bacteria and antibiotics in order to understand its behavior, and hence take preventative measures against disease. Upon experimentation with different parameter values, we have found that the mutation of the bacteria can be delayed by increasing the initial or residual values of the antibiotics, and that the resistant bacteria cells population can be decreased by either increasing the synergy or Gamma values, or decreasing the mutation rates.

Statement of Problem

We are told to always dispose of drugs and chemicals separately and to never just flush it down our drains. Have you ever wondered why though? This paper models such a scenario where antibiotics end up in the wastewater treatment plant as a result of improper disposal methods such as domestic flushing down drains, hospital waste, industrial discharge, etc. Upon such occurrence, the bacteria in the water end up reacting to the antibiotics and mutating. As a result, they grow resistant and pollute the water, since they can no longer be killed using the normal wastewater treatment methods. This then leads to the outbreak of diseases. In order to be able to predict the growth of these resistant bacteria populations before they cause an outbreak,

we model their behavior under antibiotics, taking into account various driving factors like antibiotic residue concentrations and antibiotic interaction. These findings would then be beneficial in setting policies on the standard for treatment of waste disposed into water bodies, which would then mitigate the disease outbreaks.

Model Design



In the creation of this model, we have employed multiple variables, both dependent and independent. Let us first take a look at some of the dependent variables. These are also the Stock variables in our Vensim diagram. Variables C_1 and C_2 represent the concentrations for Antibiotic

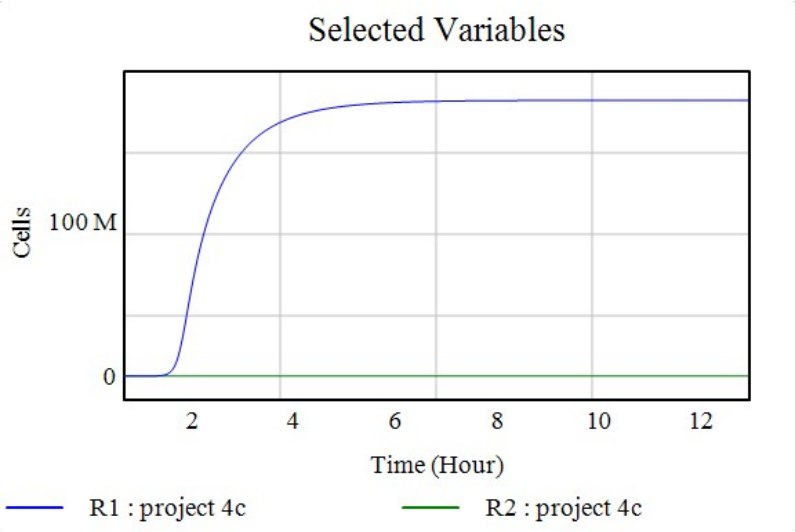
1 and Antibiotic 2 respectively. The units used for them are microgram/milliliter. Then, we have S to represent the Susceptible cells, with units being cells. R1 is the bacterial cells resistant to Antibiotic 1 due to chromosomal mutation, with cells as the units. R_2 is the bacterial cells resistant to Antibiotic 2 due to chromosomal mutation, using cells as the units again. Let us now look at the independent variables. E1 and E2 represent the environmental concentration of each Antibiotic, using microgram/milliliter/hour. 'ke' represents the decay rate of the antibiotics, with units 1/hr. Alpha S represents the growth rate of Susceptible bacteria, while Alpha R1 and Alpha R2 represent the growth rate of the Resistant bacteria. Their units are 1/hr. Nmax is the carrying capacity with units cells/ml. GS, gR1 and gR2 are the bacterial influx rates (cells/hr). KT is the bacterial efflux rate (cells/hr). 'syn' represents the synergy parameter between the Antibiotics. The units are nondimensional. Gammamax1 and Gammamax2 represent the bacterial killing rate in response to Antibiotic 1 and Antibiotic 2 (1/hr). Finally, C50-S, C50-R1 and C50-R2 represent the Antibiotic concentration where the killing action is half its maximum value. (microgram/milliliter). Let us now take a look at the differential equation:

$$\frac{dR_1}{dt} = \alpha_{R,1}\left(1 - \frac{R_m+R_1+R_2+R_p+S}{N_{max}}\right)R_1 + g_{R1} - k_T R_1 - syn * \delta_{max,1}\left(\frac{C_1}{C_1+C_{R,1}^{50}}\right)R_1 - syn * \delta_{max,2}\left(\frac{C_2}{C_2+C_S^{50}}\right)R_1 + m_1(C_1)S$$

The first term 'Alpha R1*(1-(S+R1)/Nmax)*R1' represents the Growth rate of the resistant bacteria. The second term 'gR1' represent the bacterial influx rates. The sixth term 'm1*(C1)*S' represents the mutation rate of the bacteria. These three terms are flowing into our stock variable R1. The third term 'kT*R1' represents the Natural Deaths. The fourth term 'syn*Gamma max1*(C1/(C1+"CR1-50"))*R1' represents the Cell Death by Antibiotic 1' and finally the fifth term 'syn*Gamma max2*(C2/(C2+"CS-50"))*R1' represents the Cell Death by Antibiotic 2'.

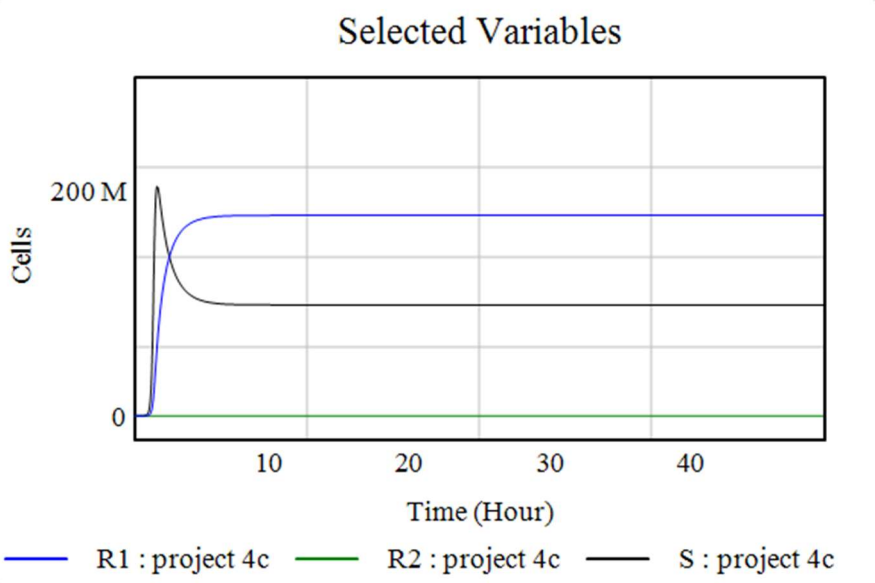
These three terms flow out of our Stock variable. The terms in the differential equations for S and R2 follow a similar pattern.

Model Solution



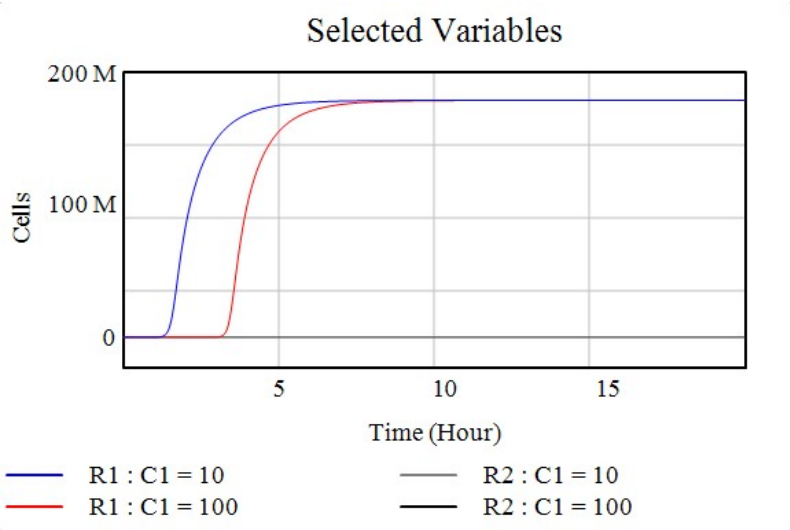
Graph 1

Graph 1 displays the behavior of the bacteria resistant to Antibiotic 1 (C1) versus the bacteria resistant to Antibiotic 2 (C2). As we can see, R1 starts mutating almost immediately and reaches the carrying capacity in just a couple of hours. R2 on the other hand stays at zero. It behaves in such a manner mainly because of the difference in the mutation rates. The mutation rate for R1 is $2.35 * 10^{-6}$, whereas that for R2 it's $2.35 * 10^{-8}$. This difference is what causes the drastic variation in behavior we see between R1 and R2. R2's mutation rate isn't high enough to resist the antibiotic's effect and hence always remains at zero.



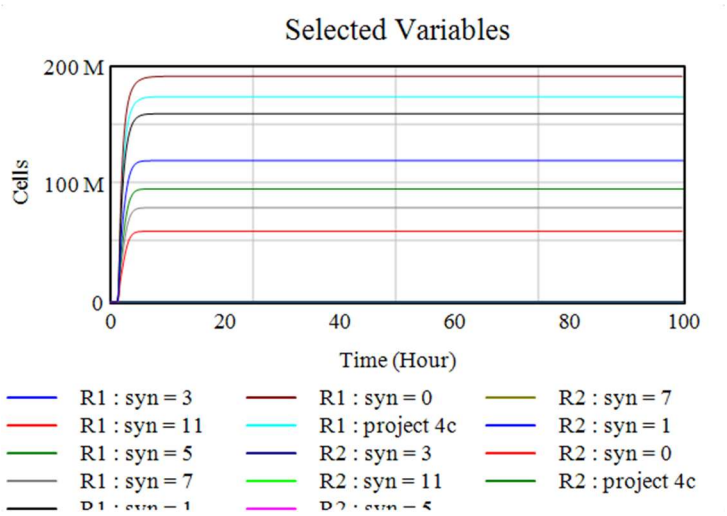
Graph 2

Graph 2 shows the Susceptible cells (S) against R1 And R2 over time. Initially, we see that S grows really fast due to its large growth rate. However, since the Susceptible cells aren't resistant to either Antibiotic 1 or Antibiotic 2, there is a drastic drop in their population. As given in the equation for susceptible population, the term 'syn*Gamma max1*(C1/(C1+"CS-50"))*S display the death due to Antibiotics 1. With the value of CS-50 being so low, it increases the death rate. The same form repeats with the deaths due to Antibiotic 2 as well. Thus, it explains the drastic drop in S given that S has no resistance against the antibiotics. Notice how the behavior of the Susceptible cells is in direct contrast to that of the Resistant cells. As the S drop, the R1 is mutating rapidly. Once the Resistant cells reach their carrying capacity though, the Susceptible cells stabilize.



Graph 3

Graph 3 shows the behavior of the mutation of the bacteria with different starting values for the antibiotics. R2 doesn't show much of a difference since it's always at zero regardless of the initial antibiotic amount. What we see with R1 is interesting though. It shows that the more antibiotics we start with, the longer it takes for the bacteria to start mutating. This is because it allows the effect of the antibiotics to last longer and kill the bacteria, before the effect eventually wears off and the bacteria start mutating rapidly again. A similar effect is also achieved through increasing the E1 and E2 values.



Graph 4

Finally, Graph 4 compares the bacteria mutating to become resistant, at different energy levels. Synergy is the interaction between the different types of antibiotic residues present in the wastewater. When we take this energy level between our two antibiotics to be less than one, then it induces an antagonistic interaction. This means that the effects of the two antibiotics clash, causing its killing effect on the bacteria to decrease further. Therefore, we see that the lesser these energy levels, the greater the resistant population is. When we take this energy to be greater than one though, the antibiotics complement each other, enhancing their killing rate of the bacteria. In real life, this would mean that we'd need to watch out for not just the effect antibiotics have on the bacteria in the wastewater, but also on each other.

A similar graph to the synergy graph is also achieved through varying the Gamma values. We tried to experiment by changing the values for different parameters in order to find the point at which the resistant bacterial population would decrease for R1. We presumed that increasing the killing rate of Antibiotic 1 would lead to a decrease in the resistant cells. However, no matter how we changed the values of Gammamax1, The R1 population wouldn’t seem to change. Upon

closer analysis of the differential equation for R1 though, we came to find that R1 being resistant to C1, wouldn't be affected no matter how high we made Gammamax1. Furthermore, the ‘death from Antibiotic 1’ having such a high value of C50-R1 in the denominator, renders the term almost equal to zero. Instead, upon making Gammamax2 have a high value, we found R1 to display a significant decrease.

Results and Conclusions

Through the analysis of the various graphical relations of the model, we have found that the mutation of the bacteria can be delayed by increasing the initial or residual values of the antibiotics, and that the resistant bacteria cells population can be decreased by either increasing the synergy or Gamma values, or decreasing the mutation rates. Due to these findings, we now have a better understanding of the behavior of bacteria, upon exposure to antibiotics. This information can then aid in public-health policy making decisions and standardizing requirements for the treatment of waste discharge.

An important point to keep in mind, however, is that this model is in no way a perfect representation of the real-life system yet. We have made several assumptions in the making of it. One such assumption is that the bacteria remain still and constant in our wastewater treatment plant. In reality though, the water is constantly flowing in and out. We have represented the susceptible and resistant bacterial inflow and outflow rates as gS, gR1 and gR2. However, since their values for wastewater settings are not known, we just assume it’s value to be zero. Should future studies find accurate data for these though, it would serve to make our model more accurate.

Another assumption we make is that any given bacteria can only turn resistant to one type of Antibiotic. However, that isn't necessarily the case. A bacteria could develop resistance towards multiple types of Antibiotics. We also need to consider that there would be multiple bacterial species as well. This would greatly change the results of our model. Furthermore, we assume that the bacteria grow at constant rates. We don't take into account factors like nutrients, sunlight, or even dormancy of the cell. Finally, not all our parameter values have been derived from field data and experimental validation. Given the time and resources, these factors would need to be addressed to make a truly accurate model.

Appendices

Variable	Formula	Units
Alpha R1	4.99	1/hr
Alpha R2	4.99	1/hr
Alpha S	13.66	1/hr
Antibiotic Concentration Rate 1	E1-ke*C1	
Antibiotic Concentration Rate 2	E2-ke*C2	
C1	0	microgram/mL
C2	0	microgram/mL
CR1-50	200	microgram/mL
CR2-50	100	microgram/mL
CS-50	12.5	microgram/mL
Death by Antibiotic 1	Syn*Gamma max1*(C1/(C1+CR1-50))*R1	
Death by Antibiotics	Gamma max1*(C1/(C1+CS-50))*S	
Death by Antibiotics 1	Syn*Gamma max1*(C1/(C1+CS-50))*R2	
Death by Antibiotics 2	Syn*Gamma max2*(C2/(C2+CR2-50))*R2	
Death by Antibiotic 2	Syn*Gamma max2*(C2/C2+CS-50))*R1	
Death Constant (kT)	0.8	1/hr

E1	30	microgram/mL/hr
E2	0.25	microgram/mL/hr
FINAL TIME	100	Hour
Gamma max1	27.14	1/hr
Gamma max2	27.14	1/hr
INITIAL TIME	0	Hour
ke	1.97	1/hr
Mutation Constant(m1)	$2.35*10^{-6}$	1/hr
Mutation Constant(m2)	$2.35*10^{-8}$	1/hr
Mutation Rate 1	Mutation Constant(m1)+C1+S	1/hr
Mutation Rate 2	Mutation Constant(m2)+C2+S	1/hr
Natural Deaths	Death Constant (kT)*S	Cells
Natural Deaths 1	Death Constant (kT)*R1	Cells
Natural Deaths 2	Death Constant (kT)*R2	Cells
Nmax	$3*10^{8}$	Cells/mL
R1	1000	Cells
R1 Growth Rate	Alpha R1*(1-(S+R1)/Nmax)R1	
R2	1000	Cells
R2 Growth Rate	Alpha R2*(1-R1+R2+S)/Nmax)*R2	
S	1000	Cells
SAVEPER	TIME STEP	Hour
Susceptible Cells Rate	Alpha S*(1-(S+R1)Nmax)*S	
syn	11	Nondimensional
TIME STEP	0.0078125	Hour

Bibliography

Sutradhar, Indorica, et al. “Computational Model To Quantify the Growth of Antibiotic-Resistant Bacteria in Wastewater.” *American Society for Microbiology,* vol. 6, no. 3, June 8, 2021. pp. 1-12.