Mathematical Modelling of Drug Interaction on Evolution of Antibiotic Resistance: An Analytical Approach

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Mathematical Modelling of Drug Interaction on Evolution of Antibiotic Resistance: An Analytical Approach

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Statements and Declarations

Competing Interests

The authors declare no competing interests.

Author Contributions

R.N. and S.K. conceived of the presented idea. R.N. and S.K. developed the theory and performed the computations. R.N., M.S. and S.K. verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

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Abstract

The rapid spread of antibiotic-resistant pathogens has prompted drug combinations to maintain clinical efficacy and combat the development of resistance. Drugs interact to increase (synergistic) or decrease (antagonistic) the effect of the combined therapy. Furthermore, the interactions of the two drugs can change as the bacteria evolves from the wild type (W.T.) to the mutant type (M.T.). Experimental studies have shown that the evolution of resistance is impeded if drugs interact antagonistically in the W.T. In contrast, other studies have shown that antagonistic interactions in the M.T. speed up the resistance's emergence. Theoretical works investigated the effect of W.T. drug interactions on resistance. A fundamental question is how the different combination of drug interactions in W.T. and M.T. influences antimicrobial resistance. Here we analyze a mathematical model that captures any combination of drug interactions in W.T. and the M.T. The novelty of this work is to examine the association between synergistic and antagonistic interaction of antibiotics for wild-type (sensitive bacteria) and mutants (resistant bacteria) on the growth rate of resistant strains. The most important contribution is to clarify that antagonistic interaction against the wild type has a more critical role in slowing the growth rate of resistant bacteria. The antagonistic interaction in the M.T. speeds up evolution but minimally. Our results suggest that it would be more appropriate to consider the nature of the drug interactions for the W.T. when designing combination therapy.

Keywords: Antibiotic resistance, Drug interaction, ordinary differential equation, equilibrium solutions

1. Introduction

Antibiotic-resistant bacteria are a complex and growing public health problem worldwide. Infections caused by them are more challenging to treat than infections caused by non-resistant bacteria and can lead to more extended hospital stays, additional costs, and increased mortality. One of the mechanisms by which bacteria acquire antibiotic resistance is through mutation in the chromosomal gene (Pirommas Techitnutsarut and Farida Chamchod 2021). Mutations in infectious diseases lead to antibiotic resistance during treatment with a single drug (Jean-Baptiste Michel et al. 2008). Therefore, multidrug treatment can increase the efficacy of the therapy (Jean-Baptiste Michel et al. 2008). Unfortunately, some multidrug-resistant bacteria gain resistance to a multidrug cocktail (Jean-Baptiste Michel et al. 2008, Charlotte Genestet et al. 2018). Treating the disease caused by multidrug-resistant (MDR) bacteria became more complicated and increased patient mortality (Bin Zhao et al. 2016). The challenge is to identify the factors that lead to the evolution of single-drug and multidrug-resistant bacteria and develop drug combinations strategies to maintain clinical efficacy and combat the development of resistance (Joseph Peter Torella et al. 2010).

When the two drugs are combined, they can interact and enhance each other's inhibitory effects compared to their respective inhibitory effects. This is called the synergistic interaction (Jean-Baptiste Michel et al. 2008, Joseph Peter Torella et al. 2010, Pamela J. Yeh et al. 2010) between the two drugs. In synergistic interactions, the drug can more effectively inhibit or kill wild-type (W.T.) bacteria in the same amount as used alone (Pamela J. Yeh et al. 2010). On the other hand, drugs interact and reduce each other's inhibitory effects compared to their respective inhibitory effects. This is called the antagonistic interaction (Jean-Baptiste Michel et al. 2008, Joseph Peter Torella et al. 2010, Pamela J. Yeh et al. 2010) between the two medications. In this interaction, the drug cannot effectively suppress or kill W.T. bacteria in the same amount as when used alone [6]. The interaction is additive if the drugs interact and have the same effect as when used individually [6].

Moreover, the interactions of the two drugs can change as the bacteria evolves from the wild type to the mutant type (M.T.). At the beginning of combination therapy, drugs can synergistically affect the W.T. Nevertheless, when the bacteria evolve from W.T. to M.T., the interaction can change from synergistic to antagonistic (Rafael Pena-Miller et al. 2013).

Experimental (Jean-Baptiste Michel et al. 2008, Joseph Peter Torella et al. 2010), numerical (Jean-Baptiste Michel et al. 2008, Joseph Peter Torella et al. 2010, Rafael Pena-Miller et al. 2013, Remy Chaitet al. 2007, Matthew

Hegreness 2008), and analytical [Pirommas Techitnutsarut and Farida Chamchod 2021, Eduardo Ibargüen-Mondragón et al. 2014] studies have been done to investigate how the evolution of antibiotic resistance is affected by synergistic and antagonistic interaction of drugs in the case of multidrug therapy. Hegreness M. et al. (Matthew Hegreness 2008) and Remy Chait et al. (Remy Chaitet al. 2007) accomplished that the evolution of antibiotic resistance is faster in synergistic drug combinations than the antagonistic combination. They find that a synergistic interaction for the W.T. accelerates antibiotic resistance's development due to the selective advantage of resistance mutation. They concluded that an antagonistic interaction on the W.T. slows down the particular benefit of resistance mutation. However, Pena-Miller et al. (Rafael Pena-Miller et al. 2013) showed that synergistic interaction changes to antagonistic interaction. It can be seen from their work that antagonistic interactions in the M.T. speed up the resistance's emergence. However, it is unclear if the beneficial effect of antagonism in W.T. is more critical than the detrimental effect of antagonism in M.T.

In this study, we purposed a mathematical model to analyze the impact of drug interaction on the evolution of antibiotic resistance. The main objective is to examine the presence of which interaction type; thus, the growth rate of resistant bacteria decreases. The novelty of this work is to analytically explore the association between the interaction of antibiotics for the W.T. and the M.T. on the antibiotic resistance.

2. Materials and Methods

2.1 Model formulation

We model a situation where an individual receives a multidrug treatment against bacteria (as with Mycobacterium tuberculosis (Eduardo Ibargüen-Mondragón et al. 2014, Charlotte Genestet et al. 2018). Let us denote by $S(t)$ and $R(t)$ the population sizes of sensitive (or W.T.) and resistant (or M.T.) bacteria to M and N antibiotics at time t, respectively; and by $C_1(t)$, $C_2(t)$ the concentration of the M and N antibiotics, respectively. We assume that bacteria follow a logistic growth (cell competition) with carrying capacity K. Let β_s, β_r represent the birth rate of sensitive and resistant bacteria, respectively. Specific mutations that confer resistance to chemical control have an inherent fitness cost which may be manifested through reduced reproductive capacity or competitive ability (Justino Alavez-Ramírez et al. 2006). We measure fitness cost as a reduction in the reproduction rate of the resistant strain. Therefore $\beta_r \leq \beta_s$. Resistant bacteria can emerge due to mutations of sensitive bacteria, and we model this situation by the terms $q_1 S$ and $q_2 S$, where q_1 and q_2 are the mutation rate of sensitive bacteria, respectively. The immune system kills sensitive and resistant bacteria with rates μ_s and μ_r , respectively. Sensitive and resistant bacteria also die due to the action of antibiotics. To consider the combined effect of antibiotic M and N interaction to kill sensitive and resistant bacteria, we incorporate a simple function as follows

$$\overline{X_s} = (\overline{\alpha_{11}} C_1 + \overline{\alpha_{12}} C_2 + \lambda_1 \overline{\alpha_{11}}\, \overline{\alpha_{12}} C_1 C_2)$$
$$\overline{X_r} = (\overline{\alpha_{21}} C_1 + \overline{\alpha_{22}} C_2 + \lambda_2 \overline{\alpha_{21}}\, \overline{\alpha_{22}} C_1 C_2) \quad (1)$$

where

$$\overline{\alpha_{11}} = \frac{E_{max}^{M,s}}{IC_{50}^{M,S}}\ ,\ \overline{\alpha_{12}} = \frac{E_{max}^{N,s}}{IC_{50}^{N,S}} \tag{2}$$

and

$$\overline{\alpha_{21}} = \frac{E_{max}^{M,r}}{IC_{50}^{M,R}}\ ,\ \overline{\alpha_{22}} = \frac{E_{max}^{N,r}}{IC_{50}^{N,R}} \tag{3}$$

In here, $E^{M.,S}{}_{max}$ and $E^{N,S}{}_{max}$ represent the maximal killing rate of antibiotic *M* and *N* of sensitive bacteria. $E^{M.,R}{}_{max}$ and $E^{N,R}{}_{max}$ represent the maximal killing rate of antibiotic *M* and *N* of resistant bacteria. $IC^{M,S}{}_{50}$ and $IC^{N,S}{}_{50}$ signify the half-maximal inhibitory concentration of the antibiotic *M* and N for sensitive bacteria. $IC^{M,S}{}_{50}$ and $IC^{N,S}{}_{50}$ denote the half-maximal inhibitory concentration of the antibiotic *M* and *N* for resistant bacteria.

The λ_1 and λ_2 are the interaction parameters for sensitive and resistant bacteria, respectively. They range between *-1.5* and *1.5 ($-1.5 \leq \lambda_1, \lambda_2 \leq 1.5$)* (Joseph Peter Torella et al. 2010), where negative values indicate antagonistic interactions, positive values indicate synergistic interactions. Finally, the *M* and *N* antibiotic concentration is supplied at a constant rate θ_1 and θ_2 , and are removed at a constant per capita rate μ_1 and μ_2, respectively.

Under the assumptions revealed above, we obtain the following system of ordinary differential equations:

$$\begin{aligned}
\frac{dS}{dt} &= \beta_s S\left(1 - \frac{S+R}{K}\right) - (q_1+q_2)S - (\overline{X_s} + \mu_s)S \\
\frac{dR}{dt} &= \beta_r R\left(1 - \frac{S+R}{K}\right) + (q_1+q_2)S - (\overline{X_r} + \mu_r)R \\
\frac{dC_1}{dt} &= \theta_1 - \mu_1 C_1 \\
\frac{dC_2}{dt} &= \theta_2 - \mu_2 C_2
\end{aligned} \tag{4}$$

With the following change of variables

$$s = \frac{S}{K}\ ,\ \ r = \frac{R}{K}\ ,\ \ c_1 = \frac{C_1}{\theta_1/\mu_1}\ ,\ \ \ c_2 = \frac{C_2}{\theta_2/\mu_2} \tag{5}$$

the system (1) is written as

$$\begin{aligned}
\frac{ds}{dt} &= \beta_s s(1-(s+r)) - (q_1+q_2)s - (X_s + \mu_s)s \\
\frac{dr}{dt} &= \beta_r r(1-(s+r)) + (q_1+q_2)s - (X_r + \mu_r)r \\
\frac{dc_1}{dt} &= \mu_1 - \mu_1 c_1
\end{aligned} \tag{6}$$

$$\frac{dc_2}{dt} = \mu_2 - \mu_2 c_2$$

Where,

$\alpha_{1i} = \overline{\alpha_{1i}}(\theta_i/\mu_i)$,

$\alpha_{2i} = \overline{\alpha_{2i}}(\theta_i/\mu_i)$

for *i=1,2* and

$$X_s = (\alpha_{11}c_1 + \alpha_{12}c_2 + \lambda_1\alpha_{11}\,\alpha_{12}c_1c_2)$$
$$X_r = (\alpha_{21}c_1 + \alpha_{22}c_2 + \lambda_2\alpha_{21}\,\alpha_{22}c_1c_2) \quad (7)$$

The region of biological interest of system (6) is given by

$$\Omega = \{(s, r, c_1, c_2)\, \epsilon\, R_+^4 :\ 0 \le s, r, c_1, c_2 \le 1,\ \ 0 \le s + r \le 1\} \quad (8)$$

The following lemma assures that system (6) is well-posed because solutions with initial conditions in *Ω* remain there for all $t \geq 0$.

Lemma 2.1. The set *Ω* defined in (8) is positively invariant for the system (6). See (Eduardo Ibargüen-Mondragón et al. 2019) for proof of the above lemma.

2.2 Qualitative analysis of the model

This part will find the equilibrium points, analyze their stability, and give some numerical simulation.

2.2.1 Equilibrium solutions

The equilibria of the system (6) are given by the solutions of the system of algebraic equations

$$\beta_s s(1-(s+r)) - (q_1+q_2)s - ((\alpha_{11}c_1 + \alpha_{12}c_2 + \lambda_1\alpha_{11}\,\alpha_{12}c_1c_2) + \mu_s)s = 0$$
$$\beta_r r(1-(s+r)) + (q_1+q_2)s - ((\alpha_{21}c_1 + \alpha_{22}c_2 + \lambda_2\alpha_{21}\,\alpha_{22}c_1c_2) + \mu_r)r = 0 \quad (9)$$
$$\mu_1 - \mu_1 c_1 = 0$$
$$\mu_2 - \mu_2 c_2 = 0$$

From the last two equations of system (9), we have $c_1 = c_2 = 1$. Replacing c_1 and c_2 in the first two equations of system (9), we obtain

$$\beta_s s(1-(s+r)) - (q_1+q_2)s - ((\alpha_{11} + \alpha_{12} + \lambda_1\alpha_{11}\,\alpha_{12}) + \mu_s)s = 0$$
$$\beta_r r(1-(s+r)) + (q_1+q_2)s - ((\alpha_{21} + \alpha_{22} + \lambda_2\alpha_{21}\,\alpha_{22}) + \mu_r)r = 0 \quad (10)$$

It holds from the first equation of (10) that *s=0* or

$$\beta_s(1-(s+r))-m-((\alpha_{11}+\alpha_{12}+\lambda_1\alpha_{11}\,\alpha_{12})+\mu_s)=0$$

where

$$m=q_1+q_2 \tag{12}$$

Assume *s=0* replacing this value in the second equation of system (10) we obtain

$$\beta_r r-\beta_r r^2-((\alpha_{21}+\alpha_{22}+\lambda_2\alpha_{21}\,\alpha_{22})+\mu_r)r=0 \tag{13}$$

which implies *r=0* or

$$r=\frac{R_r-1}{R_r} \tag{14}$$

where

$$R_r=\frac{\beta_r}{(\alpha_{21}\alpha_{22}\lambda_2+\ \alpha_{21}+\ \ \alpha_{22})+\mu_r} \tag{15}$$

Therefore, we obtain the solutions equilibrium solutions

$$P_0=(0,0,1,1)$$
$$P_1=\left(0,\frac{R_r-1}{R_r},1,1\right) \tag{16}$$

From (14), it follows that a necessary and sufficient condition for the biological sense of P_1 is

$R_r > 1$. Now, for $s\neq 0$ the first equation of (10) is reduced to

$$s=\frac{R_s-1}{R_s}-r \tag{17}$$

where

$$R_s=\frac{\beta_s}{m+(\alpha_{11}+\alpha_{12}+\lambda_1\alpha_{11}\,\alpha_{12})+\mu_s} \tag{18}$$

From (17), it is concluded that a necessary condition for the existence of sensitive and resistant bacteria is $R_s > 1$

Also, sufficient condition for s to be positive is

$$\frac{R_s-1}{R_s}>r \tag{19}$$

Substituting (17) in the second equation of (10) and solving for *r* we obtain

$$r=\frac{m\left(\frac{R_s-1}{R_s}\right)}{\beta_r\left(\frac{1}{R_r}-\frac{1}{R_s}\right)+m} \tag{20}$$

Replacing r defined by (20) in the inequality (19), it is easy to verify that $s > 0$ is equivalent to

$$R_s > R_r \tag{21}$$

Further, $r > 0$ if

$$\frac{1}{R_r} > \frac{1}{R_s} \tag{22}$$

Therefore, a necessary condition for s and r to be positive is $R_s > R_r$ These results are summarized in the following proposition.

Proposition 2.2.1.1: Model (6) always has the infection-free equilibrium $P_0 = (0.0,1,1)$ contained in Ω. If $R_r > 1$, $P_1 = (0, (R_r - 1) / R_r, 1, 1)$ is a second equilibrium in Ω. When $R_s > 1$ and $R_s > R_r$ in addition to P_0, and P_1 there exists a third equilibrium in Ω, $P_2 (\bar{s}, \bar{r}, 1, 1)$ where $\bar{s}$ and $\bar{r}$ are given by the right-hand side of equations (17) and (20).

2.2.2 Stability of equilibria points

In this section, we determine the local asymptotic stability of the equilibrium solutions of the system (6). To this end, let us start with the trivial equilibrium $P_0 = (0.0,1,1)$. Linearization of system (6) around P_0 is given by

$$\vec{x}' = J(p)\vec{x},$$

where

$$\vec{x} = (s, r, c_1, c_2)^T$$

and the matrix J evaluated at P is

$$J(P) = \begin{bmatrix} j_{11}(P) & -\beta_s s & -(c_2\lambda_1\alpha_{11}\alpha_{12} + \alpha_{11})s & -(c_1\lambda_1\alpha_{11}\alpha_{12} + \alpha_{12})s \\ -\beta_r r + m & j_{22}(P) & -(c_2\lambda_2\alpha_{21}\alpha_{22} + \alpha_{21})r & -(c_1\lambda_2\alpha_{21}\alpha_{22} + \alpha_{22})r \\ 0 & 0 & -\mu_1 & 0 \\ 0 & 0 & 0 & -\mu_2 \end{bmatrix} \tag{23}$$

with

$$\begin{aligned} j_{11}(p) &= \beta_s\big(1 - (s + r)\big) - \beta_s s - m - \big((\alpha_{11}c_1 + \alpha_{12}c_2 + \lambda_1\alpha_{11}\alpha_{12}c_1c_2) + \mu_s\big) \\ j_{22}(p) &= \beta_r(1 - (s + r)) - \beta_r r - ((\alpha_{21}c_1 + \alpha_{22}c_2 + \lambda_2\alpha_{21}\,\alpha_{22}c_1c_2) + \mu_r) \end{aligned} \tag{24}$$

By evaluating the (23) Jacobian J in P_0 we obtain

$$J(P_0) = \begin{bmatrix} j_{11}(P_0) & 0 & 0 & 0 \\ m & j_{22}(P_0) & 0 & 0 \\ 0 & 0 & -\mu_1 & 0 \\ 0 & 0 & 0 & -\mu_2 \end{bmatrix} \tag{25}$$

The eigenvalues of $J(P_0)$ are given by

$$\varphi_1 = j_{11}(P_0) = \beta_s - m - \left((\alpha_{11} + \alpha_{12} + \lambda_1\alpha_{11}\alpha_{12}) + \mu_s\right) = \beta_s\left(\frac{R_s - 1}{R_s}\right)$$

$$\varphi_2 = j_{22}(P_0) = \beta_r - ((\alpha_{21} + \alpha_{22} + \lambda_2\alpha_{21}\,\alpha_{22}) + \mu_r) = \beta_r\left(\frac{R_r - 1}{R_r}\right) \quad (26)$$

$$\varphi_3 = -\mu_1$$

$$\varphi_4 = -\mu_2$$

Since φ_1 and φ_2 are negative for $R_s < 1$ and $R_r < 1$ respectively, then P_0 is locally and asymptotically stable. Since α_{11}, α_{12}, μ_s, *and* β_s are positive, there are three conditions for $R_s < 1$ if $\lambda_1 > 0$. $\lambda_1 < 0$, or $\lambda_1 = 0$. If $\lambda_1 > 0$, $\lambda_1 < 0$ the necessary condition for $R_s < 1$ is

$$\beta_s - \mu_s - m < \alpha_{11} + \alpha_{12} + \lambda_1\alpha_{11}\,\alpha_{12}$$

and if $\lambda_1 = 0$, the necessary condition is

$$\beta_s - \mu_s - m < \alpha_{11} + \alpha_{12}$$

Analogously, since α_{21}, α_{22}, μ_r, *and* β_r are positive, there are three condition for $R_r > 1$, if $\lambda_2 > 0$, $\lambda_2 < 0$, or $\lambda_2 = 0$. If $\lambda_2 > 0$, $\lambda_2 < 0$ the necessary condition for $R_r > 1$ is

$$\beta_r - \mu_r < \alpha_{21} + \alpha_{22} + \lambda_2\alpha_{21}\,\alpha_{22}$$

and if $\lambda_2 = 0$ the necessary condition is

$$\beta_r - \mu_r < \alpha_{21} + \alpha_{22}$$

This result is summarized in the following proposition.

Proposition 2.2.2.1: If $R_s < 1$ and $R_r < 1$, then the trivial equilibrium P_0 is locally and asymptotically stable in Ω. If $R_s > 1$ or $R_r > 1$, then P_0 is unstable.

Now, we will determine the conditions for which the equilibrium P_1 is locally and asymptotically stable. To this end, let us observe that the Jacobian given in (23) evaluated in P_1 is given by

$$J(P_1) = \begin{bmatrix} j_{11}(P_1) & 0 & 0 & 0 \\ -\beta_r\left(\frac{R_r - 1}{R_r}\right) + m & j_{22}(P_1) & -(\lambda_2\alpha_{21}\alpha_{22} + \alpha_{21})\frac{R_r - 1}{R_r} & -(\lambda_2\alpha_{21}\alpha_{22} + \alpha_{22})\frac{R_r - 1}{R_r} \\ 0 & 0 & -\mu_1 & 0 \\ 0 & 0 & 0 & -\mu_2 \end{bmatrix} \quad (27)$$

The eigenvalues of $J(P_1)$ are given by

$$\omega_1 = j_{11}(P_1) = \beta_s\left(1 - \frac{R_r - 1}{R_r}\right) - m - \mu_s - (\alpha_{11} + \alpha_{12} + \lambda_1\alpha_{11}\alpha_{12}) = \beta_s\left(\frac{1}{R_r} - \frac{1}{R_s}\right)$$

$$\omega_2 = j_{22}(P_1) = \beta_r\left(1 - \frac{R_r - 1}{R_r}\right) - \beta_r\left(\frac{R_r - 1}{R_r}\right) - \mu_r - (\alpha_{21} + \alpha_{22} + \lambda_2\alpha_{21}\,\alpha_{22}) = \beta_r\left(\frac{1 - R_r}{R_r}\right) \quad (28)$$

$\omega_3 = -\mu_1$

$\omega_4 = -\mu_2$

We see that $\omega_1 < 0$ if and only if $R_r > R_s$ and that $\omega_2 < 0$ if and only if $R_r > 1$. Since α_{21}, α_{21}, μ_r, and β_r are positive, there are three conditions for $R_r > 1$, if $\lambda_2 > 0$, $\lambda_2 < 0$, or $\lambda_2 = 0$. If $\lambda_2 > 0$, $\lambda_2 < 0$ the necessary condition for $R_r > 1$ is

$\beta_r - \mu_r > \alpha_{21} + \alpha_{22} + \lambda_2 \alpha_{21}\, \alpha_{22}$

and if $\lambda_2 = 0$ the necessary condition is

$\beta_r - \mu_r > \alpha_{21} + \alpha_{22}$

From the above we have the following proposition

Proposition 2.2.2.2: If $R_r > R_s$ and $R_r > 1$, then the equilibrium P_1 is locally and asymptotically stable in Ω. If $R_r < R_s$ or $R_r < 1$, then P_1 is unstable.

Now, we will determine the conditions for which the equilibrium P_2 is locally and asymptotically stable. To this end, let us observe that the Jacobian given in (23) evaluated in P_2 is given by

$$J(P_2) = \begin{bmatrix} j_{11}(P_2) & -\beta_s \bar{s} & -(\lambda_1 \alpha_{11}\alpha_{12} + \alpha_{11})\bar{s} & -(\lambda_1 \alpha_{11}\alpha_{12} + \alpha_{12})\bar{s} \\ -\beta_r \bar{r} + m & j_{22}(P_2) & -(\lambda_2 \alpha_{21}\alpha_{22} + \alpha_{21})\bar{r} & -(\lambda_2 \alpha_{21}\alpha_{22} + \alpha_{22})\bar{r} \\ 0 & 0 & -\mu_1 & 0 \\ 0 & 0 & 0 & -\mu_2 \end{bmatrix} \tag{29}$$

where

$$\begin{aligned} j_{11}(P_2) &= \beta_s\big(1 - (\bar{s} + \bar{r})\big) - \beta_s \bar{s} - m - \mu_s - (\alpha_{11} + \alpha_{12} + \lambda_1 \alpha_{11}\alpha_{12}) \\ j_{22}(P_2) &= \beta_r(1 - (\bar{s} + \bar{r})) - \beta_r \bar{r} - \mu_r - (\alpha_{21} + \alpha_{22} + \lambda_2 \alpha_{21}\, \alpha_{22}) \end{aligned} \tag{30}$$

From (11), it follows

$$j_{11}(P_2) = \beta_s\big(1 - (\bar{s} + \bar{r})\big) - \beta_s \bar{s} - m - \mu_s - (\alpha_{11} + \alpha_{12} + \lambda_1 \alpha_{11}\alpha_{12}) = -\beta_s \bar{s} \tag{31}$$

and from the second equation of (10), we have

$$j_{22}(P_2) = \beta_r(1 - (\bar{s} + \bar{r})) - \beta_r \bar{r} - \mu_r - (\alpha_{21} + \alpha_{22} + \lambda_2 \alpha_{21}\, \alpha_{22}) = -\frac{1}{\bar{r}}(\beta_r \bar{r} + m\bar{s}) \tag{32}$$

Substituting (31) and (32) in (29), $J(P_2)$ becomes

$$J(P_2) = \begin{bmatrix} -\beta_s \bar{s} & -\beta_s \bar{s} & -(\lambda_1 \alpha_{11}\alpha_{12} + \alpha_{11})\bar{s} & -(\lambda_1 \alpha_{11}\alpha_{12} + \alpha_{12})\bar{s} \\ -\beta_r \bar{r} + m & -\frac{1}{\bar{r}}(\beta_r \bar{r} + m\bar{s}) & -(\lambda_2 \alpha_{21}\alpha_{22} + \alpha_{21})\bar{r} & -(\lambda_2 \alpha_{21}\alpha_{22} + \alpha_{22})\bar{r} \\ 0 & 0 & -\mu_1 & 0 \\ 0 & 0 & 0 & -\mu_2 \end{bmatrix} \tag{33}$$

The eigenvalues of $J(P_2)$ are

$$\tau_1 = -\mu_1$$
$$\tau_2 = -\mu_2 \quad (34)$$

and the eigenvalues of the matrix

$$A = \begin{bmatrix} -\beta_s \bar{s} & -\beta_s \bar{s} \\ -\beta_r \bar{r} + m & -\frac{1}{\bar{r}}(\beta_r \bar{r}^2 + m\bar{s}) \end{bmatrix} \quad (35)$$

Since

$$Trace(A) = -\beta_s \bar{s} - \frac{1}{\bar{r}}(\beta_r \bar{r}^2 + m\bar{s}) < 0 \quad (36)$$

and

$$Det(A) = \frac{1}{\bar{r}}(\beta_s\, \beta_r\, \bar{s}\, \bar{r}^2 + \beta_s m\, \bar{s}^2) + \beta_s\, \beta_r\, \bar{s}\, \bar{r} - \beta_s\, \bar{s}\, m > 0 \quad (37)$$

the eigenvalues of *A* have a negative real part. We resume the above results in the following proposition.

Proposition 2.2.2.3: If $R_s > 1$ and $R_s > R_r$, the equilibrium P_2 is in Ω, and it is locally and asymptotically stable.

2.2.3 Biological interpretation of equilibrium solutions and the stability analysis

The R_s represent the average number of sensitive bacteria produced by the fraction of sensitive bacteria that escape the action of the combination of antibiotics and the immune system. R_s defined in (18) is rewritten as

$$R_s = \frac{\mu_s}{m + (\alpha_{11} + \alpha_{12} + \lambda_1 \alpha_{11}\, \alpha_{12}) + \mu_s} N_s \quad (38)$$

Where N_s is defined as the product of the reproduction rate of sensitive bacteria β_s and the average life span of sensitive bacteria $1/\mu_s$.

$\alpha_{1i} = \overline{\alpha_{1i}}(\theta_i/\mu_i)$,

for $i=1,2$ are the rate at which the *M* and *N* antibiotics eliminate sensitive bacteria at their equilibrium level.

$$\frac{\mu_s}{m + (\alpha_{11} + \alpha_{12} + \lambda_1 \alpha_{11}\, \alpha_{12}) + \mu_s}$$

The term represents a fraction of sensitive bacteria that do not present spontaneous mutations and escape to the action of the combination of antibiotics and the immune system. The R_r represent the average number of resistant bacteria produced by the fraction of resistant bacteria that escape the action of the combination of antibiotics and the immune system. R_r defined in (15) is rewritten as

$$R_r = \frac{\mu_r}{(\alpha_{21} + \alpha_{22} + \alpha_{21}\alpha_{22}\lambda_2\ ) + \mu_r} N_r$$

Where N_r is defined as the product of the reproduction rate of resistant bacteria β_r and the average life span of sensitive bacteria $1/\mu_r$. The (39)

$$\alpha_{2i} = \overline{\alpha_{2i}}(\theta_i/\mu_i),$$

for $i=1,2,$ are the rate at which the M and N antibiotics eliminate resistant bacteria at their equilibrium level. The

$$\frac{\mu_r}{(\alpha_{21} + \alpha_{22} + \alpha_{21}\alpha_{22}\lambda_2) + \mu_r}$$

the term represents a fraction of resistant bacteria that do not present spontaneous mutations and escape to the action of the combination of antibiotics and the immune system

When antibiotics eliminate the sensitive bacteria, and the resistant bacteria prohibit the proliferation of resistant bacteria, in this case, both bacteria die out. In this situation, suppose resistant bacteria produce, on average more than one, and the reproduction capacity of sensitive bacteria is lower than resistant bacteria. In that case, only resistant bacteria live, and sensitive bacteria die out. When sensitive bacteria produce more than one bacterium, and the reproduction capacity of sensitive bacteria is more than resistance, then both sensitive and resistant bacteria live. Although the reproduction capacity of resistant bacteria is lower than sensitive, the spontaneous mutation of sensitive bacteria makes them live.

2.2.4 Numerical simulations

This section gives some numerical simulations and graphs that illustrate the above results. The values of the parameters used in the simulations are constant and were determined based on data from Table 1. For numerical simulation, we consider an individual with a disease caused by bacteria that develop resistance to antibiotics M and N. The drug interaction parameter for sensitive and resistant bacteria are (λ_1) and (λ_2) respectively. The λ equals to 0 for additive interaction (Joseph Peter Torella et al. 2010), 1 for synergistic interaction (Joseph Peter Torella et al. 2010), and -1 for antagonistic interaction (Joseph Peter Torella et al. 2010). Our simulation follows three scenarios. In the first scenario, antibiotics interact additively with the sensitive and the resistant bacteria ($\lambda_1=\lambda_2=0$). In the second scenario, antibiotics interact synergistically with the sensitive bacteria, but they interact antagonistically with resistant bacteria ($\lambda_1=1, \lambda_2=-1$). In the third scenario, antibiotics interact antagonistically with sensitive bacteria but synergistically with resistant bacteria ($\lambda_1=-1, \lambda_2=1$).

Table 1: Interpretation and considered values of the parameters for the model (6). Data are deduced from the literature.

Parameter	Description	Value	Units	Ref.
K	Bacteria carrying capacity	10^9	Cells	[23]

β_s	The growth rate of sensitive bacteria	1	h^{-1}	[23]
β_r	The growth rate of resistant bacteria	0.65	h^{-1}	[23]
μ_s	The natural death rate of sensitive bacteria	0.5	h^{-1}	[23]
μ_r	The natural death rate of resistant bacteria	0.5	h^{-1}	[23]
m	The mutation rate of sensitive bacteria	$10^{-8} + 10^{-6}$	$mut \times gen$	[3]
$E_{max}^{M,S}$	The maximal kill rate of sensitive bacteria with the antibiotic *M*	1.5	h^{-1}	[3]
$E_{max}^{N,S}$	The maximal kill rate of sensitive bacteria with the antibiotic *N*	1.5	h^{-1}	Hypothesis
$E_{max}^{M,R}$	The maximal kill rate of resistant bacteria with the antibiotic *M*	1.1	h^{-1}	[23]
$E_{max}^{N,R}$	The maximal kill rate of resistant bacteria with the antibiotic *N*	1.1	h^{-1}	Hypothesis
$IC_{50}^{M,S}$	The concentration of the antibiotic *M*, which has a half-maximum effect on sensitive bacteria	0.25	$\mu g/ml$	[23]
$IC_{50}^{N,S}$	The concentration of the antibiotic *N*, which has a half-maximum effect on sensitive bacteria	0.25	μg/ml	Hypothesis
$IC_{50}^{M,R}$	The concentration of the antibiotic *M*, which has a half-maximum effect on resistant bacteria	5	μg/ml	[23]
$IC_{50}^{N,R}$	The concentration of the antibiotic *N*, which has a half-maximum effect on resistant bacteria	5	μg/ml	Hypothesis

Table 1: Interpretation and considered values of the parameters for the model (6). Data are deduced from the literature (continues)

θ_1	hourly dose of antibiotic	0.21	mg/h	[3]
θ_2	hourly dose of the antibiotic *N*	0.42	mg/h	[3]

μ_1	The degradation rate of the antibiotic *M*	0.0025	h^{-1}	[3]
μ_2	The degradation rate of the antibiotic *N*	0.0021	h^{-1}	[3]
λ_1	Interaction parameter between the antibiotics *M* and *N* for sensitive bacteria	variese between [-1.5 1.5]	-	[12]
λ_2	Interaction parameter between the antibiotics *M* and *N* for resistant bacteria	variese between [-1.5 1.5]	-	[12]

Here, for simplicity, we assume that antibiotics *M* and *N* have the same maximum kill rate *(E_{max})* on sensitive and resistant bacteria. We also assume that the IC_{50}'s of M and N antibiotics are the same for simplicity.

Fig. 1 shows that the system (6) solution approaches the trivial equilibrium P_0 point since $R_s < 1$ and $R_r < 1$ in all scenarios (Fig. 1a-1c). On the other hand, when $R_s < R_r$ and $R_r > 1$, the solutions approach the equilibrium P_1 point, as seen in Fig. 2a-2c. Here, the resistant bacteria evade the combined effect. Finally, when $R_s > R_r$ and $R_s > 1$ system (6 solution approaches the equilibrium point P_2 (Fig. 3a-3c). The less fit resistant bacteria is stabilized by the influx of mutations from the sensitive.

The number of bacteria produced by a sensitive bacteria (R_s) equals to 0.58, 0.48, and 0.74 for the first, second, and third scenarios, respectively. Anagolosly, The number of bacteria produced by a resistant bacteria (R_r) equals 0.69, 0.72, and 0.65 for the first, second, and third scenarios, respectively. It is inferred from Fig. 1a-1b that when the drug interaction is synergistic, the sensitive and resistant bacteria have a lower reproductive number than when the drug interactions are additive. Conversely, when the drug interaction is antagonistic, the sensitive and resistant bacteria have a bigger reproduction number than when the drug interactions are additive.

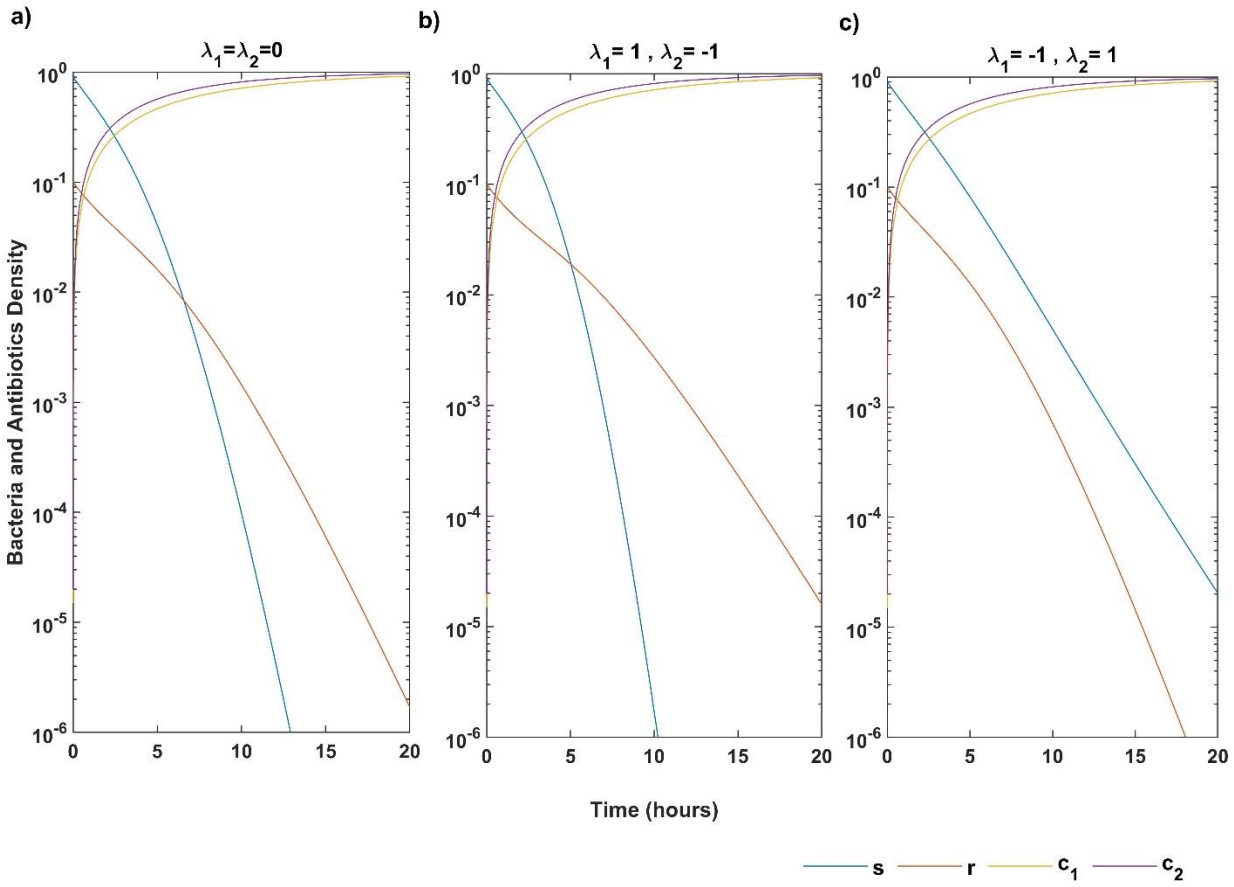

Fig. 1 Temporal course of sensitive (s) and resistant (r) bacteria population through three scenarios of antibiotics interaction effect: **a)** additive ($\lambda_1=\lambda_2=0$) effect on both the sensitive and the resistant bacteria, **b)** synergistic ($\lambda_1=1$) effect on the sensitive bacteria, and antagonistic ($\lambda_2=-1$) effect on resistant bacteria, **c)** antagonistic ($\lambda_1=-1$) effect on the sensitive, and synergistic ($\lambda_2=1$) effect on the resistant bacteria. In here c_1 and c_2 are the concentration of antiobitics, M and N. Simulations are done using parameter values in Table 1 and bacteria and antibiotic densities (y-axises) given in the log plot**.** In all three cases $R_s < 1$ and $R_r < 1$ which implies that the solution of the system (6) approach P_0.

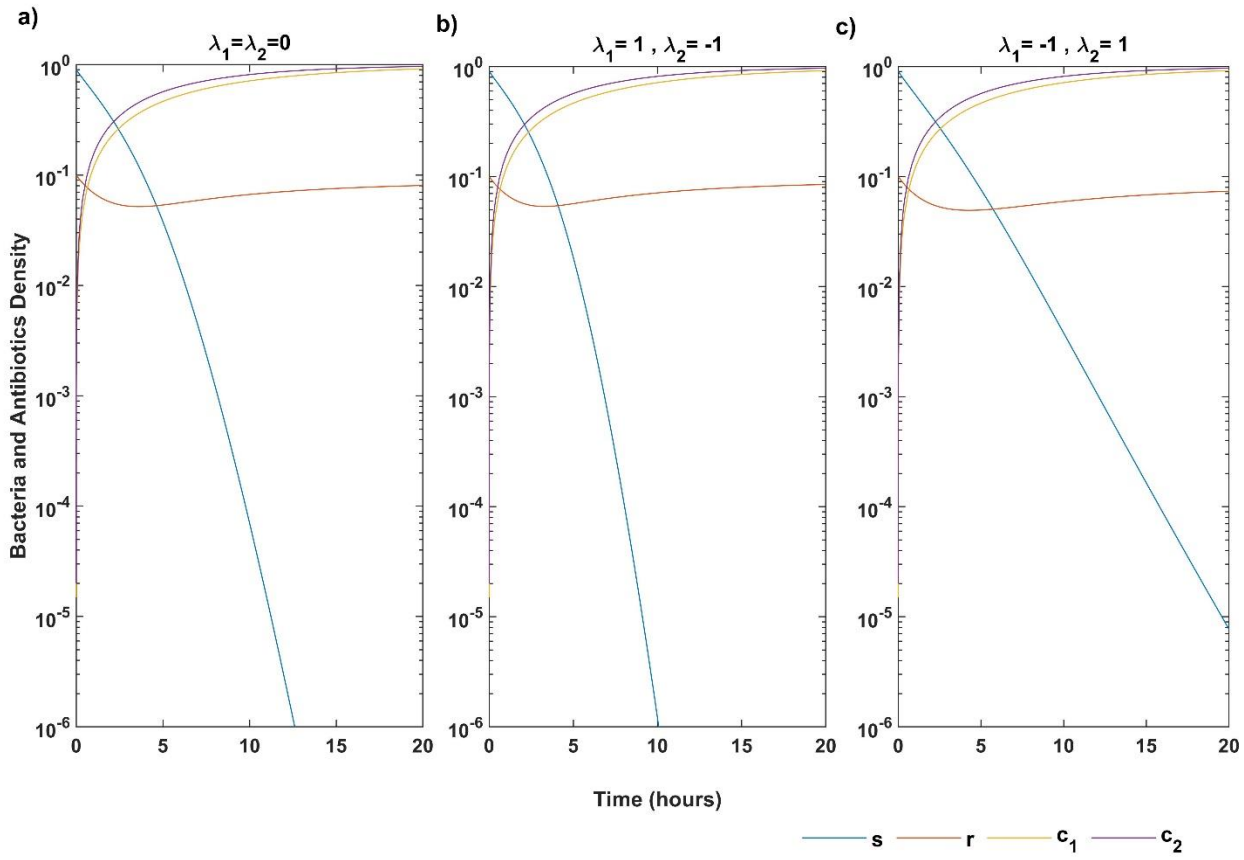

Fig. 2 Temporal course of sensitive (s) and resistant (r) bacteria population through three scenarios of antibiotics interaction effect: **a)** additive ($\lambda_1=\lambda_2=0$) effect on both the sensitive and the resistant bacteria, **b)** synergistic ($\lambda_1=1$) effect on the sensitive bacteria, and antagonistic ($\lambda_2=-1$) effect on resistant bacteria, **c)** antagonistic ($\lambda_1=-1$) effect on the sensitive and synergistic ($\lambda_2=1$) effect on the resistant bacteria. In here c_1 and c_2 are the concentration of antibiotics, *M* and *N*. Simulations are done using parameter values in Table 1 and bacteria and antibiotic densities (y-axises) given in the log plot**.** In all three cases $R_s < 1$ and $R_r > 1$, which implies that the solution of the system (6) approach P_1.

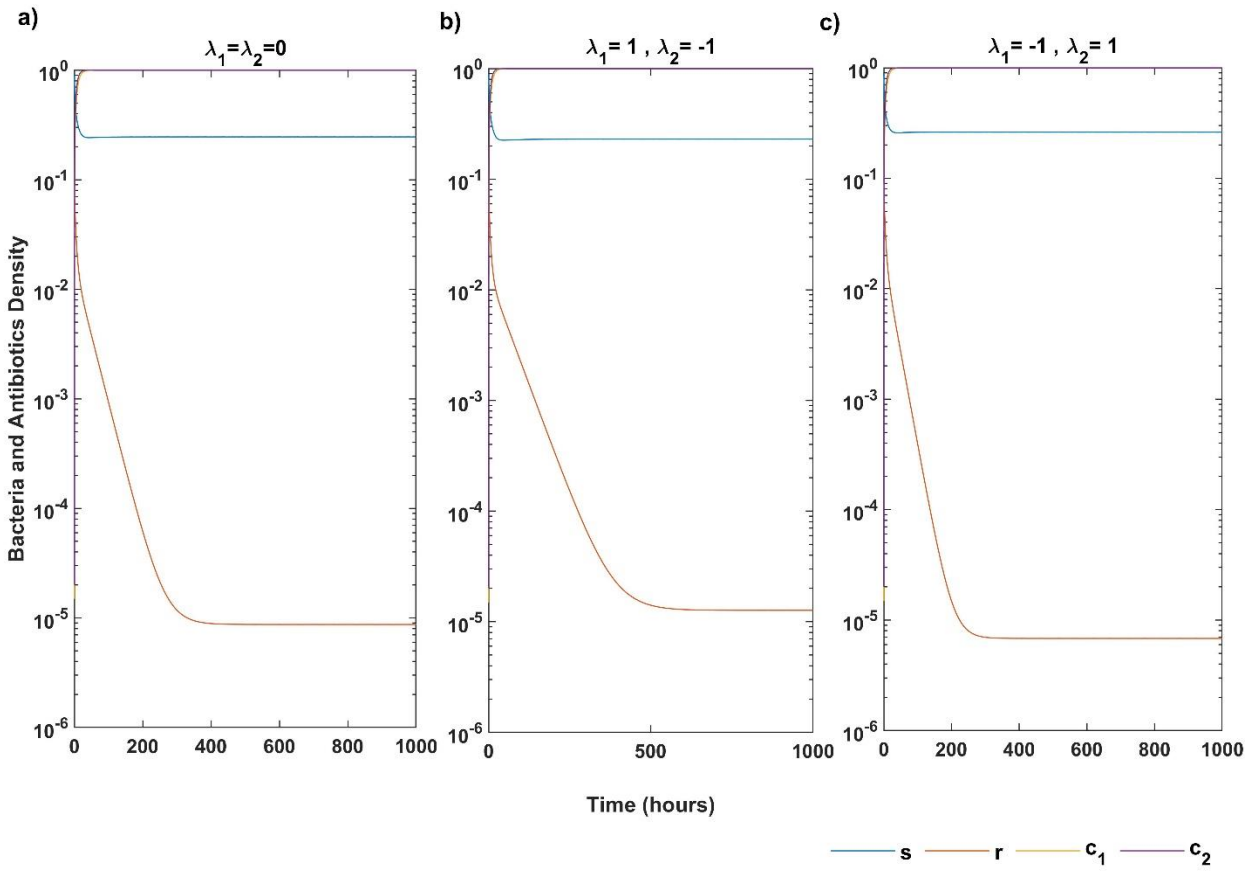

Fig. 3 Temporal course of sensitive (s) and resistant (r) bacteria population through three scenarios of antibiotics interaction effect: **a)** additive ($\lambda_1=\lambda_2=0$) effect on both the sensitive and the resistant bacteria, **b)** synergistic ($\lambda_1=1$) effect on the sensitive bacteria, and antagonistic ($\lambda_2=-1$) effect on resistant bacteria, **c)** antagonistic ($\lambda_1=-1$) effect on the sensitive and synergistic ($\lambda_2 = 1$) effect on the resistant bacteria. In here c_1 and c_2 are the concentration of antibiotics, *M* and *N*. Simulations are done using parameter values in Table 1 and bacteria and antibiotic densities (y-axises) given in the log plot**.** In all three cases $R_s > 1$ and $R_r < R_r$, which implies that the solution of the system (6) approach P_2.

3. Results

Here we will inspect the relationship between the interaction parameters of drugs for wild-type (λ_1) and mutants (λ_2) minimum inhibitory concentration (MIC). The MIC is the lowest concentration of an antimicrobial that will inhibit

the visible growth of a microorganism after overnight incubation (J M Andrews 2001, 2002). Antibiotic that has lower MIC is more effective in killing per dose. First, we analytically investigate the influence of (λ_2) on the MIC of the mutant bacteria .

Therefore, we equate R_r =1, for no visible growth, in the equation (15)

$$R_r = \frac{\beta_r}{(\alpha_{21}\alpha_{22}\lambda_2 + \alpha_{21} + \alpha_{22}) + \mu_r} = 1 \tag{40}$$

Solving for (λ_2) we get

$$\lambda_2 = \frac{\beta_r - \mu_r - \alpha_{21} - \alpha_{22}}{\alpha_{21}\alpha_{22}} = \frac{\beta_r - \mu_r - \left(\frac{E^r_{max}}{IC^{M,R}_{50}}\frac{\theta_1}{\mu_1}\right) - \left(\frac{E^r_{max}}{IC^{N,R}_{50}}\frac{\theta_2}{\mu_2}\right)}{\left(\frac{E^r_{max}}{IC^{M,R}_{50}}\frac{\theta_1}{\mu_1}\right)\left(\frac{E^r_{max}}{IC^{N,R}_{50}}\frac{\theta_2}{\mu_2}\right)} \tag{41}$$

for simplicity, we assume that both antibiotics have the same minimal inhibitory concentration ($IC^{M,R}{}_{50} = IC^{N,R}{}_{50} = IC^{R}{}_{50}$) and maximum kill rate ($E^{M,R}{}_{max} = E^{N,R}{}_{max} = E^{R}{}_{max}$) for resistant bacteria. Here we will investigate only the condition where $\mu_1 = \mu_2$ and $\theta_1 = \theta_2$. then (41) written as

$$\lambda_2 = \frac{\beta_r - \mu_r - 2\left(\frac{E^r_{max}}{IC^{R}_{50}}\frac{\theta_1}{\mu_1}\right)}{\left(\frac{E^r_{max}}{IC^{R}_{50}}\frac{\theta_1}{\mu_1}\right)^2} \tag{42}$$

We found (see appendix A for writing $IC^{R}{}_{50}$ as function of MIC_r)

$$IC^{R}_{50} = \frac{E^r_{max}\, MIC_r}{\beta_r - \mu_r} \tag{43}$$

where MIC_r is the MIC for resistant bacteria treated with a single antibiotic. Substituting (43) to (42), we get

$$\lambda_2 = \frac{MIC_r{}^2\theta_1{}^2 - 2\, MIC_r\mu_1\theta_1}{(\,(\beta_r - \mu_r)\,\mu_1)^2} \tag{44}$$

The (44) equation relates the λ_2 to MIC_r . Specifically, it relates λ_2 to how effective the drugs used in combination should be, at a minimum, when used alone. The minimum effectiveness translates to the maximum MIC value. We have plotted the graph of the (44) equation in Fig. 4 by using values in Table 1. Figure 4 shows that as the interactions change from antagonistic to synergistic, the antibiotics are allowed to have higher MIC values. Synergism can make up for the inefficiency of a single drug. The drugs that interact antagonistically must be very effective as a single drug.

In other words, if the interactions are synergistic, it is speculated that we do not need to use drugs with high inhibitory effects, as the synergistic interactions enhance their inhibitory effects. Similarly, as antagonistic interactions between drugs against resistant strains decrease ($\lambda_2 > 0$), the minimum concentration for inhibiting resistant strains reduce. When the interactions are antagonistic, we need to use drugs with high inhibitory effects, as antagonistic interactions reduce their inhibitory effects. From bacteria's point of view, the resistant strain is better-

off if the drugs interact antagonistically. Antagonistic drugs have to be very efficient; otherwise, they cannot control bacterial growth. The same argument extends to sensitive bacteria as well.

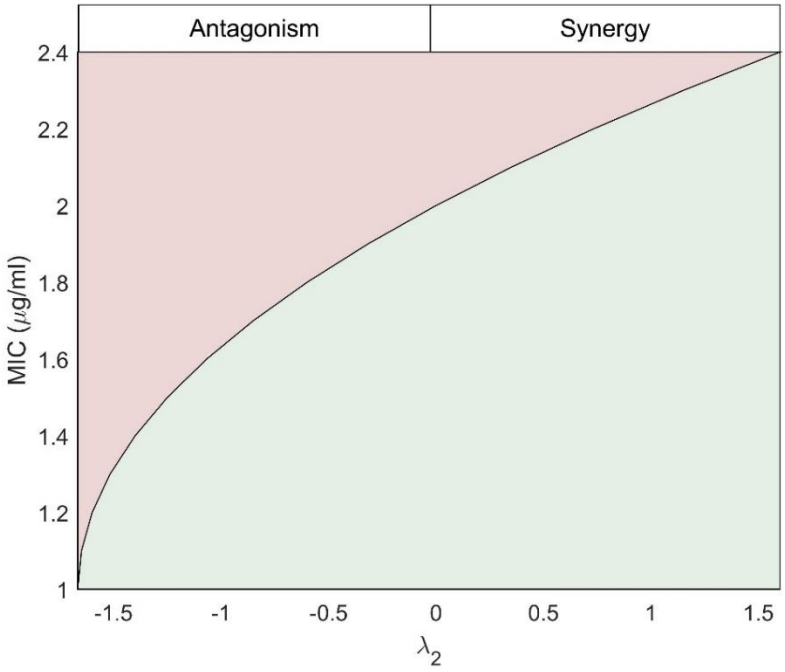

Fig. 4 Effect of interaction parameter between antibiotics for resistant bacteria (λ_2) on minimum inhibitory concentration (MIC) level of drugs.

After establishing that the antagonistic interactions benefit both the sensitive and resistant bacteria, we would like to consider the evolutionary aspects. To consider evolution, we have to compare the growth rate of the sensitive and resistant bacteria. A larger growth advantage would lead to faster evolution (Seyfullah Enes Kotil and Kalin Vetsigian 2018).

Now we suppose a quasi-stable condition for concentrations, and we compute the maximum growth rate of sensitive and resistant bacteria when *s* and *r* are close to goes to zero in (6) system. To calculate the growth rate of sensitive and resistant bacteria, we divide the first and second equation of (6) with s and r, respectively. We get

$$G_s = \beta_s - m - ((\alpha_{11}\alpha_{12}\lambda_1 + \alpha_{11} + \alpha_{12}) + \mu_s)$$

$$G_r = \beta_r - ((\alpha_{21}\alpha_{22}\lambda_2 + \alpha_{21} + \alpha_{22}) + \mu_r) \quad (45)$$

where G_s and G_r are the growth rates of sensitive and resistant bacteria, respectively, in quasi-stable condition, for interaction parameters. For simplicity, we assume that both antibiotics have same minimal inhibitory concentration for resistant bacteria ($IC^{M,R}{}_{50} = IC^{N,R}{}_{50} = IC^{R}{}_{50}$) and sensitive bacteria ($IC^{M,S}{}_{50} = IC^{N,S}{}_{50} = IC^{S}{}_{50}$). We also assume that both antibiotics have same maximum kill rate for resistance ($E^{M,R}{}_{max} = E^{N,R}{}_{max} = E^{R}{}_{max}$) and sensitive ($E^{M,S}{}_{max} = E^{N,S}{}_{max} = E^{S}{}_{max}$) bacteria. So that

$$\alpha_{11} = \frac{E^S_{max}}{IC^S_{50}} \frac{\mu_1}{\theta_1}, \qquad \alpha_{12} = \frac{E^S_{max}}{IC^S_{50}} \frac{\mu_2}{\theta_2}$$

and

$$\alpha_{21} = \frac{E^R_{max}}{IC^R_{50}} \frac{\mu_1}{\theta_1}, \alpha_{22} = \frac{E^R_{max}}{IC^R_{50}} \frac{\mu_2}{\theta_2}$$

In here we will investigate only the condition where $\mu_1 = \mu_2$ and $\theta_1 = \theta_2$. Consequently, $\alpha_{11} = \alpha_{12}$ and $\alpha_{21} = \alpha_{22}$ then (45) became

$$G_s = \beta_s - m - ((\alpha_{11}{}^2 \lambda_1 + 2\,\alpha_{11}) + \mu_s)$$
$$G_r = \beta_r - ((\alpha_{21}{}^2 \lambda_2 + 2\,\alpha_{21}) + \mu_r) \tag{46}$$

Since antibiotics have less effect on resistant bacteria than sensitive, so we can write $\alpha_{11} = \delta\,\alpha_{12}$ for some $\delta \epsilon R$, substituting this in second equation of (46) we get

$$G_r = \beta_r - (\delta^2\,\alpha_{11}{}^2 \lambda_2 + 2\,\delta\,\alpha_{11}) + \mu_r) \tag{47}$$

solving first equation of (46) for α_{11} we get

$$\alpha_{11} = \frac{-1 + \sqrt{-\lambda_1 \mu_s + \lambda_1 \beta_s - \lambda_1 G_s - \lambda_1 m + 1}}{\lambda_1} \tag{48}$$

substituting (48) in to (47)

$$G_r = \beta_r - \frac{\delta^2 \left(-1 + \sqrt{-\lambda_1 \mu_s + \lambda_1 \beta_s - \lambda_1 G_s - \lambda_1 m + 1}\right)^2 \lambda_2}{\lambda_1{}^2} - \frac{2\,\delta \left(-1 + \sqrt{-\lambda_1 \mu_s + \lambda_1 \beta_s - \lambda_1 G_s - \lambda_1 m + 1}\right)}{\lambda_1} - \mu_r) \tag{49}$$

We plot the surface of the growth rate of resistance strains (G_r) as a function of the interaction parameter of antibiotics for wild-type λ_1 and mutants λ_2 Fig. 5. We set the parameters to the values given in Table 1. The (G_s) is set to 0, to get solutions when the drug is used inhibit the sensitive bacteria. Figure 5 illustrates, as expectedly, that the G_r decreases as increasing antagonism with the sensitive bacteria, while G_r increases as increasing antagonism with the resistant bacteria. More importantly, the G_r depends more on λ_1 compared to λ_2. The simulation of temporal course of resistant bacteria, which is shown in Fig. 6, confirms our analytical conclusion in Fig. 5.

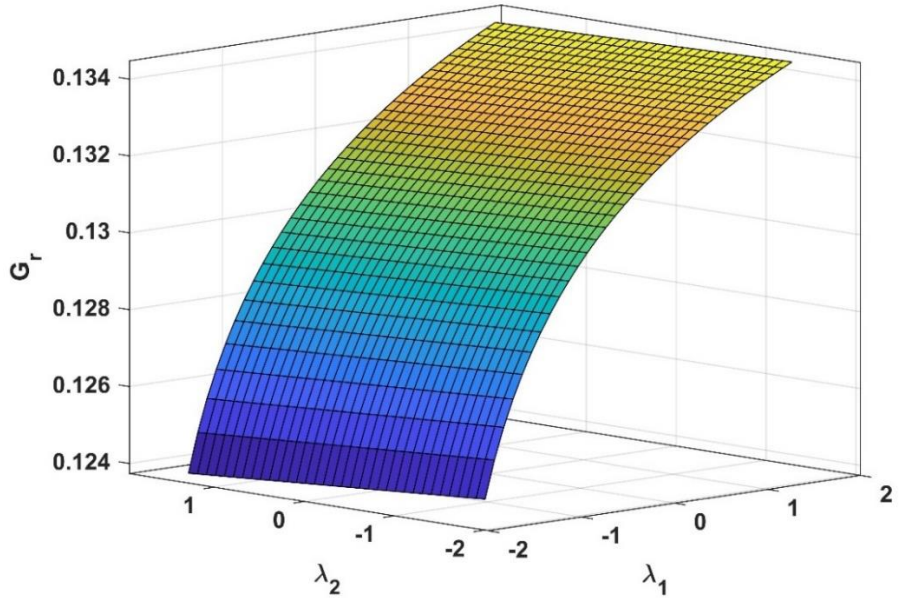

Fig. 5 Association between interaction parameters of antibiotics for wild-type λ_1 and mutants λ_2. on the growth rate of resistant strains.

Fig. 6a simulates the temporal course of sensitive and resistant strains under four different scenarios of the combined effect of antibiotics *M* and *N*. We select scenarios for the combined action of antibiotics according to the four corners of the graph in Fig. 5. In the first scenario, antibiotics *M* and *N* have an antagonistic effect on the wild type and a synergistic effect on the mutant. In the second scenario, the antibiotics *M* and *N* have a synergistic effect on the wild type and an antagonistic effect on the mutant type. The third is that antibiotics *M* and *N* have antagonistic effects on both wild type and mutant. In the fourth scenario, antibiotics *M* and *N* act synergistically on both wild-type and mutant.

As seen in Fig. 6a, the resistant bacteria population density has a reasonable interpretation with Fig.5 growth rate of resistant bacteria when the concentration of antibiotics is in maximum density (see Fig. 6b). Fig. 6a shows that the resistant bacteria have maximum population density when the $\lambda_1=1$ (synergistic). Fig. 5 also interpreted that the growth rate of resistant bacteria increases when sensitive bacteria are inhibited by the synergistic effect. In addition, in Fig. 5 and Fig. 6a, the λ_2 has not significantly contributed to the growth rate of resistant bacteria. Inversely, resistant bacteria have minimum population density for the scenario when λ_2 (antagonistic).

4. Discussion

The rapid spread of antibiotic-resistant pathogens has prompted drug combinations to maintain clinical efficacy and combat the development of resistance. Unfortunately, the emergence of multidrug-resistant bacteria causes treatment to be more complicated and increases mortality. It is important to investigate the factor that causes multidrug resistance in combined therapy. In this work, we develop a mathematical model using a system of ODE incorporated

with a pharmacodynamic model to examine the impact of drug interaction on the evolution of resistance during multidrug therapy. Qualitative analysis shows that there exists bacteria-free equilibrium, P_0, resistant bacteria equilibrium. P_0, resistant bacteria equilibrium. P_1, and endemic equilibrium, P_2, where sensitive and resistant bacteria both co-exist. We found two parameters R_s and R_r, which determine the existence and stability of equilibrium points. R_s define as the number bacteria generated by a fraction of sensitive bacteria that survive the effect of combined antibiotics and immune system response. R_r, define as the number bacteria generated by a fraction of resistant bacteria that survive the effect of combined antibiotics and immune

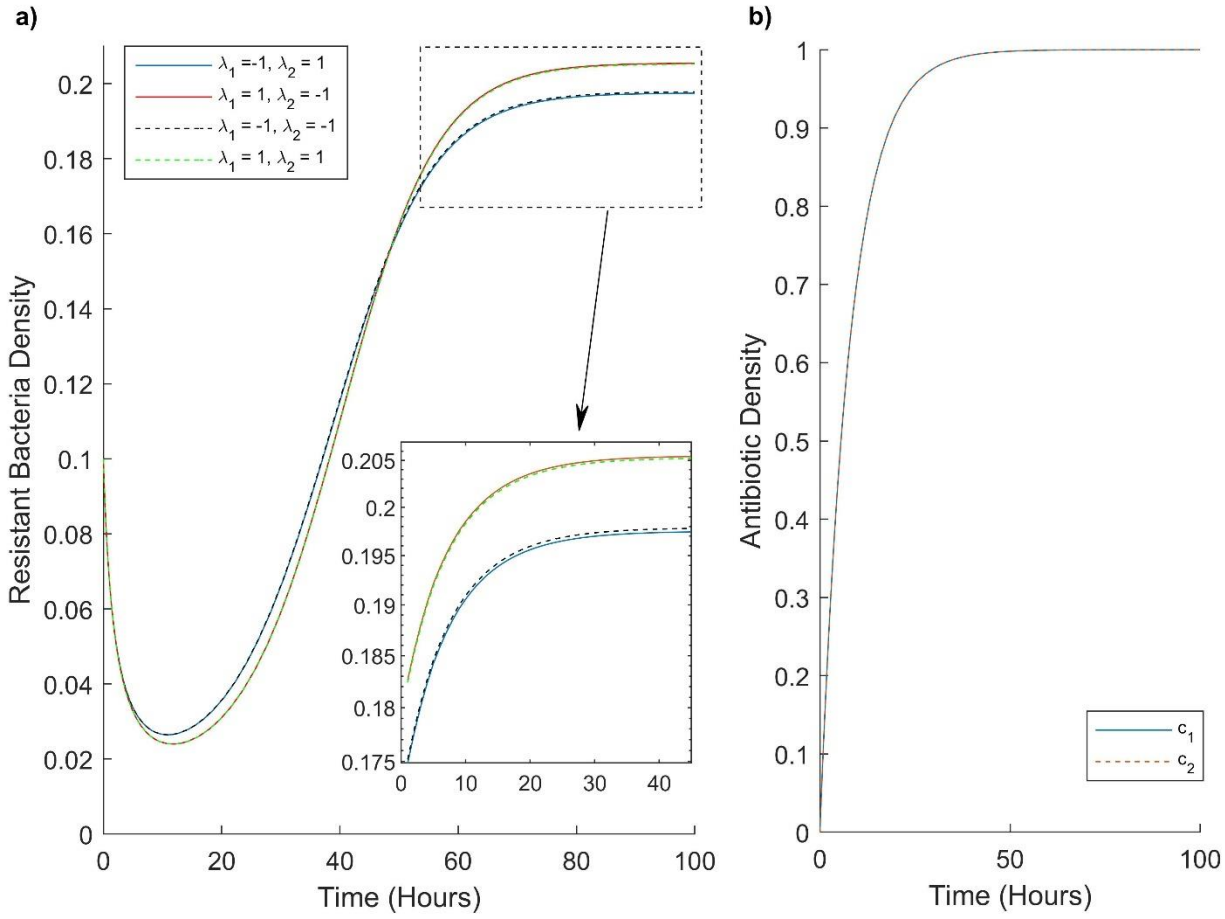

Fig. 6 Temporal course of resistant (*r*) bacteria population under different combination scenarios. **(a)** shows resistance bacteria density over time **(b)** shows antibiotic M (blue line) and N (red dash line) density over time. In **(a)** Blue line shows synergistic (λ_1=1) effect of M and N antibiotics on sensitive bacteria, and antagonistic (λ_2=-1) effect of M and N antibiotics on resistant bacteria. The Red line shows antagonistic (λ_1=-1) effect of M and N antibiotics on sensitive bacteria, and synergistic (λ_2=1) effect of M and N antibiotics on resistant bacteria. Blackline shows antagonistic (λ_1=-1, λ_2=-1) effect of M and N antibiotics on sensitive and resistant bacteria. Greenline synergistic (λ_1=1, λ_2=1) effect of M and N antibiotics on sensitive and resistant bacteria. The rectangular dash point out the resistant bacteria density when the concentration of M and N antibiotics are at their maximum level *(c_1 = c_2 =1)*

system response. When R_s <1 and R_r <1 the solution of system (6) approach bacteria-free equilibrium means that sensitive and resistant bacteria are eliminated by combined antibiotic and immune system response. This result tells as that the net growth rate of bacteria is less than the combined antibiotic inhibitory effect when the interaction between drugs is synergistic $(\lambda_1 > 0, \lambda_2 > 0)$, antagonistic $(\lambda_1 < 0, \lambda_2 < 0)$, or additive $(\lambda_1 = 0, \lambda_2 = 0)$. When $R_s < R_r$ and R_r >1, solution of system (6) approach resistant bacteria equilibrium P_1, means infection caused only by resistant bacteria. When R_s >1 and $R_s > R_r$ the system (6) solution approaches endemic equilibrium P_2.

Hegreness M. et al. (Matthew Hegreness et al. 2008) and Remy Chait et al. [Remy Chait et al. 2007] found that the evolution of antibiotic resistance grows slower if drugs interact antagonistically with the wild-type. Pena-Miller et al. (Rafael Pena-Miller et al. 2013) conclude that synergistic interaction changes to antagonistic interaction. Thus, antagonistic interaction in the mutants are preferred. Our results clarify that antagonistic relation with the wild-type has a more critical role in the therapy outcome than the mutant (see Fig, 5).

In addition, Hegreness M. et al. (Matthew Hegreness et al. 2008) found that the evolution of antibiotic resistance grows faster during synergistic interaction. This acceleration is the selective advantage for resistance mutations during synergistic interaction. Our results confirm their findings since we show that at the minimum inhibitory concentration (MIC) level, the growth rate of resistant bacteria increases due to the enhancement of the synergistic effect of antibiotics on wild-type bacteria and the antagonistic effect on mutants (see Fig. 5).

Moreover, Torella et al. (Joseph Peter Torella et al. 2010) found that the synergistic interaction shortens the clearing time for a population of susceptible bacteria but increases the competitive advantage for resistant bacteria. Although the antagonistic interaction increases the purification time, it decreases the competitive advantage of resistant bacteria. Our results in Fig. 5 show that mutants under the pressure of antagonistic antibiotics outperform competitively resistant strains.

In this study, we investigate the association between synergistic and antagonistic interaction of antibiotics for wild-type (sensitive bacteria) and mutants (resistance bacteria) on the growth rate of resistant strains. The most important contribution of our work is to clarify that antagonism against wild type has a more critical role. Our analytical results suggest that it would be more appropriate to develop combined therapy strategies against wild type (sensitive bacteria).

The best multidrug therapy that would stand the test time should include very effective drugs that interact antagonistically with the sensitive bacteria. If possible, interact synergistically with the mutant bacteria.

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