An Agent-Based Model Exploring Antibiotic Resistance in Wastewater Systems

Pranavi Rohit
University of Washington Seattle, United States of America

Abstract:- Antibiotic resistance poses a critical global health threat as bacteria evolve to withstand antibiotics. Apart from severely impacting individuals, often patients of antibiotic-resistant diseases, antibiotic resistance also uniquely affects communities given their relation to wastewater systems. This impact is particularly noteworthy in connection to wastewater systems, which remain integral to urban areas, where the purification of wastewater is essential. Unfortunately, these systems are acknowledged as notable reservoirs for antibiotic-resistant bacterial growth. The potential entry of a resistant pathogen into the community post-wastewater treatment can spark outbreaks, impacting thousands within a city. Recognizing the urgency to comprehend antibiotic resistance emergence in detail and work towards prevention, this study employs agent-based modeling. This approach is crucial in light of the challenges associated with collecting real-world data, including time, expense, and logistical constraints. The developed model provides valuable insights into bacterial population dynamics and the mechanisms fueling antibiotic resistance, encompassing phenomena such as horizontal gene transfer and chromosomal mutations. Multiple simulations conducted with the model confirmed previous findings and uncovered insights into the impact of bacteria population sizes at varying antibiotic concentrations. These insights have the potential to extend to applications in the real world, including added filtration systems and better legislature around the disposal and usage of antibiotics.

Keywords:- Antibiotic Resistance, Agent-based Modeling, Wastewater Systems.

I. INTRODUCTION

Antibiotic resistant diseases are responsible for 750,000 deaths annually [1]. These resistant strains emerge and circulate when bacteria cease to respond to antibiotic treatments, as their genome has evolved over time to resist death by the antimicrobial treatment [2]. In the past decade, antibiotic resistance has spread through numerous bacteria colonies at an alarming rate, rendering staple antibiotics ineffective to a growing number of diseases. Little data is available about the growth and dynamics of these bacteria—data that is essential to preventing the origin and transmission of resistant disease strains in our world. One of the largest issues in this field is that collecting such experimental data is extremely time consuming and expensive. However, utilizing models that simulate these bacterial interactions offer a solution to many of the roadblocks faced in obtaining this data.

Mathematical and computational modeling can utilize known bacterial growth, death, interaction, and mutation rates to make predictions about antibiotic resistance. In addition to this, modeling can provide similar data to experimental data, while being far cheaper to collect. A wide range of control measures to prevent the growth of resistance in bacterial colonies can be explored with modeling as well, affording the researcher greater control and flexibility over the data they collect. These programs can be a powerful resource in predicting antibiotic resistance in numerous future environments.

Wastewater systems are one example—they are habitats where antibiotic resistance proliferates and spreads between species [3]. Antibiotics often enter wastewater systems through deposition of fecal matter, improper disposal of medication, and the runoff from manure and other waste-based fertilizers [4]. As a result, the wastewater system becomes a reservoir for bacterial exposure to antibiotics, increasing the evolutionary fitness of resistant bacteria. These resistant bacteria can go on to infect human and non-human hosts, potentially feeding into an outbreak resistant to all current antibiotic treatment.

However, many environments with high potential of harboring growth of antibiotic resistant bacteria are unfit for data collection. There are huge difficulties in collecting such data at microscopic levels in the real-world, rendering the task close to impossible. This leaves a gaping hole in our understanding of antibiotic resistance, as well as difficulty in predicting the effectiveness of control measures that may slow its spread. From a healthcare perspective, this leads to higher medical costs, prolonged hospital stays, and increased mortality for the patients who contract infections stemming from resistant pathogens. Agent-based models provide a way to predict population futures using the limited known data. Previously, models grounded in mathematics have been used to quantify the growth of antibiotic resistant bacteria [5]. Many of these studies utilize differential equations to track characteristics of a bacterial population over time, including bacterial growth and death, antibiotic resistance acquisition, and antibiotic pressures.

However, these studies are often flawed in their representation of interactions between bacteria, many of which are responsible for the spread and acquisition of antibiotic resistance within a bacterial colony. The model presented here more accurately captures the spatial interactions and unique individual properties within a

bacteria colony to predict the establishment of resistance with greater accuracy.

II. LITERATURE REVIEW

Antibiotic resistance is acquired by bacteria through two common mechanisms: chromosomal mutations during DNA replication, or horizontal gene transfer.

➤ *Horizontal Gene Transfer*

Horizontal gene transfer (HGT) is the transfer of genetic material from one organism to another, independent of genetic inheritance through reproduction. Three mechanisms allow for HGT to occur between bacteria: transformation, conjugation, and transduction [6]. Transformation begins when bacteria release genomic fragments, called plasmids, into the environment. These plasmids are picked up by other bacteria who integrate them into their own genetic material. Conjugation refers to the direct exchange of genes from one bacteria to another when they are within close proximity. Finally, transduction involves bacteriophages, or viruses that infect bacteria, moving genes from bacteria to bacteria.

➤ *Current Models and Limitations*

A recent mathematical model synthesizes known information about bacterial growth, HGT, and antibiotic concentrations in wastewater systems using ordinary differential equations [5]. The model incorporates multiple bacterial, environmental and antibiotic parameters. Overall, it seeks to gain a better understanding about the susceptible and antibiotic resistant bacterial population when exposed to one or multiple antibiotics. It offers quantitative values for the drivers of resistant population growth—a novel approach in the field at the time of publication.

However, as the use of this model and others like it have grown, clear limitations emerge. These models rely on parameter values, drawn from literature and not experimentally validated in wastewater systems, which likely decreases the accuracy of the model. In addition, only a small subset of antibiotics and bacterial species are incorporated, resulting in an inaccurate representation of a wastewater environment, which is likely teeming with a multitude of both.

Furthermore, this model relies on differential equations, where homogeneous mixing in the environment is a core assumption. This implies that all bacterial interactions are equally likely regardless of location or species—an inaccurate and potentially destructive assumption. Lastly, these models are deterministic, meaning the randomness of unique individual attributes and random interactions between bacteria are ignored, misrepresenting the uncertainty of model results.

➤ *Advantages of Agent-Based Models*

Using an agent-based model for this study can mitigate many pitfalls identified above. These versatile models depict the unique properties of individuals in the system, interactions between said individuals, and environmental factors, while respecting the spatial limitations of all interactions. Centered around stochasticity, agents—in this instance, bacteria—in the model are randomly assigned certain attributes and given a set of simple rules to follow, with each rule influencing a key behavior. The model adheres to a simple algorithm at each time step, representative of a unit of time, terminating the program after a certain number of steps have passed. At every time step, agents assess their individual situation and follow their set of simple rules, which may be dependent on that agent's unique attributes. The actions of individuals in the simulation mirror their behavior in the real world closely in highly accurate agent-based models.

III. PURPOSE

➤ *The Study Seeks to Expand our Understanding of Bacterial Resistance in Wastewater Systems by Answering the following Questions:*

• *Bacterial Populations:*
Will the reduction of the bacterial population or antibiotic concentration have a greater impact on decreasing antibiotic resistance?

• *Antibiotic Influx Frequency:* Can short influxes of antibiotics, as opposed to continuous inflow, reduce the occurrence of resistance?

• *Bacteriophages:* Can bacteriophages be used to reduce antibiotic resistance in wastewater systems?

IV. METHODOLOGY

➤ *Model Overview*

This work utilizes the Mesa library [7] in Python to implement an agent-based model[1]. Its purpose is to simulate a wastewater system with bacteria experiencing antibiotic pressures, where each bacteria has unique attributes and interacts locally with other bacteria in the environment.

The model initializes a world with a width and length of 25 units, with the measurements of each patch, referenced as a grid square, directly proportional to the distance an agent, a bacteria, can travel within one hour—the length of a model step. Global properties of the model, which apply to all agents within the model, include inflow and outflow rates in addition to the antibiotic concentration of the system. These are designated as global properties as they would apply equally to all bacteria in a wastewater system, hence their establishment within the agent-based model.

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[1] Complete program code for the model can be found at the specified, publicly accessible repository:

https://github.com/pranavi-rohit/antibiotic-resistance-in-wastewater-systems/tree/main

> *Agent Properties*

The agents in the model reflect bacteria with similar properties to *Escherichia coli*, but their properties and interactions are general enough to encompass the behavior of most bacterial species. Each bacteria can be categorized by its susceptibility to the two antibiotics incorporated in the model (i) susceptible to both, (ii) resistant to one or the other, or (iii) resistant to both. Susceptible bacteria reproduce faster than resistant bacteria because they do not have to replicate an additional part of their genome—the plasmids that house resistant genes. While susceptible bacteria have a reproductive advantage, as they do not have to expend energy replicating these plasmids, they also have a higher chance of death when exposed to antibiotics [5].

Agent properties are initialized with multiple unique properties, beginning with their resistance to antibiotics 1 and 2. Agents have a 4.875% chance of having resistance to antibiotic 1 or 2, and less than a 0.25% chance of having resistance to both at model initialization. If they initialize with neither, they are classified as susceptible and make up approximately 90% of the bacteria in the initialized population. However, the proportion of a population that initializes with resistance varies from simulation to simulation, as this value is dependent on a random number generator embedded within the program.

> *Agent Interactions*

One model step, a time step, represents one hour in the real-world, with numerous agent interactions occurring during each time step. In a given time step, each agent has the opportunity to move in a random-walk to any neighboring grid cell. Agents can move to any of their surrounding grid squares or stay within the same patch, with this movement determined by a random generator. The width and length of a grid is scaled approximately to the distance bacteria can travel in one hour: 1 inch.

If a resistant bacterium is located on the same patch as a susceptible bacterial agent, horizontal gene transfer can occur. A bacterium with resistance to any antibiotic(s) will randomly select a cellmate, any other bacteria within the same patch. With some probability of failure, accounted for by a random number generator in the program code, the other bacteria may now gain resistance to the antibiotic associated with the original bacteria. The variable denoting their resistance to the said antibiotic will change to reflect this updated resistance status.

Though not yet incorporated, a model extension includes adding two additional agent types: bacteriophages and plasmids. These agents would account for transduction and transformation, the other two mechanisms through which horizontal gene transfer occurs [6]. Once plasmids are initialized, there is a small probability agents with any type of resistance can expel a plasmid into the same grid patch. Plasmid movement mirrors bacterial movement, and a plasmid will inherit its resistance from its parent bacteria. Any other bacteria without this resistance, once within the same patch as the plasmid, has a chance of taking it up and therefore gaining a new form of antibiotic resistance.

Transduction involves adding a second agent, bacteriophages, to facilitate plasmid movement. When resistant agents are within the same patch as bacteriophages, there is a chance bacteriophages can transfer a plasmid from one bacterium to another, following the same movement function as bacteria. If in a patch with a bacteria not already resistant to the antibiotic, there is a chance the bacteriophage will transfer the plasmid to that bacteria. This results in the bacteria becoming resistant to an antibiotic.

In addition to acquiring ABR through HGT, agents can gain resistance through chromosomal mutations. At any given time, an agent has a $2.35 \cdot 10^{-6}$ chance of acquiring resistance to antibiotic 1 and a $2.35 \cdot 10^{-8}$ chance of acquiring resistance to antibiotic 2, should they not already have this property [5]. This function is independent of agent reproduction.

A reproduction function governs the initialization of new bacteria at every model step, ensuring each new bacteria mirrors their parent's properties, most notably antibiotic susceptibility. The same function applies to any agent, but the distinction between susceptible and resistant bacteria stems from their reproduction rates and probability of death when exposed to antibiotics. Susceptible bacteria produce two to four offspring every hour, while resistant bacteria produce one to three, and in a wastewater system free of antibiotics, susceptible bacteria have an evolutionary advantage due to higher reproductive rates [5].

A carrying capacity of 625,000 bacteria[2] is set for every simulation. Once the population crosses this threshold, reproduction for all bacteria ceases until the population lower than its carrying capacity again. This carrying capacity provides an opportunity for the current resistant bacteria to stabilize within the population, since the reproductive advantage of susceptible bacteria has less value when the population is already at carrying capacity. Both susceptible and resistant bacteria are offered a window for reproduction, and the proportion of the bacterial colonies are expected to vary as well.
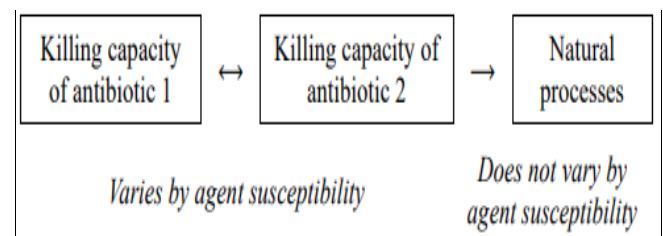

Fig 1 Process to Identify Cause of Death for Agent, with the Potential to be any Three

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[2] This value is not experimentally validated, rather a constraint of the computing power available. With more agents within the simulation, running a simulation with an adequate amount of model steps takes and unreasonable period of time.

Bacterial death becomes a factor after a bacterium has completed at least one cycle of reproduction. As referenced in Fig. 1, two causes of death are incorporated into the model: death due to either antibiotic and death due to natural processes.
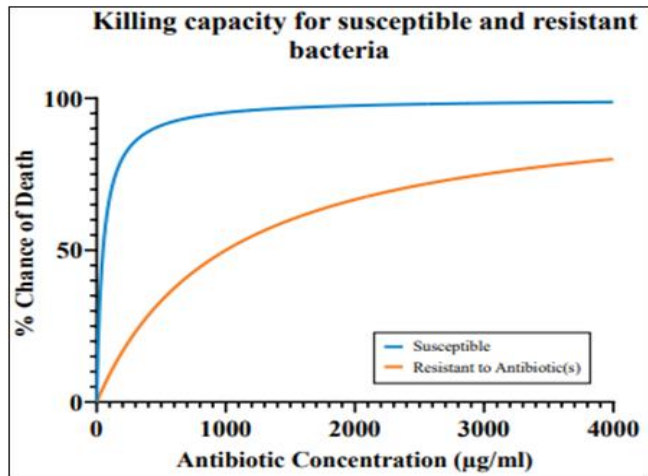

Fig 2 Chance of Bacterial Death, Contingent on the Antibiotic Concentration Present and Classification of Bacteria. Modified Hill's Equation Graphed for Both Groups

At every time step, bacteria are exposed to all three, but one of the antibiotics first. Which antibiotic they are "exposed to" first is purely random, as to ensure data is not skewed by the order of this process. If a bacterium does not die from either antibiotic, it can die from natural processes. Natural death rates are constant across all bacteria, regardless of susceptibility. The likelihood of death every hour, for any agent, is 0.4%. This value was derived from the average life span of bacteria in cattle slurry, often a common deposit in wastewater systems [8].

The probability of death in a given hour due to antibiotics is described by a non-linear function of: (i) the bacteria's susceptibility to a given antibiotic, (ii) the given antibiotic's killing capabilities, known as half killing capacity, and (iii) the current antibiotic concentration.

The half killing capacity parameter denotes the concentration at which the antibiotic has a 50% chance of killing bacteria in a given hour. The function is a simplified version of the Hill equation [5].

$$P = \frac{[C]}{[C] + K_d}$$

In this context, P is the probability of bacterial death, C is the concentration of the antibiotic, and K_d is the constant of resistance. For susceptible bacteria, K_d is 49.1 μg/mL while in resistant bacteria K_d is 1000 μg/mL [5]. Lower values of K_d indicate that the bacteria are more susceptible to the antibiotic, while higher values indicate greater resistance.

A. Model Execution

To initialize a model simulation, six initial conditions must be established: initial bacterial population, grid size (both width and length), concentrations for both antibiotics, and finally, the number of model steps (hours). Other parameters, such as the population's carrying capacity, must also be characterized. The rates and data for multiple parameters used in the first model are derived from the mathematical model presented in the literature review and can be found in Table 1. This model exhibits a stable and constant rate of antibiotic residue, as the concentration of any antibiotic does not change over time.

Table 1 Model Initialization and Parameters

Description	Value	Units	Reference
Model initialization			
Resistant to antibiotic 1	≈ 4.875	%	–
Resistant to antibiotic 2	≈ 4.875	%	–
Resistant to antibiotic 1 & 2	≈ 0.25	%	–
Model parameters			
Bacterial carrying capacity	$6.25 \cdot 10^5$	bacteria	–
Horizontal gene transfer rate	$0.01 – 10^{-9}$	bacteria/hour	[8]
Susceptible division rate	2–4	bacteria/hr	[8]
Resistant division rate	1–3	bacteria/hr	[8]
Natural death rate	0.4	bacteria/hr	[5]
Susceptible half killing capacity	49	μg/mL	[5]
Resistant half killing capacity	1000	μg/mL	[5]

Stable antibiotic concentration	Smaller initial bacterial population	Varied antibiotic concentration
Bacteria population initialized at 6250	Bacteria population initialized at 3125, half of control population	Bacteria population initialized at 6250 Antibiotic concentration starts at zero, increases at a constant rate every hour
Antibiotic concentration stays constant throughout simulation	Antibiotic concentration stays constant throughout simulation	Reaches the designated concentration by end of simulation
High Medium Low None	High Medium Low None	High Medium Low

Fig 3 A Total of 11 Simulations Completed, Assigned to One of Three Overarching Categories

Though not an exhaustive list, multiple conditions can be pursued with this agent-based model in addition to the stable antibiotic concentration model. They are (i) low, medium, and high antibiotic pressure, (ii) small starting bacterial population, and lastly, (iii) varying antibiotic concentration.

Exploring the effects of a smaller bacterial population and varying concentrations of antibiotics over time have the greatest application to the real world, and are relatively easy to implement in the existing model. Adding a greater number of antibiotics or bacterial species is indicative of a real wastewater system, but requires a large addition of code to the existing model. However, with more time and access to more computational power, they prove to be worthwhile future scenarios to pursue. The following analyses explore bacterial resistance under the following scenarios: (i) low, high, and medium antibiotic pressure, (ii) small starting bacterial population, and (iii) varying antibiotic resistance.

Table 2 Antibiotic Concentration Classifications

Classification	Concentration (µg/mL)
None	0
Low	25
Medium	50
High	500

Antibiotic concentration is one of the greatest evolutionary pressures leading to antibiotic resistance, and studying it in relation to each scenario is essential to understand more about bacterial populations in a variety of scenarios. Data collected during each simulation includes the proportion of each bacterial population, with the populations characterized by their antibiotic susceptibility. This same measure is also tracked across all bacterial deaths. As the agents within the model interact for a defined time frame , the acquisition of antibiotic resistance—the percentage of the total bacterial population that gained antibiotic resistance from HGT or chromosomal mutations—is also logged over time.

V. RESULTS AND DISCUSSION

The following section explores how the three scenarios [3]presented above impact the growth of resistant bacterial populations over a 24-hour simulation period. Note that this is a small period of time due to the computationally intensive model and time constraints of this program, but demonstrates the general dynamics seen within a wastewater system.

➢ *Smaller Initial Bacterial Population*
The simulations within this scenario are initialized in a 25 by 25 grid with an initial bacterial population of 3125. This is half of the initial bacterial capacity, 6250. Four simulations with these parameters are conducted, each with different antibiotic concentrations to better understand how a smaller bacterial population is affected.

In the control simulation with no antibiotics present, a large increase in the susceptible population is observed at the beginning of the simulation, but a slow decline in the population begins from hour 4. This is inversely tied to the proportion of antibiotic resistant bacteria, which decrease rapidly within the first hours but increase gradually after. At the end of 24 hours, the susceptible bacteria population is 97.3% of all bacteria present in the system. Aligned with the killing capacity of antibiotics when none are present, the probability of death from antibiotics is 0% for both populations. Susceptible and resistant populations have an equal chance of death from natural processes, but as reproduction rates are lower for resistant bacteria, an overall increase is seen in the proportion of the susceptible bacterial population.

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[3] Complete data for every simulation can be found at the specified, publicly accessible repository:

https://github.com/pranavi-rohit/antibiotic-resistance-in-wastewater-systems/tree/main/simulation_data
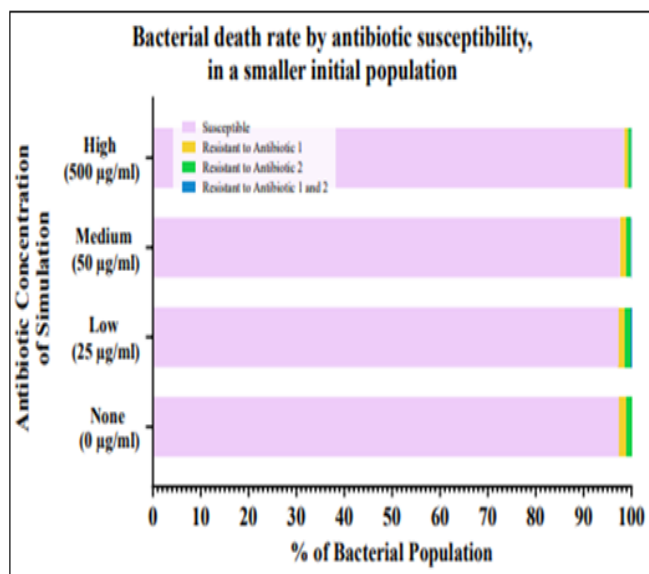
Fig 4 Comparisons of the Proportion of Susceptible and Resistant Bacteria at the of 24 Hours, in Across Four Simulations with Different Antibiotic Concentrations

In contrast, with a stable, low antibiotic concentration of 25 μg/mL per antibiotic, the susceptible bacterial population peaks slightly later (in hour 5) and begins to decrease shortly after. This decrease becomes much more pronounced by the last three hours of the simulation, reducing the proportion of susceptible bacteria to 87.8%. This is the lowest final proportion of susceptible bacteria in any of the scenarios assessed, likely amplified by the larger initial population— the timeframe for the bacterial population to reach carrying capacity decreased, reducing the beneficial reproductive advantage susceptible bacteria had.
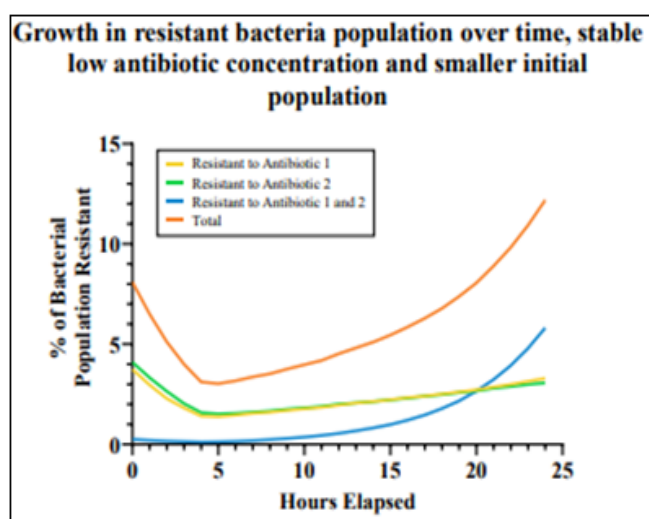

Fig 5 Increasing Antibiotic Resistance for a Smaller Initial Bacterial Population

Exposed to a medium antibiotic concentration, changes to population of susceptible bacteria continue in a similar pattern. However, the scenario terminates with a greater proportion of susceptible bacteria than in the previous scenario, which had a low concentration of antibiotics. Susceptible bacteria still have a much higher chance of dying

from antibiotics, but resistant bacteria now have higher death rates due to the increased pressure from antibiotics than experienced at the low concentration. This causes a shift in the final proportions, but resistant bacterial dominance is less pronounced than previously seen at the low antibiotic concentration.

When both populations were exposed to a total of 500 μg/mL of antibiotics (the highest concentration), the proportion of susceptible bacteria at the end of simulation was greater than any other concentration acting on a small bacterial population. Again, this is likely because both types of bacteria have an almost equal chance of dying from antibiotics—simply because the concentration is so high. This pushes the bacterial population well below carrying capacity, allowing the reproductive advantage of susceptible bacteria to quickly grow their population, even with a high mortality rate from antibiotics.

➤ *Stable Antibiotic Concentration*

Simulations conducted as part of this scenario were initialized in a 25 by 25 grid with an initial bacterial population of 6250 over the span of 24 hours. Though there is variation in the ending proportions of bacterial populations due to stochasticity, the overarching trends about resistant and susceptible populations still hold true. A high starting value for susceptible populations, peaking early and a gradual decline (with a difference in gradient, depending on the antibiotic concentration) is observed in this scenario as well. The inverse, with the resistant population declining sharply early into the simulation and then eventually increasing also appears. The largest difference between respective simulations occurs with a high antibiotic concentration. The simulation with no antibiotics present had the highest proportion of susceptible bacteria by approximately 2.3%, in comparison to the simulation with the highest concentration of antibiotics. With the smaller bacterial population, the proportion of susceptible bacteria was slightly higher in the high antibiotic concentration simulation.

➤ *Varying Antibiotic Concentration*

The last scenario considers varying the concentration of antibiotics, which may represent discrete influxes of wastewater inflow containing antibiotics as opposed to a continuous concentration of antibiotics. In this scenario, antibiotic concentration is initialized at zero, and increases at a constant rate every hour—so as it reaches the designated concentration low, medium, or high concentration noted in Table 2—by hour 24.
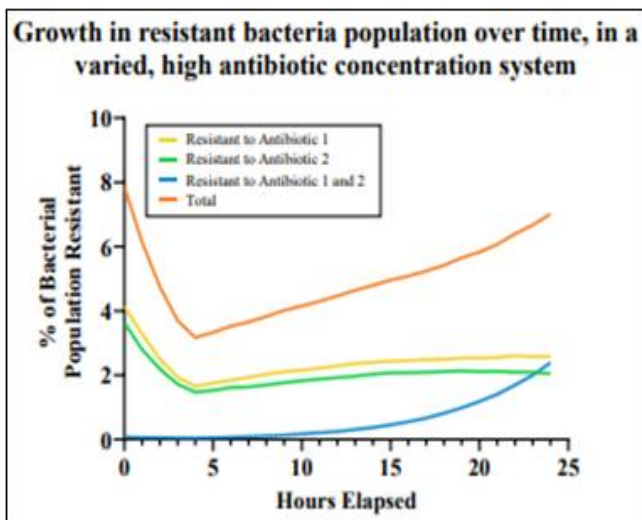
Fig 6 Increasing Antibiotic Resistance in a Bacterial Population Exposed to High Levels of Antibiotics, with Exposure Increasing over Time

The greatest proportion of susceptible bacteria exists within the initial hours of the simulation, and begins to decrease after hours 4-6 in low, medium, and high varied antibiotic concentrations. The lowest proportion of susceptible bacteria, and therefore highest proportion of resistant bacteria, (out of all the scenarios involving varied antibiotics) is produced when bacteria are exposed to the high antibiotic concentration. This is due to the rapidly increasing chance of death associated with susceptible bacteria—once the simulation passes hour 4, the antibiotic concentration easily shifts from a lower killing capacity—essentially chance of death—to high one for susceptible bacteria. This change is present within the resistant population as well, but is far more gradual and allows them to evade a high probability of death until the final hours of the simulation.

➢ *Scenario Overview*
Out of all the scenarios tested, a smaller bacterial population exposed to no antibiotics is most preferable. Successful scenarios end with a very low resistant bacteria population and ideally, demonstrate decreasing resistance over time in hopes to slow the spread of antibiotic resistance.

This simulation was successful because antibiotics were not present to provide an evolutionary advantage to resistant bacteria. This condition produces the greatest proportion of susceptible bacteria after 24 hours, more so than any other antibiotic state, because of the difference in reproduction rates for each group. Susceptible bacteria reproduce at a greater rate than resistant bacteria, hence why their population rapidly increases within the first hours, peaks, and then stabilizes. Susceptible bacteria continue to proliferate until the system reaches carrying capacity and the population comes close to stabilizing.

Second, a smaller initial population produces a greater proportion of susceptible bacteria than a larger initial population. Should the carrying capacities of the both systems are same as assumed in this study, the smaller bacterial population has a greater window of time to reproduce because they will not reach the carrying capacity as rapidly. Paired with their faster reproduction rate, this causes a large uptick in the proportion of the population and a decrease in the resistant population.

Another intriguing conclusion was gleaned from the simulation exposing the smaller initial and control bacterial populations to a high antibiotic concentration. The population of resistant bacteria in an established wastewater system was predicted to decrease even more when exposed to a very high concentration of antibiotic residue, than if all antibiotics in the system were eliminated. The death rate is almost the same for both the susceptible and resistant populations, greatly reducing the advantage of resistant bacteria, but the reproduction rate is greater for susceptible bacteria. Many bacteria are dying from antibiotics, opening a window for both populations to reproduce below the population's carrying capacity. The reproductive advantage of susceptible bacteria quickly drives their population count up, out-competing resistant bacteria as time passes.

However, it is crucial to remember there are a multitude of bacteria and types of antibiotics present in wastewater systems—each bacteria and antibiotic has their own unique characteristics, as well as killing capacities. Predicting the necessary concentration and type of antibiotics to employ— in hopes to decrease the population of antibiotic resistant bacteria as much as possible—could be a risky and lengthy process, requiring great amounts of care and attention to detail.

One of the most unfavorable scenarios is within the same, small initial population, but initializing the population with a low antibiotic concentration. The low amount of antibiotic concentration is not enough to severely harm the resistant bacterial population occupying the reservoir, but leads to a high mortality rate in the susceptible population. As seen in Fig. 2, susceptible bacteria have a very high probability of death in a given time step at this concentration, while resistant bacteria are most likely to survive. With more time passing, and far more new generations of resistant bacteria inhabit the system, the evolutionary fitness of resistant bacteria increases greatly.

VI. CONCLUSION

Grounded in agent-based modeling, this study gained valuable insight about the growth of antibiotic resistant bacteria. Using a computational model, agents represented the attributes and behavior of bacteria within a wastewater system—recognized as an environment where a large amount of bacteria can gain antibiotic resistance. Three scenarios were tested—the constant and varying presence of antibiotics, in addition to a smaller initial bacteria population. Under each scenario, simulations with a small, average, or high concentration of antibiotics were run to compare with the control model with no antibiotics.

The first is (i) situations where susceptible and resistant bacteria are affected equally by antibiotic residue produce the greatest proportion of susceptible bacteria.

The next is (ii) a smaller initial population produces a greater proportion of susceptible bacteria than a larger initial population if the carrying capacities of both populations are identical.

The third is (iii) the constant presence of antibiotic residue, at a low value, promotes the greatest amount of resistant bacteria growth.

The fourth is (iv) the population of resistant bacteria in an established wastewater system will decrease even more when exposed to a very high concentration of antibiotic residue, than if all antibiotics in the system were eliminated. Insights from the model accurately displayed how antibiotic resistance spreads within a colony, providing information that can be used to build policy around the issue—predicting antibiotic resistance decreases the likelihood of it occurring as it offers strategies for prevention.

Such insights can become the basis for informing best practices in wastewater facilities.

➢ *Additional Levels of Filtration*
Stemming from the second conclusion statement, adding a filtration step before depositing waste can decrease the amount of bacteria entering the wastewater reservoir. With a smaller initial population, the reproductive advantage of susceptible bacteria can take hold for longer before the wastewater system reaches carrying capacity—likely resulting in higher proportions of susceptible bacteria, hence inversely lower proportions of bacteria resistant to antibiotics.

➢ *Reducing the Frequency of Wastewater Influx*
Gleaned from the third conclusion statement, reducing the frequency of a wastewater influx indicates a more stable antibiotic concentration. This lessens the chances of a large proportion of resistant bacteria emerging by potentially limiting—if not eliminating—the window of time the system harbors a low antibiotic concentration.

➢ *Raising Awareness about Best Disposal Practices for Antibiotics*
Preemptively aiming for none or minimal amounts of antibiotics to enter the wastewater system 14 supports the beneficial outcome detailed in the first conclusion statement. In an environment with no antibiotics present, both bacteria are equally affected (truly, unaffected) by antibiotic residue, and susceptible bacteria can out compete resistant bacteria due to their increased rate of reproduction.

Even as the model stands currently, it has the capacity to predict the emergence of antibiotic resistance in a wastewater system given the antibiotic concentration and an estimate of the bacterial population—two factors that exert great influence. With the addition of new parameters and a transition to a user-friendly interface in the future, this model could be used as a predictive tool in any wastewater system—forecasting with speed and accuracy how the emergence of antibiotic resistant bacteria would change with the implementation of different wastewater practices. This

knowledge could inform decisions that protect the health of entire cities—hundreds of thousands of individuals.

REFERENCES

[1]. S. Harbarth and A. Stewardson, "Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050?" PLoS Medicine, vol. 13, no. 11, 2016. [Online].Available: https://doi.org/10.1371/journal. pmed.1002184

[2]. C. for Disease Control and Prevention, "Antibiotic and Antimicrobial Resistance," 2019. [Online]. Available: https://www.cdc.gov/drugresistance/ about.html

[3]. "Antibiotic Resistance is Spreading from Wastewater Treatment Plants," 2019. [Online]. Available: https://www.sciencedaily.com/releases/ 2019/03/19 0306125330.htm

[4]. M. P. C. Agency, "Antibiotics and the environment," 2017.[Online]. Available: https://www.pca.state.mn.us/ featured/antibiotics- and-environment

[5]. I. Sutradhar, C. Ching, D. Desai, and M. Suprenant, "Computational Model to Quantify the Growth of Antibiotic Resistance Bacteria in Wastewater," mSystems, vol. 29, no. 6, 2021. [Online]. Available: https://journals.asm.org/doi/full/10.1128/mSystems .00360-21

[6]. A. Burmeister, "Horizontal Gene Transfer," Oxford Academic, vol. 193, no. 4, 2015. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/26224621/

[7]. B. Paul and K. Whitwell, "Introductory Tutorial," 2018.[Online]. Available: https://mesa.readthedocs.io/ en/master/tutorials/intr otutorial.html

[8]. M. Baker and J. L. Hobman, "Mathematical Modeling of Antimicrobial Resistance in Agricultural Waste Highlights Importance of Gene Transfer Rate," Oxford Academic, vol. 92, no. 4, 2016. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/26906100/