Computational Model to Quantify the Growth of Antibiotic Resistant Bacteria in Wastewater

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Abstract

Though wastewater and sewage systems are known to be a significant reservoir of antibiotic resistant bacterial populations and periodic outbreaks of drug resistant infection, there is little quantitative understanding of the drivers behind resistant population growth in these settings. In order to fill this gap in quantitative understanding of outbreaks of antibiotic resistant infections in wastewater, we have developed a mathematic model synthesizing many of the known drivers of antibiotic resistance in these settings in order to predict the growth of resistant populations in different environmental scenarios. A number of these drivers of drug resistant infection outbreak including antibiotic residue concentration, antibiotic interaction and synergy, chromosomal mutation and horizontal gene transfer, have not previously been integrated into a single computational model. Our integrated model shows that low levels of antibiotic residues present in wastewater can lead to the increased development of resistant populations, and the dominant mechanism of resistance acquisition in these populations is horizontal gene transfer rather than chromosomal mutations. Additionally, we found that synergistic antibiotic interactions can cause increased resistant population growth. Our study shows that the effects of antibiotic interaction are observable even at the low antibiotic concentrations present in wastewater settings. These findings, consistent with recent experimental and field studies, provide new quantitative knowledge on the evolution of antibiotic resistant bacterial reservoirs, and the model developed herein can be adapted for use as a prediction tool in public health policy making, particularly in low income settings where water sanitation issues remain widespread and disease outbreaks continue to undermine public health efforts.

Significance

The rate at which antimicrobial resistance (AMR) has developed and spread throughout the world has increased in recent years, and it is suggested that at the current rate, several million people may die by 2050 due to AMR. One major reservoir of resistant bacterial populations that has been linked to outbreaks of drug resistant bacterial infections, but is not well understood, is in wastewater settings, where antibiotic pollution is often present. Using ordinary differential equations incorporating several known drivers of resistance in wastewater, we find that interactions between antibiotic residues and horizontal gene transfer significantly affect the growth of resistant bacterial reservoirs.

Introduction

Wastewater and sewage systems are a major reservoir of resistant bacterial populations due to the collection of antibiotic waste from humans and animals, inappropriate drug disposal, and effluent from drug manufacturers, hospitals and agricultural/veterinary settings (1). In some cases, environmental concentrations can reach, or even exceed, minimal inhibitory concentrations of certain antibiotics (2). This problem is particularly pertinent in low- and middle-income countries (LMICs) where cases of antibiotic resistant infections have been rising and 70% of sewage produced is estimated to enter the environment untreated (1). For example, in 2016, an outbreak of extensively drug resistant (XDR) typhoid cases emerged in in the southern Sindh province of Pakistan, and geospatial mapping revealed that the XDR *S.* Typhi infections spread around sewage lines in the city of Hyderabad (3). Such outbreaks put the health and safety of surrounding populations at risk and, due to the communicable nature of these infections, increases risk for populations beyond the immediate vicinity of wastewater and sewage lines. Antibiotic pollution in wastewater and sewage systems results in a complex environment with many interacting antibiotic residues (4, 5). This pollution is a major driver of resistance, as selective pressure from antibiotics in the environment is known to promote chromosomal resistance mutations (2, 6, 7). Furthermore, the interactions between different antibiotic residues may also affect resistance due to the synergistic and antagonistic effects changing the selective pressure on the bacteria (8). Additionally, the concentrations of antibiotic residues and disinfectants in sewage may be able to support and promote horizontal transfer of resistance genes among bacteria (9-12). These mobile resistance genes have also been measured at high levels in wastewater and sewage (2). Though the drivers of AMR in wastewater have been studied to some extent (2, 12), one of the largest gaps in understanding the emergence of AMR within a sewage environment is the limited quantitative understanding of the effects and interactions of the many biological and environmental mechanisms at work (13). Quantitative understanding of resistant population growth in wastewater settings is critical in order to predict the development of large resistant populations in wastewater that may pose a risk to local populations of resistant infection outbreak. Furthermore, quantitative tools for understanding of resistance can be used to develop and model strategies for preventing the emergence of large resistant populations and therefore inform policy decisions. Therefore, there is a critical need for developing quantitative methods of probing AMR in wastewater environments. Our study is a step in filling this gap in knowledge.

Mathematical modelling has been an important tool in quantitatively studying AMR development at both an epidemiologic and mechanistic level (6, 8, 12, 14-16). Epidemiological studies have included approaches that investigate bacterial population dynamics in a number of biological contexts including biofilms (14). At the population level, much of AMR modeling deals with disease states in which resistance patterns are long established, with significant gaps in the study of resistance emergence in new pathogens and the role of environmental factors in the development of AMR (15). At the mechanistic level, modelling has been used as a tool to understand the individual roles of both mutational and plasmid-mediated resistances (8, 12).

Models based on resistance developed from chromosomal mutations have been used to study the role of synergistic and antagonistic antibiotic interactions on the emergence of resistant populations, finding that increased synergy increases the likelihood of resistance acquisition (8). Additionally, studies using modelling as a tool to understand horizontal gene transfer (HGT) as a driver of AMR have found HGT to a significant mode of resistance acquisition for *E. coli* in settings including agricultural slurry (12). However, much of the work done recently has been focused on fitting data in the absence of antibiotic exposure (16). Understanding the evolutionary trajectories of bacteria after exposure to antibiotics is important to be able to develop strategies that prevent the emergence of resistance (6). Thus, there remains a need for quantitative modeling of both chromosomal mutation acquisition and HGT under selective pressure from antibiotics to fully understand AMR development in settings such as sewage and wastewater, where often antibiotic residues are often present. Here we present a model of emergence and growth of drug resistant bacteria in wastewater, integrating acquisition of resistance through both chromosomal mutations and HGT while also incorporating the effects of antibiotic interactions.

Methods

Our mathematical model of the growth of antibiotic resistant bacterial populations in wastewater builds on prior studies (8, 12, 17) and extends it to incorporate a variety of critical, but overlooked, input factors specific to the bacterial species, antibiotics and environments of interest. The inputs can be broadly classified into bacterial parameters, environmental parameters and antibiotic parameters. Bacteria specific input factors include the growth rates of antibiotic susceptible and resistant strains, mutation rates, and rates of horizontal gene transfer. The antibiotic specific inputs, such as bactericidal activity and degree of synergy, allow for the study of the effects of drug quality and antibiotic pollution on the development of resistance. Additionally, environmental inputs, including physical inflow and outflow rates and antibiotic residue concentration, allow for the modelling of resistance development in a variety of settings of interest. These input parameters can be used to model an output of resistant bacterial populations over time, thus allowing for the prediction of resistant population development (Figure 1).

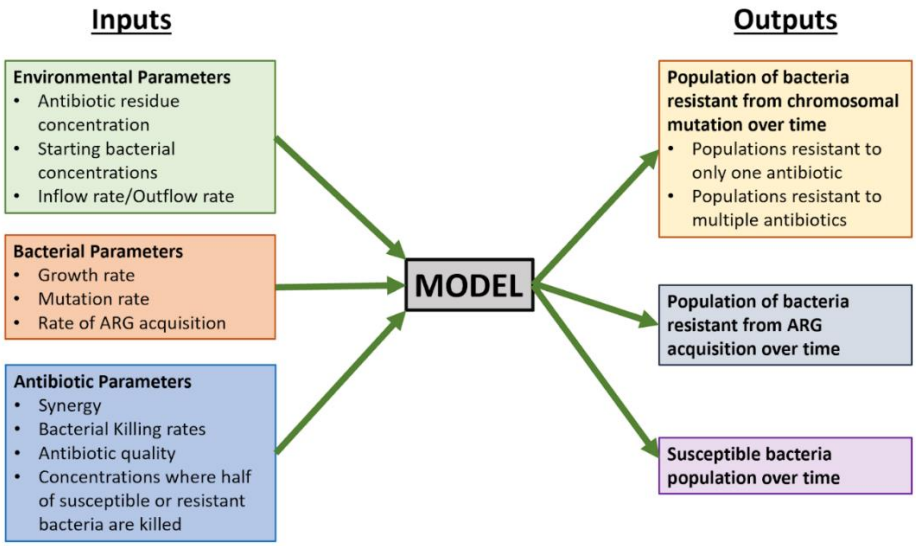

Figure 1. Inputs and outputs for preliminary model of antibiotic resistance development in a single bacterial species in a wastewater setting

The model consists of ordinary differential equations governing the concentration of two antibiotics over time as well as equations modelling the susceptible population (*S)* and populations resistant from chromosomal mutation (R_m) or from HGT (R_p) over time (Eq set 1) (Table 1). Each antibiotic is modelled with terms for the antibiotic residue concentration in the environment (E) and the antibiotic clearance rate (k_e). Each bacterial population is modelled with terms for growth rate (α), which is limited by the carrying capacity (N_{max}), as well as killing rate (δ), and bacterial inflow (g) and outflow (k_T) rates (Table 2). As susceptible and resistant bacterial inflow and outflow rates for wastewater settings are not known, the model assumes bacterial inflow and outflow rates of zero. However, these parameters may be quantified in future field studies. Additional terms for mutation rate under antibiotic pressure (m) and horizontal gene transfer (β) are included for relevant populations. Mutation rates are concentration dependent and modified by a synergy term (syn) with $syn<1$ indicating antagonistic interaction and $syn>1$ indicating synergistic interaction. Parameters governing mutation rate and antibiotic interaction are based on a previously developed model from Michel et al (8). HGT model parameters are adapted from a model of HGT in agricultural waste from Baker et al (12). Other model parameters are based on experimentally derived parameters of *E.* coli in piglet studies (17, 18). We note that the incorporation of all of these parameters, which have previously not been studied at once, into one single model can provide insights into their roles in the emergence and development of resistant populations. These equations were coded and solved in Matlab (Mathworks Inc).

1) $\frac{dC_1}{dt} = E_1 - k_e C_1$

2) $\frac{dC_2}{dt} = E_2 - k_e C_2$

3) $\frac{dS}{dt} = \alpha_S \left(1 - \frac{R_m+R_1+R_2+R_p+S}{N_{max}}\right) S + g_s - k_T S - syn * \delta_{max,1} \left(\frac{C_1}{C_1+C_{S,1}^{50}}\right) S - syn * \delta_{max,2} \left(\frac{C_2}{C_2+C_{S,2}^{50}}\right) S - \frac{\beta S R_p}{R_m+R_p+S}$

4) $\frac{dR_m}{dt} = \alpha_R \left(1 - \frac{R_m+R_1+R_2+R_p+S}{N_{max}}\right) R_m + g_R - k_T R_m - syn * \delta_{max,1} \left(\frac{C_1}{C_1+C_{R,1}^{50}}\right) R_m - syn * \delta_{max,2} \left(\frac{C_2}{C_2+C_{R,2}^{50}}\right) R_m + syn * m_T(C_1, C_2) S + R_1 m_2(C_2) + R_2 m_1(C_1)$

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

6) $\frac{dR_2}{dt} = \alpha_{R,2} \left(1 - \frac{R_m+R_1+R_2+R_p+S}{N_{max}}\right) R_2 + g_R - k_T R_2 - syn * \delta_{max,1} \left(\frac{C_1}{C_1+C_{R,1,2}^{50}}\right) R_2 - syn * \delta_{max,2} \left(\frac{C_2}{C_2+C_{R,2,2}^{50}}\right) R_2 + m_2(C_2) S$

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

Eq Set 1. Sensitive and Resistance Populations under selective pressure from antimicrobial combination therapy including chromosomal mutation and HGT resistance mechanisms

Variable	Definitions
C_1	Antibiotic 1 Concentration (ug/mL)
C_2	Antibiotic 2 Concentration (ug/mL)
S	Susceptible (cells)
R_m	Resistant to Antibiotic 1 and Antibiotic 2 from Chromosomal Mutation (cells)
R_1	Resistant to Antibiotic 1 from Chromosomal Mutation (cells)
R_2	Resistant to Antibiotic 2 from Chromosomal Mutation (cells)
R_p	Resistant to Antibiotic 1 and Antibiotic 2 from MDR Plasmid (cells)
E	Environmental Concentration of Antibiotic((ug/mL)/hr)
syn	Synergy parameter (non-dimensional)

Table 1. Model Variables and Definitions

Parameter	Value	Description	Source
k_e	1.97	Antibiotic Clearance (1/hr)	17
α_S	13.66	Growth rate of susceptible bacteria (1/hr)	17
α_{Rm}	1.9	Growth rate of bacteria resistant from mutation (1/hr)	17
α_{Rp}	2.1	Growth Rate of bacteria resistant from plasmid (1/hr)	17, 12
N_{max}	10^{11}	Carrying capacity (cells)	12
g_s, g_{Rm}, g_{Rp}	0	Bacterial Influx rates (cells/hr)	N/A
k_T	0	Bacterial Efflux rate (1/hr)	N/A
δ_{max}	27.14	Bacterial Killing rate (1/hr)	17
C_S^{50}, C_R^{50}	49.1, 1000	Antibiotic concentration where the killing action is half its maximum value (ug/mL)	17
$m_1(C_1)$	$2.35*10^{-6}$ (C_1)	Mutation frequency under Ab1 (1/hr)	8
$m_2(C_2)$	$2.35*10^{-8}$ (C_2)	Mutation frequency under Ab2 (1/hr)	8
β	.001	Gene transfer rate = (1/hr)	12

Table 2. Model Parameter Values and Descriptions

Results and Discussion

Model Validation

Our model was first validated by comparing our results with known scenarios under low antibiotic pressure. In the scenario of no horizontal gene transfer, as expected, only mutational resistance was observed (Figure 2a). Likewise, for a scenario in which there is no chromosomal mutation (Chromosomal Mutation Rate = Initial Mutant Population = 0), only resistance from horizontal gene transfer was observed and due to a higher growth rate for MDR plasmids, this resistance overtook the susceptible population at a higher rate (Figure 2b). Furthermore, no resistance was observed in the case of no antibiotic residues, initial resistant population, or mutation rate.

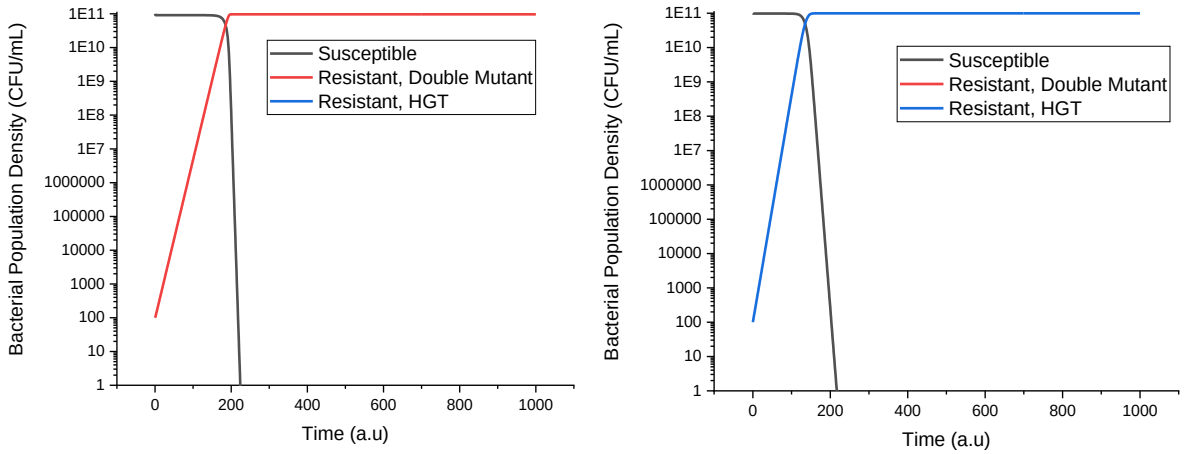

Figure 2. Sensitive and Resistant Populations under **a.)** resistance only from chromosomal mutations; **b.)** resistance only from the horizontal gene transfer of resistance conferring plasmids (MDR plasmid)

Very low antibiotic residue concentration can promote resistant population growth

We then turned our attention to simulating realistic scenarios in wastewater and sewage. We studied the effects of changing antibiotic residue concentration on the development of antibiotic resistance (Figure 3). Low subinhibitory concentrations (0.25 - 1 ug/ml), as would be present in wastewater settings (4, 5), were studied. These concentrations are an order of magnitude lower than the minimum inhibitory concentrations of each of the two antibiotics, which is 98.2 ug/ml. Increased antibiotic residue concentration increased the rate of resistance development and decreased the time to resistant population dominance (defined as time when $R_m + R_p < S$). This is in agreement with several prior studies linking subtherapeutic antibiotic levels with both chromosomal resistance mutation development and horizontal gene transfer (6, 7, 9-12). Furthermore, horizontal gene transfer was observed to be the dominant mode of resistance, in agreement with patterns reported in studies of *E. coli* in other settings (19).

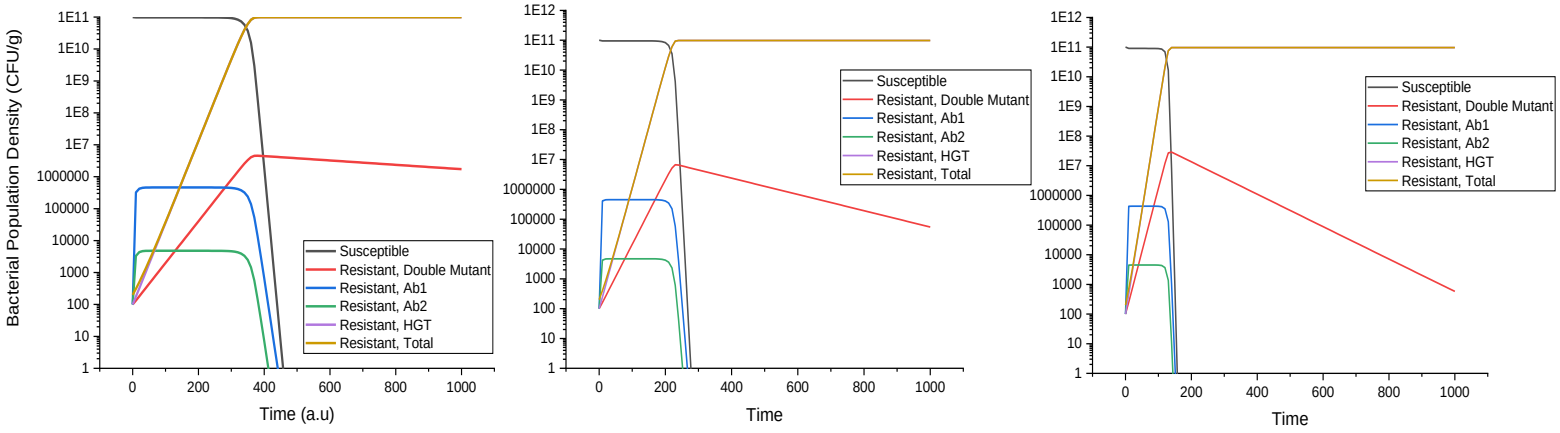

Figure 3. Sensitive and Resistant Populations under Antibiotic Residue Concentrations of a.) 0.25 ug/ml Antibiotic 1 + 0.25 ug/ml Antibiotic 2; b.) 0.5 ug/ml Antibiotic 1 + 0.5 ug/ml Antibiotic 2; c.) 1 ug/ml Antibiotic 1 + 1 ug/ml Antibiotic 2

Increase in Horizontal Gene Transfer Rate increases resistance at low concentrations

Additionally, the effect of horizontal gene transfer was modelled by increasing the horizontal gene transfer rate, β (Figure 4). Prior studies have indicated that HGT rate is a less significant driver of resistance frequencies (20). However, our model shows that at very low subinhibitory concentrations of antibiotic, increased HGT rate significantly decreases the time to resistant population domination. This result indicates that increasing HGT rate allows resistance to be acquired at very low selective pressures where there are infrequent chromosomal mutations. This result is significant as it shows that decreasing antibiotic levels in wastewater to low levels is not sufficient in preventing the growth of resistant populations. At very low concentrations of antibiotics, the HGT rate of antibiotic resistance genes in wastewater can significantly the proliferation of resistant populations in bacteria with high gene transfer rates. As the HGT rates of the multitude of bacterial species in wastewater is not a commonly monitored parameter, this may be a factor to be mindful of in wastewater surveillance, as these rates can have a significant effect on the evolution of resistant population reservoirs even at low antibiotic concentrations.

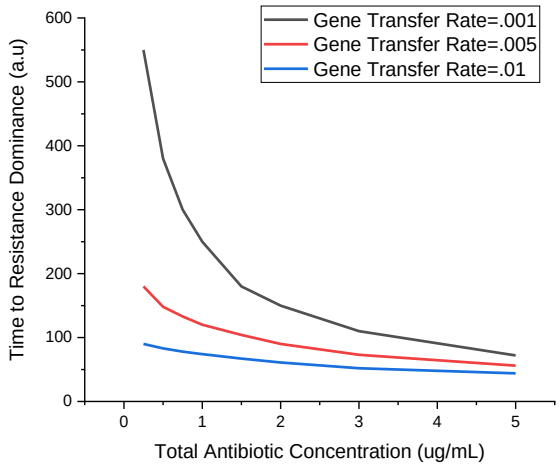

Figure 4 Effect of Horizontal Gene Transfer rate on Time to Resistant Population Dominance for Subinhibitory Antibiotic Residue Concentrations (defined as time when $R_m+R_p< S$)

Effect of Bacterial Killing Rate

Because wastewater settings can often have low concentrations of antibiotics from various polluting factors (4, 5), we probed the specific effects of antibiotic residues on the development of resistant populations. Individual antibiotics have differing killing rates based on factors such as their modes of action. Thus, we investigated the effect of bacterial killing rate on resistant population growth (Figure 5). Increased killing rate was observed to decrease the time it takes for the resistant population to dominate. This result is in agreement with previous studies showing increased selective pressure from antibiotics at subinhibitory concentrations can increase resistance development (6, 7).

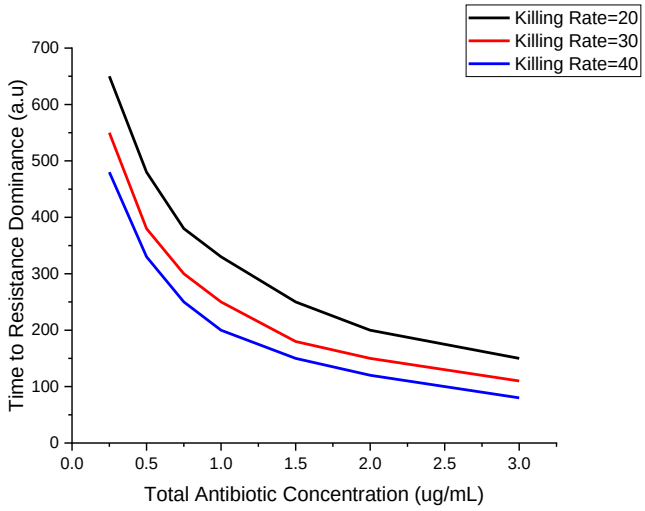

Figure 5 Effect of Bacterial Killing Rate on Time to Resistant Population Dominance for Subinhibitory Antibiotic Residue Concentrations (defined as time when $R_m+R_p< S$)

Synergistic antibiotic interactions increase resistant population growth

The interaction between two antibiotics have previously been shown to affect resistance acquisition (21). Synergy is the interaction of multiple drugs to have a greater killing action than the sum of their parts. This synergy has also been shown to increase the likelihood of resistance acquisition at subtherapeutic doses (8). However, the effects of antibiotic interaction on the growth of resistant populations in wastewater settings, where many antibiotic residues can be present, has not previously been observed or modeled. In order to fill this gap, we have probed the effects of synergistic effects between antibiotic residues on the system by varying the synergy parameter, *syn*. Results from our model show that increased synergy between different antibiotics resulted in a decrease in the time to resistant population elimination at suprainhibitory concentrations (Figure 6a). This result may initially seem counterintuitive, but is in fact consistent with previous studies. This is because the synergistic action between the antibiotics increases the killing action to the point where it is effective on the resistant populations at lower concentrations. Hence, the synergy between the two antibiotics allows for greater bactericidal activity at lower concentrations. However, at sub-inhibitory concentrations, increasing synergy between antibiotics decreased the time to resistant population dominance (Figure 6b). This is in agreement with other models in which synergy between antibiotics was observed to increase resistance acquisition (8). Our model further shows that these effects of synergy are observable even with low levels of antibiotic, such as the antibiotic residue concentrations present in wastewater. While the effects of antibiotic interaction on resistance acquisition have been previously modeled, low subinhibitory antibiotic concentrations such as those found in wastewater have not been well studied and are critical for understanding resistance development in wastewater settings (16).

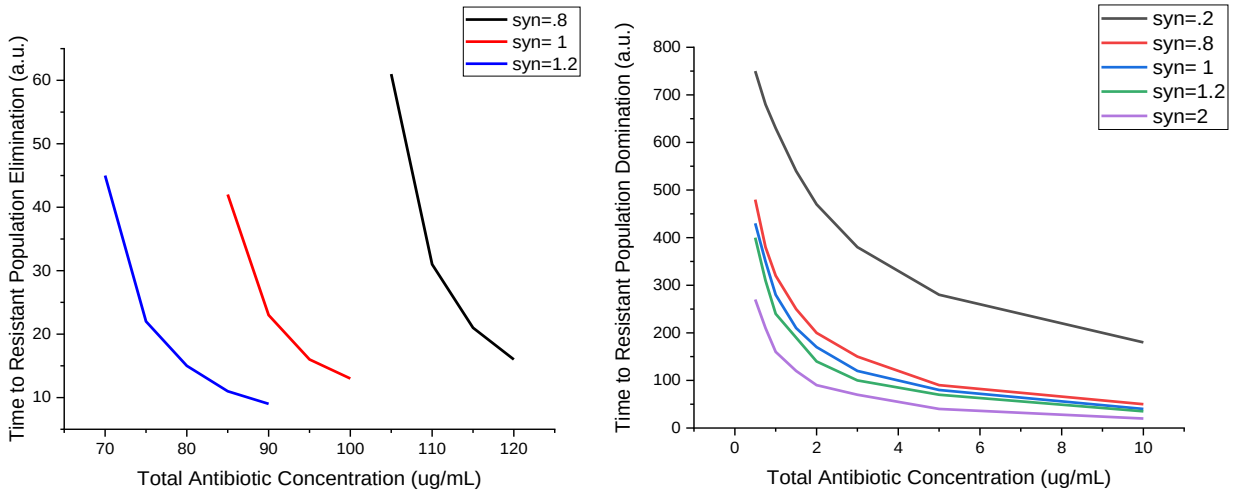

Figure 6 Effect of Synergy of Antibiotic Combinations on **a.)** Time to Resistant Population Elimination for Suprainhibitory Antibiotic Residue Concentrations **b.)** Time to Resistant Population Dominance for Subinhibitory Antibiotic Residue Concentrations (defined as time when $R_m+R_p< S$)

Conclusion

Though we have been able to draw a number of conclusions on the relative effect of a variety of factors affecting resistance development in wastewater, we note that our model does have limitations. First, our model is based on parameter values from literature, not all of which have been experimentally validated against wastewater conditions. Additionally, our model does not account for multiple bacterial species or a multitude of antibiotic residues, as would be present in a complex environment like wastewater. Improvements to the accuracy and robustness of the model could be made through parameter initialization from field data, and experimental validation of model inputs and outputs under specific wastewater conditions.

That said, despite these limitations, our model provides new, and important quantitative insight on the evolution of resistant bacterial reservoirs. It also provides an integrated framework to incorporate several aspects of resistance acquisition and growth previously lacking from models focused on AMR in wastewater settings. In terms of results, we have shown that HGT rate can be a significant driver of resistant population growth at very low antibiotic concentrations. This indicates that HGT rates of bacteria in wastewater may be important to monitor in addition to antibiotic residue concentration. We have also been able to show that synergy between the antibiotic residues present in wastewater can increase the rate of resistant population growth, even at the low concentrations present in wastewater. Thus, antibiotic residues in wastewater may pose a greater risk than might be expected without taking these antibiotic interactions into consideration. This has important implications for determining acceptable antibiotic levels in wastewater post treatment, as determining levels without considering antibiotic interactions may lead to overestimating the permissible level of antibiotic and allow for the proliferation of antibiotic resistant bacterial populations. This model can be adapted for use as a prediction tool for public health policy makers and be used to predict resistant population emergence in different sewage and wastewater conditions. Additionally, it can be expanded to be used to model different resistant outbreak prevention strategies in sewage and wastewater treatment.

Author Contributions

I. Sutradhar designed the model and analyzed the data. C. Ching, D. Desai provided guidance on model design and verification. M. Suprenant provided guidance on data analysis. I. Sutradhar and M. H. Zaman wrote the article.

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