Mathematical Biosciences and Engineering

http://www.aimspress.com/journal/MBE

MBE, 16(6): 6623–6653.
DOI: 10.3934/mbe.2019330
Received: 02 May 2019
Accepted: 15 July 2019
Published: 19 July 2019

Research article

Mathematical models for within-host competition of malaria parasites

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Abstract: In this paper, we formulate two within-host infection models to simulate dynamics of the drug sensitive and drug resistant malaria parasites, where the first model solely considers the within-host competition between these two strains, and the second model further considers the immune response. Detailed theoretical analysis of the second model are made, including the existence, stability and bifurcation of the equilibrium, which have also been verified by numerical simulations. Both theoretical and numerical results show that competition or chronic control of drug sensitive parasites could inhibit the evolution of drug resistant ones to some extent. However, if the immune response is considered, periodic solution could be observed, and they will persist for all relatively small treatment rate. This may lead to the recurrence of resistance for the chronic control strategy, even though it could delay the resistance emergence. In addition, global sensitivity analysis is implemented to provide the information on the significance of model parameters on the state variables.

Keywords: within-host model; drug resistance; competition; immune response; sensitivity analysis

1. Introduction

According to the World Malaria Report 2017 [1], an estimated 216 million cases of malaria occurred worldwide, and an estimated 445,000 deaths resulted from malaria globally. Reducing mortality and morbidity rates is a great challenge in malaria control. Currently, the principle tools for malaria eradication are antimalarial drugs and their combinations. Chloroquine and pyrimethamine, the two key roles of antimalarial drugs, have become less effective with monotherapy treatment during the last few decades in many countries. Artemisinin, an alternative antimalarial drug, shows a high eliminate rate of malaria parasites, based on its strong ability to kill almost all of the asexual stages of parasite development in the blood. However, drug resistance is still inescapable. Therefore, it is critical to balance the relationship between the drug use and the evolution of resistance effectively, thereby extending the useful lifespan of antimalarial drugs.

The effective lifespan of antimalarial drugs relies not only on the probability of the emergence

of drug resistance from the very beginning, but also on the spread rate of drug resistant parasites in the population [2]. High-dose of antimalarial chemotherapy, which aims at cleaning the whole sensitive parasites as soon as possible, is a clearly feasible regimen [3]. However, it may be a risky strategy once some resistant parasites survive, because the antimalarial drugs could help drug resistant parasites to remove drug sensitive competitors [4]. This may result in competitive release of drug resistant parasites [5, 6]. Competition between drug sensitive and drug resistant parasites, if it occurs, would be a pivotal factor affecting the spread of drug resistance. The reason is that the fitness cost of resistance in untreated hosts and benefits of resistance in treated hosts increase significantly by competitive interaction [7, 8]. Low-dose of antimalarial chemotherapy, allowing several drug sensitive parasites alive to compete with resistant ones for suppressing the rise of them, may be a viable treatment to better manage the drug resistance. The hardest part of the treatment is to find a critical point that could keep a balance between felicitously alleviating symptoms and suppressing resistant parasites [3].

Additionally, it is worthwhile to focus on the host immunity that contributes to the process of parasites clearance and resistance management [9]. Immune response may work on narrowing down the mutant-selection window and cleaning resistant parasites [10]. As a vital part of immunity, antibodies can target the sporozoite stage, decrease the infection rate and parasite proliferation rate as well as the number of blood-stage parasites [9]. In the absence of the immune response, the selection of resistant mutants may increase to a higher level [11]. Yet, a quantitative understanding which treats host immunity as a player in optimal treatment of resistant infections remains under-developed [12].

Mathematical models often show their convenience and flexibility as a way to characterize interactions between different state variables. In [13], Mackinnon et al. presented an epidemiological model to explore the drug resistance in malaria sexual cycle(i.e. from one host to the next) by tracking the relative size of host population infected with resistant parasites. The model not only incorporated two types of parasites (drug sensitive and drug resistant), but also considered the effect of drug treatment.The influence of treatment on drug resistance was further analyzed in [14]. Moreover, in order to explore whether the competitive release could contribute to the spread of resistance, Hansen et al. [15] presented a general, between-host epidemiological model that explicitly took into account the effect of coinfection and competitive release. For the sake of studying the effect of immunity, Chiyaka et al. [16] considered two intra-host models of malaria: with and without immune response, and extended the two models incorporating the antimalarial drug treatment to analyze the relationship between the drug efficacy level and infection elimination. And Li et al. [17] studied the blood-stage dynamics of malaria in an infected host by considering the immune effector. Plenty of theoretical analysis and numerical simulations were made to reveal how immune cells interacted with infected red blood cells and merozoites. In addition, Bushman et al. [18] modeled immune responses by developing a nested individual-based model consisted of a population of human hosts. They concluded that within-host competition was a key factor in shaping the evolution of drug resistance in P. falciparum.

In this paper, we develop mathematical models to understand the evolution of drug resistance within an infected host by considering competition (which is between drug sensitive parasites and drug resistant parasites), drug treatment, and immune response. In order to explicit the effect of immunity, we construct two models with and without considering the immune response, and make a detailed theoretical analysis of the two systems, including the existence of equilibrium and their stability as well as Hopf bifurcation for the system with immunity. Later, these results are verified by numerical simulations, indicating that competition could inhibit the evolution of drug resistant parasites to some

extent. Therefore, appropriate treatment, which allows some sensitive parasites to remain to suppress resistant ones, would delay the emergence of resistance. If the aggressive drug treatment is adopted, a completely opposite result that the competitive release of drug resistant parasites is obtained. These results are in line with the experimental outcomes from [8]. However, when the immune response is considered, periodic solution, caused by Hopf bifurcation, are observed for relatively small treatment rates. Moreover, numerical simulations indicate that the direction of Hopf bifurcation is backward and the Hopf branch can be extended for all the parameter values less than the critical bifurcation value. This implies that the recurrence of resistance may happen for the chronic control strategy, even though it could delay the spread of resistance. Hence, a reasonable and appropriate level of the adjustment of drug dose is of great importance in practice. Finally, sensitivity analysis is performed to identify the relative significant parameters on outcome variables, which can provide a reference to make an optimal policy on resistance management and disease control.

The paper is organized as follows. Two mathematical models are formulated and analyzed in section 2. In particular, we perform detailed analysis for the system with immunity, finding out that Hopf bifurcation could occur under appropriate conditions. Theoretical results are numerically verified in section 3. Other than this, global sensitivity analysis, the global extension of Hopf bifurcation branch are also carried out. The main results of this paper and the potential application are discussed in section 4. In section 5, we list some long but tedious formulas for the coefficients that are used in the stability analysis.

2. Model formulation

We construct two within-host dynamical models of malaria parasite infection. The first model aims to study issues related to the evolution of drug resistance in the absence of immunity. The second model explores the effect of the immune response on the spread of drug resistance completely based on the first model. It is normally supposed that the parasites population consists of diversiform of strains, which are classified as sensitive and resistant from the phenotype. An individual host could have different types of infections, for instance, a single strain is comprised of all the same types of parasites, hence, infection with one identical type is regarded as a single strain infection, while two identical types are regarded as mixed strain infection.

2.1. Within-host model in the absence of immune response

The ODEs system is employed to describe the dynamics of within-host infection, including three cell populations: uninfected red blood cells, $S(t)$; drug sensitive malaria parasites, $I_s(t)$; drug resistant malaria parasites, $I_r(t)$. The ODEs for the within-host model is as follows:

$$\begin{aligned} S'(t) &= \Lambda - \beta_1 S I_s - \beta_2 S I_r - d_1 S, \\ I_s'(t) &= \alpha\beta_1 S I_s + p\alpha\beta_2 S I_r - \gamma_1 I_s I_r - (d_2 + \mu) I_s, \\ I_r'(t) &= (1-p)\alpha\beta_2 S I_r - \gamma_2 I_s I_r - d_3 I_r, \end{aligned} \tag{2.1}$$

The model (2.1) assumes that new RBCs have a production rate is Λ with a natural life expectancy of $1/d_1$ days. The uninfected RBCs become infected by drug sensitive parasites and drug resistant parasites with rates of β_1 and β_2 respectively. Merozoites released from the liver cells invade RBCs to

intake nutrients for fissure reproduction, eventually growing up to a mature schizont to burst RBCs and releasing a number of merozoties by a single infected red blood cell. The burst size is measured by α. Unlike the bacteria, the parasite is genetically unstable from one generation to the next so that a red blood cell infected by resistant stain may produce offspring which are drug sensitive [6]. The parameter p is the proportion of drug sensitive parasites released from the red blood cell infected by resistant parasites. Since resources and ecology space are limited within a host, hence, strains in a mixed infection have to compete with each other. Our model also depicts the influence of competition by using $\gamma_1 I_s(t)I_s(t)$ and $\gamma_1 I_s(t)I_s(t)$, which is convenient for us to explore how the competition influences the evolution of drug resistance. The drug resistant parasites and sensitive parasites die at a rate of d_2 and d_3. When malaria is treated by antimalarial drugs, the intensity of antimalarial drug use is measured by μ. Parameters and their biological interpretations are given in Table 1. Moreover, it is worthwhile to note that most within-host dynamical models take infected red blood cells into account [17, 19], and the dynamic behavior of the within-host system can be described in more detail in this way. However, our paper mainly focuses on the interaction between the drug resistant parasites and drug sensitive parasites, hence for the simplification, the infected red blood cells are not involved in our model.

Table 1. Parameters in model.

Para	Definition	Estimate value	Ref
α	number of merozoites that an infected RBC can produce	30 /cell	[20]
β_1	infection rate of drug sensitive malaria parasites	9.2×10^{-10} μl/cell/day	[20]
β_2	infection rate of drug resistant malaria parasites	9.52×10^{-9} μl/cell/day	[20]
γ_1	Competitive coefficient	varies	
γ_2	Competitive coefficient	varies	
Λ	production rate of RBC	4.15×10^4 cells/μl/day	[17]
d_1	decay rate of RBC	8.33×10^{-3}/day	[21]
d_2	decay rate of drug sensitive malaria parasites	0.15/day	[21]
d_3	decay rate of drug resistant malaria parasites	0.17/day	[21]
μ	the level of drug treatment	varies	
p	proportion of drug sensitive parasites released from an infected RBC by drug-resistance parasites	0.42	estimated
b_1	removal rate of drug sensitive parasites by immune system	5×10^{-9} μl/cell/day	[17]
b_2	removal rate of drug resistant parasites by immune system	5×10^{-9} μl/cell/day	[17]
c_1	proliferation rate of immune cells by drug sensitive parasites	4.5×10^{-5} μl/cell/day	[17]
c_2	proliferation rate of immune cells by drug resistant parasites	5×10^{-6} μl/cell/day	[17]
θ	$1/\theta$ half saturation constant for drug sensitive parasites	10^{-4} μl/cell	[17]
δ	$1/\delta$ half saturation constant for drug sensitive parasites	10^{-4} μl/cell	[17]
d_4	decay rate of immune cells	$0.01/day$	[21]

To determine the interior equilibrium $P^* = (S^*, I_s^*, I_r^*)$ of system (2.1), we set the right hand side of (2.1) be zero. It then follows from the last two equation of (2.1) that

$$I_s^* = \frac{(1-p)\alpha\beta_2 S^* - d_3}{\gamma_2} \tag{2.2}$$

and

$$I_r^* = \frac{(\alpha\beta_1 S^* - d_2 - \mu)[(1-p)\alpha\beta_2 S^* - d_3]}{\gamma_1[(1-p)\alpha\beta_2 S^* - d_3] - p\alpha\beta_2\gamma_2 S^*}. \tag{2.3}$$

Substituting I_r^* and I_s^* into the first equation of (2.1), we get that S^* satisfies

$$f(S^*) := k_1 S^{*3} + k_2 S^{*2} + k_3 S^* + k_4 = 0, \tag{2.4}$$

where

$$\begin{aligned}
k_1 =& -\alpha^2\beta_1\beta_2^2(1-p)^2(\gamma_1+\gamma_2),\\
k_2 =& \alpha\beta_2(1-p)[2\beta_1\gamma_1 d_3 + \beta_1\gamma_2 d_3 + \beta_2\gamma_2(d_2+\mu)] - \alpha\beta_2\gamma_2 d_1[\gamma_1(1-p) - p\gamma_2],\\
k_3 =& \Lambda\alpha\beta_2\gamma_2[\gamma_1(1-p) - p\gamma_2] - \beta_1\gamma_1 d_3^2 - \beta_2\gamma_2 d_3(d_2+\mu) + \gamma_1\gamma_2 d_1 d_3,\\
k_4 =& -\Lambda\gamma_1\gamma_2 d_3.
\end{aligned}$$

2.2. Within-host model in the presence of immune response

Host immunity against malaria parasite is complicated and stage-specific. Many interdependent players, such as different cell types and cytokines, participate at varying degrees [10]. For the sake of simplicity, pathogen-immune interactions are typically treated as predator-prey interactions, which has been studied in many literatures [22, 23]. The two types of parasites played the prey role against a shared predator species (immune cells). Then the dynamics of the host immunity can be described as follows:

$$\begin{aligned}
S'(t) &= \Lambda - \beta_1 S I_s - \beta_2 S I_r - d_1 S,\\
I_s'(t) &= \alpha\beta_1 S I_s + p\alpha\beta_2 S I_r - \gamma_1 I_s I_r - (d_2+\mu) I_s - \frac{b_1 I_s E}{1+\theta I_s},\\
I_r'(t) &= (1-p)\alpha\beta_2 S I_r - \gamma_2 I_s I_r - d_3 I_r - \frac{b_2 I_r E}{1+\delta I_r},\\
E'(t) &= \frac{c_1 I_s E}{1+\theta I_s} + \frac{c_2 I_r E}{1+\delta I_r} - d_4 E,
\end{aligned} \tag{2.5}$$

From the above dynamical model, the function of $\frac{b_1 I_s E}{1+\theta I_s}$ and $\frac{b_2 I_r E}{1+\delta I_r}$ represent the elimination of drug sensitive parasites I_s and drug resistant parasites I_r by immune cells E respectively, where b_1 and b_2 are the removal rate of drug sensitive parasites and resistant ones, $1/\theta$ and $1/\delta$ are saturation constants that simulate immune cells to grow at half their maximum rate [17]. We assume that the net multiplication rate of immune cells stimulated by the drug sensitive parasites and sensitive ones are $\frac{c_1 I_s E}{1+\theta I_s}$ and $\frac{c_2 I_r E}{1+\delta I_r}$, where k_1 and k_2 are the multiplication rate of lymphocytes due to the interactions between immune cells and drug sensitive parasites and drug resistant parasites respectively. The immune cells have a death rate of d_4. In addition, the meaning of other terms in this model can refer to the Eq (2.1). Parameters and their biological interpretations are given in Table 1. In this case, to compute the specific expression of the interior equilibrium is extremely difficult, hence we mainly utilize the numerical method to analyze it.

2.3. Dynamical behavior of the system

In order to observe the dynamical behavior of system (2.5), we first study the existence of equilibrium of system (2.5) in R_+^4, where $R_+^4 = \{(S, I_s, I_r, E) : S \geq 0, I_s \geq 0, I_r \geq 0, E \geq 0\}$. Note that the equilibrium of system (2.5) must satisfy the following equations.

$$\begin{aligned}
&\Lambda - \beta_1 S I_s - \beta_2 S I_r - d_1 S = 0,\\
&\alpha\beta_1 S I_s + p\alpha\beta_2 S I_r - \gamma_1 I_s I_r - (d_2+\mu) I_s - \frac{b_1 I_s E}{1+\theta I_s} = 0,\\
&(1-p)\alpha\beta_2 S I_r - \gamma_2 I_s I_r - d_3 I_r - \frac{b_2 I_r E}{1+\delta I_r} = 0,\\
&\frac{c_1 I_s E}{1+\theta I_s} + \frac{c_2 I_r E}{1+\delta I_r} - d_4 E = 0,
\end{aligned} \tag{2.6}$$

Following the references[24] and [25], we obtain the basic reproduction number of our system, which is

$$R_0 = \rho(FV^{-1}) = \max\left\{\frac{\Lambda\alpha\beta_1}{d_1(d_2+\mu)}, \frac{\Lambda\alpha\beta_2(1-p)}{d_1 d_3}\right\} := \max\{R_1, R_2\}.$$

Then we can have the following Lemma.

Lemma 2.1. *System (2.5) has a unique non-negative equilibrium which is the malaria-free equilibrium* $P_0 = (\Lambda/d_1, 0, 0, 0)$, *if* $R_0 \leq 1$ *and at least two equilibria if* $R_0 > 1$. *More precisely,*

(*A*) *system (2.5) has two equilibria:* $P_0 = (\Lambda/d_1, 0, 0, 0)$ *and* $P_1 = (S_1, I_{s_1}, 0, 0)$ *if* $R_1 > 1$, *where* $S_1 = \frac{\Lambda}{d_1 R_1}$, $I_{s_1} = \frac{(R_1-1)d_1}{\beta_1}$;

(*B*) *system (2.5) has three equilibria:* $P_0 = (\Lambda/d_1, 0, 0, 0)$, $P_1 = (S_1, I_{s_1}, 0, 0)$ *and* $P_3 = (S_3, I_{s_3}, 0, E_3)$ *if* $R_1 > \max\{L, 1\}$, *where* $L = \frac{\beta_1}{d_1}(c_1 - d_4\theta) + d_4$, $S_3 = \frac{\Lambda}{\beta_1(c_1-d_4\theta)+d_1 d_4}$, $I_{s_3} = \frac{c_1-d_4\theta}{d_4}$, $E_3 = \frac{1}{b_1}(\alpha\beta_1 S_3 - (d_2+\mu))(1+\theta I_{s_3})$;

(*C*) *system (2.5) has four equilibria:* $P_0 = (\Lambda/d_1, 0, 0, 0)$, $P_1 = (S_1, I_{s_1}, 0, 0)$, $P_2 = (S_2, I_{s_2}, I_{r_2}, 0)$ *and* $P_3 = (S_3, I_{s_3}, 0, E_3)$ *if* $R_1 > \max\{L, 1\}$, $R_2 > 1$ *and* $k_1 {S_2}^3 + k_2 {S_2}^2 + k_3 S_2 + k_4 = 0$ *has a positive solution* S_2 *with*

(*H*1) $\frac{d_3}{(1-p)\alpha\beta_2} < S_2 < \min\left\{\frac{d_2+\mu}{\alpha\beta_1}, \frac{\gamma_1 d_3}{\alpha\beta_2(\gamma_1(1-p)-\gamma_2 p)}\right\}$; *or*

(*H*2) $\max\left\{\frac{d_2+\mu}{\alpha\beta_1}, \frac{d_3}{(1-p)\alpha\beta_2}\right\} < S_2 < \frac{\gamma_1 d_3}{\alpha\beta_2(\gamma_1(1-p)-\gamma_2 p)}$

where $I_{s_2} = \frac{(1-p)\alpha\beta_2 S_2 - d_3}{\gamma_2}$, $I_{r_2} = \frac{(\alpha\beta_1 S_2 - d_2 - \mu)((1-p)\alpha\beta_2 S_2 - d_3)}{\gamma_1((1-p)\alpha\beta_2 S_2 - d_3) - p\alpha\beta_2\gamma_2 S_2}$;

(*D*) *system (2.5) has five equilibria:* $P_0 = (\Lambda/d_1, 0, 0, 0)$, $P_1 = (S_1, I_{s_1}, 0, 0)$, $P_2 = (S_2, I_{s_2}, I_{r_2}, 0)$, $P_3 = (S_3, I_{s_3}, 0, E_3)$ *and* $P_4 = (S_4, I_{s_4}, I_{r_4}, E_4)$, *if* $R_1 > \max\{L, 1\}$, $R_2 > 1$ *and* $k_1 {S_2}^3 + k_2 {S_2}^2 + k_3 S_2 + k_4 = 0$ *has a positive solution* S_2 *satisfying (H1) or (H2), moreover,* $l_1 {I_{r_4}}^5 + l_2 {I_{r_4}}^4 + l_3 {I_{r_4}}^3 + l_4 {I_{r_4}}^2 + l_5 I_{r_4} + l_6 = 0$ *has a positive solution* I_{r_4} *satisfying*

(*H*3) $c_2 - d_4\delta > 0$, $\Delta < 0$ *and* $S_4 > \frac{\gamma_2 I_{r_4} + d_3}{(1-p)\alpha\beta_2}$; *or*

(*H*4) $c_2 - d_4\delta > 0$, $\Delta \geq 0$, $I_{r_{4(1,2)}} = \frac{-(\beta_2 e_2 - \beta_1 e_3 + d_1) \pm \sqrt{\Delta}}{2\beta_2}$, *and without loss of generality, let* $I_{r_{4(1)}} > I_{r_{4(2)}}$, *if* $I_{r_4} > I_{r_{4(1)}}$ *or* $I_{r_4} < I_{r_{4(2)}}$, *and* $S_4 > \frac{\gamma_2 I_{r_4} + d_3}{(1-p)\alpha\beta_2}$;

where

$$
\begin{aligned}
S_4 =& \frac{\Lambda}{\beta_1 I_{s_4} + \beta_2 I_{r_4} + d_1}\ , I_{s_4} = \frac{e_1 - e_3 I_{r_4}}{e_2 + I_{r_4}}\ , E_4 = \frac{1}{b_2}((1-p)\alpha\beta_2 S_4 - \gamma_2 I_{s_4} - d_3)(1 + \delta I_{r_4}),\\
e_1 =& \frac{d_4}{c_1\delta + c_2\theta - d_4\delta\theta},\ e_2 = \frac{c_1 - d_4\theta}{c_1\delta + c_2\theta - d_4\delta\theta},\ e_3 = \frac{c_2 - d_4\delta}{c_1\delta + c_2\theta - d_4\delta\theta},\\
\Delta =& (\beta_2 e_2 - \beta_1 e_3 + d_1)^2 - 4\beta_2(\beta_1 e_1 + d_1 e_2),
\end{aligned}
$$

and the expressions of l_1 to l_6 are shown in (2.20).

Proof. The existence of P_0 can be obtained directly from (2.6) by setting $I_s = I_r = E = 0$, the existence of P_1 can be obtained from (2.6) by setting $I_r = E = 0$, and the existence of P_3 can be obtained from (2.6) by setting $I_r = 0$. Then we would seek conditions for the existence of P_2 and P_4 of (2.6). For the existence of P_2, we have $P_2 = (S_2, I_{s_2}, I_{r_2}, 0)$ as setting $E_2 = 0$. The following formulations of I_{s_2} and I_{r_2} are obtained from (2.6), where

$$
I_{s_2} = \frac{(1-p)\alpha\beta_2 S_2 - d_3}{\gamma_2}, \tag{2.7}
$$

$$
I_{r_2} = \frac{(\alpha\beta_1 S_2 - d_2 - \mu)((1-p)\alpha\beta_2 S_2 - d_3)}{\gamma_1((1-p)\alpha\beta_2 S_2 - d_3) - p\alpha\beta_2\gamma_2 S_2}, \tag{2.8}
$$

It is clear that $I_{s_2} > 0$ and $I_{r_2} > 0$ if the following conditions hold:

$$
\begin{aligned}
&R_1 > \max\left\{\frac{\beta_1}{d_1}(c_1 - d_4\theta) + d_4, 1\right\}, R_2 > 1, S_2 > 0,\\
&(1-p)\alpha\beta_2 S_2 - d_3 > 0,\\
&\alpha\beta_1 S_2 - d_2 - \mu > 0,\\
&\gamma_1((1-p)\alpha\beta_2 S_2 - d_3) - p\alpha\beta_2\gamma_2 S_2 > 0.
\end{aligned} \tag{2.9}
$$

From (2.9), we have

$$
\begin{aligned}
&\frac{d_3}{(1-p)\alpha\beta_2} < S_2 < \min\left\{\frac{d_2+\mu}{\alpha\beta_1}, \frac{\gamma_1 d_3}{\alpha\beta_2(\gamma_1(1-p) - \gamma_2 p)}\right\} or\\
&\max\left\{\frac{d_2+\mu}{\alpha\beta_1}, \frac{d_3}{(1-p)\alpha\beta_2}\right\} < S_2 < \frac{\gamma_1 d_3}{\alpha\beta_2(\gamma_1(1-p) - \gamma_2 p)},
\end{aligned} \tag{2.10}
$$

Now, we discuss the existence of S_2. Substituting (2.7) and (2.8) into the first equation of (2.6), we obtain that

$$
k_1 {S_2}^3 + k_2 {S_2}^2 + k_3 S_2 + k_4 = 0, \tag{2.11}
$$

where

$$
\begin{aligned}
k_1 =& -\alpha^2\beta_1\beta_2^2(1-p)^2(\gamma_1 + \gamma_2) < 0,\\
k_2 =& \alpha\beta_2(1-p)[2\beta_1\gamma_1 d_3 + \beta_1\gamma_2 d_3 + \beta_2\gamma_2(d_2+\mu)] - \alpha\beta_2\gamma_2 d_1[\gamma_1(1-p) - p\gamma_2],\\
k_3 =& \Lambda\alpha\beta_2\gamma_2[\gamma_1(1-p) - p\gamma_2] - \beta_1\gamma_1 d_3^2 - \beta_2\gamma_2 d_3(d_2+\mu) + \gamma_1\gamma_2 d_1 d_3,\\
k_4 =& -\Lambda\gamma_1\gamma_2 d_3 < 0.
\end{aligned}
$$

To sum up, if $R_1 > \max\{L, 1\}$, $R_2 > 1$ and (2.11) has a positive solution S_2 with condition (2.9), then $P_2 = (S_2, I_{s_2}, I_{r_2}, 0)$ is the equilibrium of (2.6), which implies statement (C) holding. Next, we discuss the existence of the positive equilibrium $P_4 = (S_4, I_{s_4}, I_{r_4}, E_4)$ of system (2.5). Suppose that $(S_4, I_{s_4}, I_{r_4}, E_4)$ is a positive solution of (2.6). From (2.6), we have

$$\begin{aligned} S_4 &= \frac{\Lambda}{\beta_1 I_{s_4} + \beta_2 I_{r_4} + d_1} = \frac{\Lambda(e_2 + I_{r_4})}{\beta_2 I_{r_4}^2 + (\beta_2 e_2 + d_1 - \beta_1 e_3) I_{r_4} + \beta_1 e_1 + d_1 e_2}, \\ I_{s_4} &= \frac{e_1 - e_3 I_{r_4}}{e_2 + I_{r_4}}, \\ E_4 &= \frac{1}{b_2}((1-p)\alpha\beta_2 S_4 - \gamma_2 I_{s_4} - d_3)(1 + \delta I_{r_4}), \end{aligned} \tag{2.12}$$

It is clear that $S_4 > 0$, $I_{s_4} > 0$ and $E_4 > 0$ if the following conditions hold:

$$I_{r_4} > 0, \quad e_1 - e_3 I_{r_4} > 0, \tag{2.13}$$

$$(1-p)\alpha\beta_2 S_4 - \gamma_2 I_{s_4} - d_3 > 0, \tag{2.14}$$

$$\beta_2 I_{r_4}^2 + (\beta_2 e_2 + d_1 - \beta_1 e_3) I_{r_4} + \beta_1 e_1 + d_1 e_2 > 0. \tag{2.15}$$

From (2.13), we obtain that

$$0 < I_{r_4} < \frac{e_1}{e_3} = \frac{d_4}{c_2 - d_4\delta}, \tag{2.16}$$

and hence, from (2.16), we infer that

$$c_2 - d_4\delta > 0. \tag{2.17}$$

Based from (2.14), we obtain that

$$S_4 > \frac{\gamma_2 I_{r_4} + d_3}{(1-p)\alpha\beta_2}. \tag{2.18}$$

The (2.15) can be held in the following two cases, we first let

$$F(I_{r_4}) = \beta_2 I_{r_4}^2 + (\beta_2 e_2 + d_1 - \beta_1 e_3) I_{r_4} + \beta_1 e_1 + d_1 e_2.$$

Then we analyze the roots of $F(I_{r_4}) = 0$, set $\Delta = (\beta_2 e_2 - \beta_1 e_3 + d_1)^2 - 4\beta_2(\beta_1 e_1 + d_1 e_2)$,

(*I*) Note that $F(I_{r_4}) = 0$ has two real roots $I_{r_{4(1,2)}}$ if $\Delta > 0$, where

$$I_{r_{4(1,2)}} = \frac{-(\beta_2 e_2 - \beta_1 e_3 + d_1) \pm \sqrt{\Delta}}{2\beta_2},$$

when $\beta_2 e_2 - \beta_1 e_3 + d_1 > 0$, the two roots of $F(I_{r_4}) = 0$ are negative, then P_4 exists if $I_{r_4} > 0$ and both (2.17) and (2.18) hold. When $\beta_2 e_2 - \beta_1 e_3 + d_1 < 0$, the two roots of $F(I_{r_4}) = 0$ are positive, we assume that $I_{r_{4(1)}} > I_{r_{4(2)}}$, then P_4 exists if $I_{r_4} > I_{r_{4(1)}}$ or $I_{r_4} < I_{r_{4(1)}}$ and both (2.17) and (2.18) hold.

(*II*) Note that $F(I_{r_4}) = 0$ has no roots if $\Delta < 0$, then P_4 exists if both (2.17) and (2.18) hold.

Substituting (2.12) into the third equation of (2.6), we have the following equation

$$l_1 {I_{r_4}}^5 + l_2 {I_{r_4}}^4 + l_3 {I_{r_4}}^3 + l_4 {I_{r_4}}^2 + l_5 I_{r_4} + l_6 = 0, \tag{2.19}$$

where

$$\begin{aligned}
l_1 =& -\frac{\Big(b_1\beta_2\delta(d_3+\gamma_2)+b_2\beta_2\gamma_1\theta\Big)(c_2-d_4\delta)^2}{(c_1\delta+c_2\theta-d_4\delta\theta)^2},\\
l_2 =& \frac{\Big(\alpha b_1\beta_2\delta\Lambda(p-1)-\alpha b_2\beta_2\Lambda p\theta\Big)(c_2-d_4\delta)}{(c_1\delta+c_2\theta-d_4\delta\theta)}+\frac{\Big(b_1\beta_1\delta(d_3+\gamma_2)+b_2\beta_1\gamma_1\theta\Big)(c_2-d_4\delta)^3}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}\\
&-\frac{(c_2-d_4\delta)^2}{(c_1\delta+c_2\theta-d_4\delta\theta)^2}\Big(b_1\beta_2(d_3+\gamma_2)+b_2\beta_1\gamma_1+b_2\theta(d_1\gamma_1+\beta_2(d_2+\mu))+b_1d_1\delta(d_3+\gamma_2)\Big)\\
&-\Big(b_1\beta_2\delta(d_3+\gamma_2)+b_2\beta_2\gamma_1\theta\Big)\left(\frac{(c_2-d_4\delta)^2(c_1-d_4\theta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}-\frac{2d_4(c_2-d_4\delta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^2}\right)+\alpha b_2\beta_2\Lambda p,\\
l_3 =& \frac{c_2-d_4\delta}{c_1\delta+c_2\theta-d_4\delta\theta}\Big(b_2(d_1\gamma_1+\beta_2(d_2+\mu)+d_1(d_2+\mu)-\alpha\beta_1\Lambda+\beta_2\gamma_1)+\alpha b_1\beta_2\Lambda(p-1)\Big)\\
&-\frac{2d_4(c_2-d_4\delta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^2\Big(b_1\beta_2(d_3+\gamma_2)+b_2\beta_1\gamma_1+b_2\theta(d_1\gamma_1+\beta_2(d_2+\mu))+b_1d_1\delta(d_3+\gamma_2)\Big)}\\
&-\Big(\alpha b_1\beta_2\delta\Lambda(p-1)-d_2\alpha b_2\beta_2\Lambda p\theta\Big)\left(\frac{d_4}{c_1\delta}+c_2\theta-d_4\delta\theta-\frac{2(c_2-d_4\delta)(c_1-d_4\theta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^2}\right)\\
&-\frac{(c_2-d_4\delta)^2}{(c_1\delta+c_2\theta-d_4\delta\theta)^2}\Big(b_2\theta(d_1(d_2+\mu)-\alpha\beta_1\Lambda)+b_1d_1(d_3+\gamma_2)+b_2\beta_1(d_2+\mu)\Big)\\
&-\Big(b_1\beta_2\delta(d_3+\gamma_2)+b_2\beta_2\gamma_1\theta\Big)\left(\left(\frac{d_4^2}{c_1\delta}+c_2\theta-d_4\delta\theta\right)^2-\frac{2d_4(c_2-d_4\delta)(c_1-d_4\theta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}\right)\\
&+\frac{(c_2-d_4\delta)^3}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}\Big(b_1\beta_1(d_3+\gamma_2)-b_2\beta_1\theta(d_2+\mu)-3d_4(b_1\beta_1\delta(d_3+\gamma_2)+b_2\beta_1\gamma_1\theta)\Big)\\
&+\frac{(c_1-d_4\theta)}{(c_1\delta+c_2\theta-d_4\delta\theta)}\Big(3\alpha b_2\beta_2\Lambda p-(c_2-d_4\delta)^2\Big),\\
l_4 =& \frac{(c_2-d_4\delta)(c_1-d_4\theta)^2}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}-\frac{2d_4(c_1-d_4\theta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^2\Big(\alpha b_1\beta_2\delta\Lambda(p-1)-\alpha b_2\beta_2\Lambda p\theta\Big)}\\
&-\frac{(c_2-d_4\delta)^2(c_1-d_4\theta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}-\frac{2d_4(c_2-d_4\delta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^2)(b_2\theta(d_1(d_2+\mu)}-\alpha\beta_1\Lambda+b_1d_1(d_3+\gamma_2)\\
&+b_2\beta_1(d_2+\mu)-\frac{d_4}{(c_1\delta+c_2\theta-d_4\delta\theta)}-\frac{2(c_2-d_4\delta)(c_1-d_4\theta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^2b_2(d_1\gamma_1+\beta_2(d_2+\mu))}\\
&+b_2\Big(d_1(d_2+\mu)-\alpha\beta_1\Lambda\Big)+b_2\beta_2\gamma_1+\alpha b_1\beta_2\Lambda(p-1)-\frac{d_4^2}{(c_1\delta+c_2\theta-d_4\delta\theta)^2}\\
&-\frac{2d_4(c_2-d_4\delta)(c_1-d_4\theta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^3b_1\beta_2(d_3+\gamma_2)}+b_2\beta_1\gamma_1+b_2\theta\Big(d_1\gamma_1+\beta_2(d_2+\mu)\Big)+b_1d_1\delta(d_3+\gamma_2)
\end{aligned}$$

$$
\begin{aligned}
&- \frac{3d_4\Big(b_1\beta_1(d_3+\gamma_2)-b_2\beta_1\theta(d_2+\mu)\Big)(c_2-d_4\delta)^2}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}+\frac{3d_4^2\Big(b_1\beta_1\delta(d_3+\gamma_2)+b_2\beta_1\gamma_1\theta\Big)(c_2-d_4\delta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}\\
&- \frac{d_4^2\Big(b_1\beta_2\delta(d_3+\gamma_2)+b_2\beta_2\gamma_1\theta\Big)(c_1-d_4\theta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}+\frac{3\alpha b_2\beta_2\Lambda p(c_1-d_4\theta)^2}{(c_1\delta+c_2\theta-d_4\delta\theta)^2},\\
l_5 =&\frac{(c_2-d_4\delta)(c_1-d_4\theta)^2}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}-\frac{2d_4(c_1-d_4\theta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^2b_2\Big(d_1\gamma_1+\beta_2(d_2+\mu)\Big)}+b_2\Big(d_1(d_2+\mu)-\alpha\beta_1\Lambda\Big)\\
&+b_2\beta_2\gamma_1+\alpha b_1\beta_2\Lambda(p-1)-\frac{d_4^2}{(c_1\delta+c_2\theta-d_4\delta\theta)^2}-\frac{2d_4(c_2-d_4\delta)(c_1-d_4\theta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^3b_2\theta\Big(d_1(d_2+\mu)-\alpha\beta_1\Lambda\Big)}\\
&+b_1d_1(d_3+\gamma_2)+b_2\beta_1(d_2+\mu)-\frac{d_4^3\Big(b_1\beta_1\delta(d_3+\gamma_2)+b_2\beta_1\gamma_1\theta\Big)}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}-\frac{d_4\alpha b_1\beta_2\delta\Lambda(p-1)(c_1-d_4\theta)^2}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}\\
&+\frac{d_4\alpha b_2\beta_2\Lambda p\theta(c_1-d_4\theta)^2}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}+\frac{3d_4^2\Big(b_1\beta_1(d_3+\gamma_2)-b_2\beta_1\theta(d_2+\mu)\Big)(c_2-d_4\delta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}+\frac{\alpha b_2\beta_2\Lambda p(c_1-d_4\theta)^3}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}\\
&-\frac{d_4^2(c_1-d_4\theta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}\Big(b_1\beta_2(d_3+\gamma_2)+b_2\beta_1\gamma_1+b_2\theta(d_1\gamma_1+\beta_2(d_2+\mu))+b_1d_1\delta(d_3+\gamma_2)\Big),\\
l_6 =&-\frac{d_4^3\Big(b_1\beta_1(d_3+\gamma_2)-b_2\beta_1\theta(d_2+\mu)\Big)}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}-\frac{d_4^2(c_1-d_4\theta)b_2\theta}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}\Big(d_1(d_2+\mu)-\alpha\beta_1\Lambda\Big)\\
&-\frac{d_4(c_1-d_4\theta)^2}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}\Big(b_2(d_1\gamma_1+\beta_2(d_2+\mu))+b_2(d_1(d_2+\mu)-\alpha\beta_1\Lambda)+b_2\beta_2\gamma_1+\alpha b_1\beta_2\Lambda(p-1)\Big)\\
&-\frac{d_4^2(c_1-d_4\theta)}{(c_1\delta+c_2\theta-d_4\delta\theta)^3}\Big(b_1d_1(d_3+\gamma_2)+b_2\beta_1(d_2+\mu)\Big).
\end{aligned}
\tag{2.20}
$$

Therefore, according to the above analysis and inference, system (2.6) have a positive solution, which implies statement (*D*) holds. This completes the proof. □

Then we begin to analyze the stability of these equilibria of system (2.5). Computing the Jacobian matrix of system (2.5) at point $P = (S, I_s, I_r, E)$, we have

$$
J(P)=\begin{pmatrix}
-\beta_1 I_s-\beta_2 I_r-d_1 & -\beta_1 S & -\beta_2 S & 0\\
\alpha\beta_1 I_s+p\alpha\beta_2 I_r & A_1 & p\alpha\beta_2 S-\gamma_1 I_s & -\frac{b_1 I_s}{1+\theta I_s}\\
(1-p)\alpha\beta_2 I_r & -\gamma_2 I_r & (1-p)\alpha\beta_2 S-\gamma_2 I_s-d_3-\frac{b_2 E}{(1+\delta I_r)^2} & -\frac{b_2 I_r}{1+\theta I_r}\\
0 & \frac{c_1 E}{(1+\theta I_s)^2} & \frac{c_2 E}{(1+\delta I_r)^2} & \frac{c_1 I_s}{1+\theta I_s}+\frac{c_2 I_r}{1+\delta I_r}-d_4
\end{pmatrix},
$$

where $A_1=\alpha\beta_1 S-\gamma_1 I_r-d_2-\mu-\frac{b_1 E}{(1+\theta I_s)^2}$.

2.3.1. Local stability of the malaria-free equilibrium P_0

At the malaria-free equilibrium $P_0 = (\Lambda/d_1, 0, 0, 0)$, the Jacobian matrix is

$$
J(P_0)=\begin{pmatrix}
-d_1 & -\beta_1\frac{\Lambda}{d_1} & -\beta_2\frac{\Lambda}{d_1} & 0\\
0 & \alpha\beta_1\frac{\Lambda}{d_1}-d_2-\mu & p\alpha\beta_2\frac{\Lambda}{d_1} & 0\\
0 & 0 & (1-p)\alpha\beta_2\frac{\Lambda}{d_1} & 0\\
0 & 0 & 0 & -d_4
\end{pmatrix},
$$

and the characteristic equation is

$$(\Lambda + d_1)\left(\Lambda - \left(\alpha\beta_1\frac{\Lambda}{d_1} - d_2 - \mu\right)\right)\left(\Lambda - \left((1-p)\alpha\beta_2\frac{\Lambda}{d_1} - d_3\right)\right)(\Lambda + d_4) = 0. \tag{2.21}$$

According to (2.21), all eigenvalues could be negative if $R_0 < 1$, and one of the eigenvalues is positive if $R_0 > 1$. Then we can get the following lemma.

Lemma 2.2. *The malaria-free equilibrium P_0 of system (2.5) is locally asymptotically stable if $R_0 < 1$ and unstable if $R_0 > 1$.*

2.3.2. Local stability of the malaria infection equilibrium P_1

If $R_1 > 1$, the system (2.5) has a malaria infection equilibrium $P_1 = (S_1, I_{s_1}, 0, 0)$ with

$$S_1 = \frac{\Lambda}{d_1 R_1}, I_{s_1} = \frac{(R_1 - 1)d_1}{\beta_1}.$$

The Jacobian matrix at P_1 is

$$J(P_1) = \begin{pmatrix} -\beta_1 I_{s_1} - d_1 & -\beta_1 S_1 & -\beta_2 S_1 & 0 \\ \alpha\beta_1 I_{s_1} & \alpha\beta_1 S_1 - d_2 - \mu & p\alpha\beta_2 S_1 - \gamma_1 I_{s_1} & -\frac{b_1 I_{s_1}}{1+\theta I_{s_1}} \\ 0 & 0 & (1-p)\alpha\beta_2 S_1 - \gamma_2 I_{s_1} - d_3 & 0 \\ 0 & 0 & 0 & \frac{c_1 I_{s_1}}{1+\theta I_{s_1}} - d_4 \end{pmatrix},$$

and the characteristic equation is

$$\left(\lambda - \left(\frac{c_1 I_{s_1}}{1+\theta I_{s_1}} - d_4\right)\right)\left(\lambda - \left((1-p)\alpha\beta_2 S_1 - \gamma_2 I_{s_1} - d_3\right)\right)\left(\lambda^2 + m_1\lambda + m_2\right) = 0, \tag{2.22}$$

where $m_1 = \beta_1 I_{s_1} + d_1 - \alpha\beta_1 S_1 + d_2 + \mu, m_2 = \alpha\beta_1^2 S_1 I_{s_1} - (\beta_1 I_{s_1} + d_1)(\alpha\beta_1 S_1 - (d_2 + \mu))$.

By the Routh-Hurwitz criterion, the roots of (2.22) have negative real parts if and only if

$$\frac{c_1 I_{s_1}}{1+\theta I_{s_1}} - d_4 < 0, \ \ (1-p)\alpha\beta_2 S_1 - \gamma_2 I_{s_1} - d_3 < 0, \ \ m_1 > 0, \ \ m_2 > 0. \tag{2.23}$$

On the basis of above analysis and combining with the Lemma 2.1 and 2.2, we have the following theorem.

Theorem 2.1. *If $R_1 > 1$, then system (2.5) has two equilibria: $P_0 = (\Lambda/d_1, 0, 0, 0)$ and $P_1 = (S_1, I_{s_1}, 0, 0)$. Moreover, the malaria-free equilibrium P_0 is unstable, and the malaria infection equilibrium P_1 is locally asymptotically stable if the inequalities in (2.23) hold.*

2.3.3. Local stability of the malaria infection equilibrium P_2

From lemma 2.1, we know that system (2.5) has four equilibria: $P_0 = (\Lambda/d_1, 0, 0, 0)$, $P_1 = (S_1, I_{s_1}, 0, 0)$, $P_2 = (S_2, I_{s_2}, I_{r_2}, 0)$ and $P_3 = (S_3, I_{s_3}, 0, E_3)$.

The local stability of P_2 is established from the Jacobian matrix at P_2, which is given by

$$J(P_2) = \begin{pmatrix} -\beta_1 I_{s_2} - \beta_2 I_{r_2} - d_1 & -\beta_1 S_2 & -\beta_2 S_2 & 0 \\ \alpha\beta_1 I_{s_2} + p\alpha\beta_2 I_{r_2} & \alpha\beta_1 S_2 - \gamma_1 I_{r_2} - d_2 - \mu & p\alpha\beta_2 S_2 - \gamma_1 I_{s_2} & -\frac{b_1 I_{s_2}}{1+\theta I_{s_2}} \\ (1-p)\alpha\beta_2 I_{r_2} & -\gamma_2 I_{r_2} & (1-p)\alpha\beta_2 S_2 - \gamma_2 I_{s_2} - d_3 & -\frac{b_2 I_{r_2}}{1+\theta I_{r_2}} \\ 0 & 0 & 0 & \frac{c_1 I_{s_2}}{1+\theta I_{s_1}} + \frac{c_2 I_{r_2}}{1+\delta I_{r_1}} - d_4 \end{pmatrix},$$

and the characteristic equation is

$$\lambda^4 + r_1\lambda^3 + r_2\lambda^2 + r_3\lambda + r_4 = 0, \tag{2.24}$$

where the expressions of r_1, r_2, r_3 and r_4 are shown in Appendix due to their complexity.

By using the Routh-Hurwitz criterion, the roots of (2.24) have negative real parts if and only if

$$r_1 > 0, \;\; r_1 r_2 - r_3 > 0, \;\; (r_1 r_2 - r_3) r_3 - r_1^2 r_4 > 0, \;\; (r_1 r_2 - r_3) r_3 r_4 - r_1^2 r_4^2 > 0. \tag{2.25}$$

Hence, we obtain the following theorem based on above analysis and Lemma 2.1 and 2.2.

Theorem 2.2. *If $R_1 > \max\left\{\frac{\beta_1}{d_1}(c_1 - d_4\theta) + d_4, 1\right\}$, $R_2 > 1$ and $k_1 {S_2}^3 + k_2 {S_2}^2 + k_3 S_2 + k_4 = 0$ has a positive solution S_2 with $\frac{d_3}{(1-p)\alpha\beta_2} < S_2 < \min\left\{\frac{d_2+\mu}{\alpha\beta_1}, \frac{\gamma_1 d_3}{\alpha\beta_2(\gamma_1(1-p)-\gamma_2 p)}\right\}$ or $\max\left\{\frac{d_2+\mu}{\alpha\beta_1}, \frac{d_3}{(1-p)\alpha\beta_2}\right\} < S_2 < \frac{\gamma_1 d_3}{\alpha\beta_2(\gamma_1(1-p)-\gamma_2 p)}$. Then system (2.5) has four equilibria P_0, P_1, P_2 and P_3, where P_0 is always unstable, P_1 is locally asymptotically stable if the inequalities in (2.23) hold, P_2 is locally asymptotically stable if the inequalities in (2.25) hold and P_3 is locally asymptotically stable if the inequalities in (2.27) hold.*

2.3.4. Local stability of the malaria infection equilibrium P_3

From lemma 2.1, we know that system (2.5) has three equilibria: $P_0 = (\Lambda/d_1, 0, 0, 0)$, $P_1 = (S_1, I_{s_1}, 0, 0)$ and $P_3 = (S_3, I_{s_3}, 0, E_3)$.

The local stability of P_3 is established from the Jacobian matrix at P_3, which is given by

$$J(P_3) = \begin{pmatrix} -\beta_1 I_{s_3} - d_1 & -\beta_1 S_3 & -\beta_2 S_3 & 0 \\ \alpha\beta_1 I_{s_3} & \alpha\beta_1 S_3 - d_2 - \mu - \frac{b_1 E_3}{(1+\theta I_{s_3})^2} & p\alpha\beta_2 S_3 - \gamma_1 I_{s_3} & -\frac{b_1 I_{s_3}}{1+\theta I_{s_3}} \\ 0 & 0 & (1-p)\alpha\beta_2 S_3 - \gamma_2 I_{s_3} - d_3 - b_2 E_3 & 0 \\ 0 & \frac{c_1 E_3}{(1+\theta I_{s_3})^2} & c_2 E_3 & \frac{c_1 I_{s_3}}{1+\theta I_{s_3}} - d_4 \end{pmatrix}.$$

and the characteristic equation is

$$\lambda^4 + s_1\lambda^3 + s_2\lambda^2 + s_3\lambda + s_4 = 0, \tag{2.26}$$

where the expressions of s_1, s_2, s_3 and s_4 are shown in Appendix.

By employing the Routh-Hurwitz criterion, the roots of (2.26) have negative real parts if and only if

$$s_1 > 0, \;\; s_1 s_2 - s_3 > 0, \;\; (s_1 s_2 - s_3) s_3 - s_1^2 s_4 > 0, \;\; (s_1 s_2 - s_3) s_3 s_4 - s_1^2 s_4^2 > 0. \tag{2.27}$$

Hence, we obtain the following theorem based on above analysis and Lemma 2.1 and 2.2.

Theorem 2.3. *If $R_1 > \max\left\{\frac{\beta_1}{d_1}(c_1 - d_4\theta) + d_4, 1\right\}$, then system (2.5) has three equilibria P_0, P_1 and P_3, where P_0 is unstable, P_1 is locally asymptotically stable if the inequalities in (2.23) hold, and P_3 is locally asymptotically stable if the inequalities in (2.27) hold.*

2.3.5. Local stability of the malaria infection equilibrium P_4

From Lemma 2.1, we know tha system (2.5) has five equilibria: $P_0 = (\Lambda/d_1, 0, 0, 0)$, $P_1 = (S_1, I_{s_1}, 0, 0)$, $P_2 = (S_2, I_{s_2}, I_{r_2}, 0)$ and $P_3 = (S_3, I_{s_3}, 0, E_3)$, $P_4 = (S_4, I_{s_4}, I_{r_4}, E_4)$.

The local stability of P_4 is established from the Jacobian matrix at P_4, which is given by

$$J(P_4) = \begin{pmatrix} -\beta_1 I_{s_4} - \beta_2 I_{r_4} - d_1 & -\beta_1 S_4 & -\beta_2 S_4 & 0 \\ \alpha\beta_1 I_{s_4} + p\alpha\beta_2 I_{r_4} & A_1 & p\alpha\beta_2 S_4 - \gamma_1 I_{s_4} & -\frac{b_1 I_{s_4}}{1+\theta I_{s_4}} \\ (1-p)\alpha\beta_2 I_{r_4} & -\gamma_2 I_{r_4} & (1-p)\alpha\beta_2 S_4 - \gamma_2 I_{s_4} - d_3 - \frac{b_2 E_4}{(1+\delta I_{r_4})^2} & -\frac{b_2 I_{r_4}}{1+\theta I_{r_4}} \\ 0 & \frac{c_1 E_4}{(1+\theta I_{s_4})^2} & \frac{c_2 E_4}{(1+\delta I_{r_4})^2} & \frac{c_1 I_{s_4}}{1+\theta I_{s_4}} + \frac{c_2 I_{r_4}}{1+\delta I_{r_4}} - d_4 \end{pmatrix},$$

where $A_1 = \alpha\beta_1 S_4 - \gamma_1 I_{r_4} - d_2 - \mu - \frac{b_1 E_4}{(1+\theta I_{s_4})^2}$. The characteristic equation is

$$\lambda^4 + t_1\lambda^3 + t_2\lambda^2 + t_3\lambda + t_4 = 0, \tag{2.28}$$

where the expressions of t_1, t_2, t_3 and t_4 are shown in Appendix.

By employing the Routh-Hurwitz criterion, the roots of (2.26) have negative real parts if and only if

$$t_1 > 0, t_1t_2 - t_3 > 0, (t_1t_2 - t_3)t_3 - t_1^2 t_4 > 0, (t_1t_2 - t_3)t_3t_4 - t_1^2 t_4^2 > 0. \tag{2.29}$$

Hence, we obtain the following theorem on the existence and stability of positive equilibrium based on above analysis and Lemma 2.1 and 2.2.

Theorem 2.4. *If $R_1 > \max\left\{\frac{\beta_1}{d_1}(c_1 - d_4\theta) + d_4, 1\right\}$, $R_2 > 1$, and $k_1 {S_2}^3 + k_2 {S_2}^2 + k_3 S_2 + k_4 = 0$ has a positive solution S_2 satisfying (H1) or (H2), and $l_1 {I_{r_4}}^5 + l_2 {I_{r_4}}^4 + l_3 {I_{r_4}}^3 + l_4 {I_{r_4}}^2 + l_5 I_{r_4} + l_6 = 0$ has a positive solution I_{r_4} satisfying either (H3) or (H4) or (H5). Then system (2.5) has five equilibria P_0, P_1, P_2, P_3 and P_4, where P_0 is unstable, P_1 is locally asymptotically stable if the inequalities in (2.23) hold, P_2 is locally asymptotically stable if the inequalities in (2.25) hold , P_3 is locally asymptotically stable if the inequalities in (2.27) hold , P_4 is locally asymptotically stable if the inequalities in (2.29) hold.*

According to the previous analysis, we know that P_4 is an interior positive equilibrium. For this equilibrium, we are interested in when it becomes unstable and Hopf bifurcation occurs. Following the analysis of a fourth-order characteristic equation in Ruan and Wolkowicz [26], we need to satisfy conditions, which make characteristic equation (2.28) having two roots with a negative real part and a pair of conjugate purely imaginary roots, which are

$$t_1 > 0, \;\; t_4 > 0, \;\; t_1t_2 - t_3 > 0, \;\; (t_1t_2 - t_3)t_3 - t_1^2 t_4 = 0. \tag{2.30}$$

Next, we would verify the transversal condition to prove the occurrence of Hopf bifurcation at the positive equilibrium P_4. We choose μ as a bifurcation parameter. Define

$$\psi(\mu) = (t_1(\mu)t_2(\mu) - t_3(\mu))t_3(\mu) - t_1^2(\mu)t_4(\mu). \tag{2.31}$$

Suppose that there exists a $\mu^* > 0$ so that $t_1(\mu^*) > 0$, $t_4(\mu^*) > 0$, $t_1(\mu^*)t_2(\mu^*) - t_3(\mu^*) > 0$ and $\psi(\mu^*) = 0$. Then equation (2.28) has four roots, $\pm\omega i$, λ_1 and λ_2, where $\omega = \sqrt{\frac{t_3(\mu^*)}{t_1(\mu^*)}}$, $Re(\lambda_1) < 0$ and $Re(\lambda_2) < 0$.

When $0 < |\mu - \mu^*| \ll 1$,we assume that equation (2.28) has four roots, $\xi(\mu) \pm \omega(\mu)i$, $\lambda_1(\mu)$ and $\lambda_2(\mu)$, where $\xi(\mu^*) = 0$, $\omega(\mu^*) = \omega$, $\lambda_1(\mu^*) = \lambda_1$ and $\lambda_2(\mu^*) = \lambda_2$. Then we compute the derivative of $\xi(\mu)$ with respect to μ at μ^*. Note that

$$(\xi(\mu) + i\omega(\mu))^4 + t_1(\mu)(\xi(\mu) + i\omega(\mu))^3 + t_2(\mu)(\xi(\mu) + i\omega(\mu))^2 + t_3(\mu)(\xi(\mu) + i\omega(\mu)) + t_4(\mu) = 0. \quad (2.32)$$

We obtain the following result by (2.31)

$$\frac{d\xi(\mu)}{d\mu}\Big|_{\mu=\mu^*} = -\frac{t_1(\mu^*)}{2((t_1(\mu^*)t_2(\mu^*) - 2t_3(\mu^*))^2 + t_1(\mu^*)^3 t_3(\mu^*))} \frac{d\psi(\mu)}{d\mu}\Big|_{\mu=\mu^*}.$$

Hence, the transversal condition holds under some conditions. According to the Hopf bifurcation theorem, we have the following theorem about bifurcation at the positive equilibrium P_4.

Theorem 2.5. *Assume that system (2.5) has a positive equilibrium at P_4. If there exists a $\mu^* > 0$ so that $t_1(\mu^*) > 0$,$t_4(\mu^*) > 0$,$t_1(\mu^*)t_2(\mu^*) - t_3(\mu^*) > 0$, $\psi(\mu^*) = 0$, and $\frac{d\xi(\mu)}{d\mu}\Big|_{\mu=\mu^*} \neq 0$, then Hopf bifurcation occurs at $\mu = \mu^*$, and a periodic solution appears near P_4 when μ passes through μ^*.*

2.4. Sobol' sensitivity analysis method

Sobol' sensitivity analysis method is one of the global sensitivity methods, which is performed over the entire parameter space, and all parameters could vary simultaneously. The core of this method is variance decomposition to measure the sensitivity of parameters [27, 28]. Assuming a mathematical model is described by a function

$$y = f(\mathbf{x}) = f(x_1, x_2, ..., x_n) \quad (2.33)$$

where $\mathbf{x} = (x_1, x_2, ..., x_n)$ represent the input parameters, which defined on a n-dimensional unit cube $H^n = \{\mathbf{x}|0 \leq x_i \leq 1, i = 1, 2, ..., n\}$, and $y = f(x)$ is the model output variable. According to the Sobol' method, $y = f(x)$ can be decomposed into single model parameters and subitem functions of parameter interaction:

$$f(\mathbf{x}) = f_0 + \sum_{i=1}^{n} f_i(x_i) + \sum_{i=1}^{n}\sum_{i\neq j}^{n} f_{ij}(x_i, x_j) + ... + f_{1,2,...,n}(x_1, x_2, ..., x_n) \quad (2.34)$$

The number of all subitems is 2^n, and the subitem function is obtained by calculating the following multiple integrals:

$$\begin{aligned} f_0 &= \int_0^1 f(x)dx \\ f_i(x_i) &= \int_0^1 f(x)\prod_{k\neq i} dx_k - f_0 \\ f_{ij}(x_i, x_j) &= \int_0^1 f(x)\prod_{k\neq i,j} dx_k - f_0 - f_i(x_i) - f_j(x_j) \end{aligned} \quad (2.35)$$

Similarly, other subitem functions of a high order can be achieved. f_0 is a constant, and the integral of every summand over any of its own variables is zero:

$$\int_0^1 f_{i1,...,is}(x_{i1}, ..., x_{is})dx_k = 0, 1 \leq i_1 \leq i_2 \leq ... \leq i_s \leq n, 1 \leq s \leq k \quad (2.36)$$

The subitem functions in equation (2.35) satisfy equation (2.36), it can be inferred that every subitem functions in equation (2.34) is orthogonal, that is:

$$\int_{H^n} f_{i_1,...,i_s}(x_{i_1},...,x_{i_s}) f_{j_1,...,j_l}(x_{j_1},...,x_{j_l}) dx = 0, \text{ for } (x_{i_1},...,x_{i_s}) \neq (x_{j_1},...,x_{j_l}) \tag{2.37}$$

Based on the above properties, the variance of output variable $V(y)$ can be decomposed as follows:

$$V(y) = \sum_{i=1}^{n} V_i(x_i) + \sum_{i=1}^{n} \sum_{i \neq j}^{n} V_{ij}(x_i, x_j) + ... + V_{1,2,...,n}(x_1, x_2, ..., x_n) \tag{2.38}$$

where $V_{i_1,...,i_s} = \int_0^1 f^2_{i_1,...,i_s}(x_{i_1},...,x_{i_s}) dx_{i_1},...,x_{i_s}$ is the partial variance corresponding to the subitem function of equation (2.34). The Sobol global sensitivity indices are defined by

$$S_{i_1,i_2,....,i_s} = \frac{V_{i_1,i_2,....,i_s}}{V(y)} \tag{2.39}$$

For instance, $S_i = \frac{V_i}{V(y)}$ is the first order Sobol' index, and $S_{ij} = \frac{V_{ij}}{V(y)}$ is the second order Sobol' index. And the total effect sensitivity index as an extension of the Sobol sensitivity indices, which is defined as the ratio of the sum of the related sensitivity indices:

$$S_{T_i} = S_i + S_{ij(i \neq j)} + ... + S_{1...i...s} \tag{2.40}$$

Total effect indices have great significance. Parameter x_i has no impact on the outcome variable in the case $S_{T_i} = 0$ and vice versa, which indicates that the condition $S_{T_i} = 0$ is necessary and sufficient for x_i to be a noninfluential factor. Hence, the total Sobol' indices not only characterized the contribution of the concerned parameters but also their interactions. The calculation of Sobol' sensitivity indices involves the computation of multiple integrals, which are very complicated and difficult especially for the complex nonlinear models. Therefore, the Monte Carlo method is employed to approximate the multiple integral solutions.

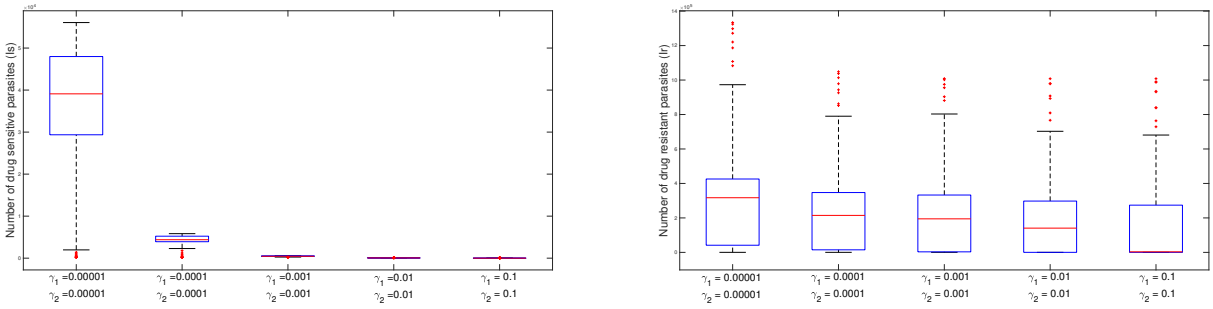
3. Numerical results

The paper illustrates some numerical results on the within-host dynamical models with and without considering the immune response.

3.1. Within-host model in the absence of immune response

Initially, we take into account the within-host dynamical model without involving the immune response. In order to examine the influence of competition, we observe the solution of I_r by varying the competitive coefficients and fixing the remaining parameters as in Table 1 (here we set $\mu = 0.1$, because it is not given in Table 1). The function of "*ode*45" provided by MATLAB software is employed to calculate the solution of I_r, where the time (days) ranges from 0 to 1500. Five different groups of competitive coefficients are chosen and sorted in ascending order. (First group : $\gamma_1 = \gamma_2 = 0.00001$, second group : $\gamma_1 = \gamma_2 = 0.0001$, third group : $\gamma_1 = \gamma_2 = 0.001$, fourth group : $\gamma_1 = \gamma_2 = 0.01$, fifth group : $\gamma_1 = \gamma_2 = 0.1$). We illustrate the results in the box-and-whisker plot, and each box-and-whisker

corresponds to a set of solutions of I_r for a given group of competitive coefficients. Figure 1 displays that the number of both drug sensitive parasites and drug resistant parasites decreases with the increasing intensity of competition. However, comparing Figure 1(a) with Figure 1(b), it is observed that the descending rate of the number of sensitive parasites is much faster than resistant ones. Moreover, the number of drug resistant parasites is roughly one order of magnitude larger than that of sensitive parasites. This phenomenon may imply that drug sensitive parasites are more susceptible to competition than resistant ones with the same competitive coefficients in each circumstance.



(a) (b)

Figure 1. Number of drug sensitive parasites (a) and drug resistant parasites (b) in different competitive intensity without considering the effect of immune response. Simulations were run in five cases of competition intensity (First group : $\gamma_1 = \gamma_2 = 0.00001$, second group : $\gamma_1 = \gamma_2 = 0.0001$, third group : $\gamma_1 = \gamma_2 = 0.001$, fourth group : $\gamma_1 = \gamma_2 = 0.01$, fifth group : $\gamma_1 = \gamma_2 = 0.1$). Box-and-whisker plots show median, interquartile range, and maximum/minimum.

Now, fix one of the competitive coefficients γ_1 and vary another one γ_2 to observe the number of two types of parasites, and the remaining parameters are as in Table 1 (here we set $\mu = 0.1$, because it is not given in Table 1). Figure 2 (a) and (b) illustrate that the number of resistant parasites constantly declines with the reduction of competitive ability. Especially, when the competitive ability of resistant parasites is under sensitive ones, the number of resistant parasites will decline sharply by about five orders of magnitude. The number of sensitive parasites is precious few comparing with resistant ones as their competitive ability is lower than resistant ones (Figure 2 (c)). But they would exceed resistant parasites once they develop a higher competitive ability (Figure 2 (d)). It can be deduced that sensitive parasites are able to competitively suppress resistant parasites when the sensitive population achieves a higher competitive ability. Especially, the higher the competitive ability of sensitive parasites are, the more the competitive suppression on resistant parasites are, and the fewer the number of resistant population is. Wale et al. demonstrated that a parasite nutrient could mediate competition between a drug resistant and drug sensitive strain of the malaria parasite. Hence, they tried to intensify the competitive suppression on drug resistant parasites by reducing the availability of the nutrient in a host environment, and results show that with resistant parasites struggling to replicate, susceptible parasites outcompeted them before they can emerge [29]. These experimental findings are consistent with our numerical results.

The model also reproduces the relationship between drug treatment and the spread of drug resistance. Figure 3 depicts the trend of the number of drug resistant parasites with the ranging treatment

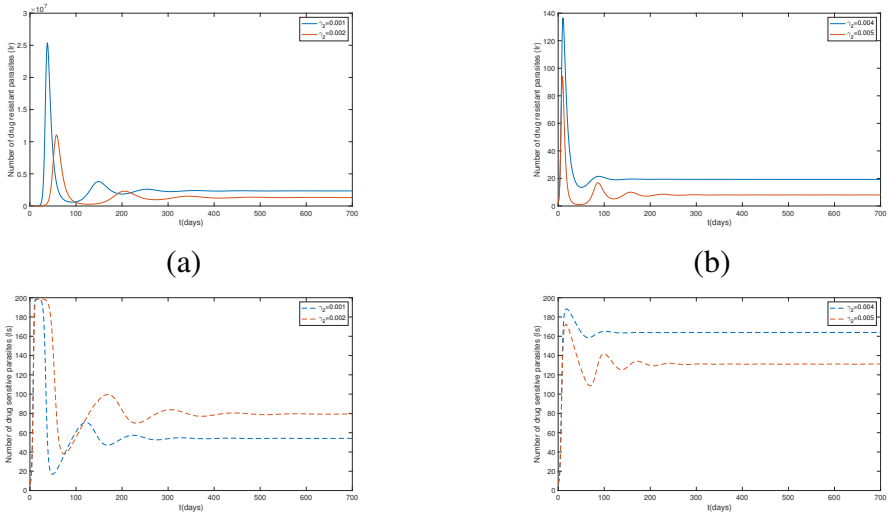


Figure 2. Number of drug resistant parasites(I_r) (solid lines) and drug sensitive parasites(I_s) (dotted line) in four different levels of competitive coefficient γ_2. The competitive coefficient $\gamma_1 = 0.003$ is fixed, and γ_2 is varying from lower than γ_1 ($\gamma_2 = 0.001$ and $\gamma_2 = 0.002$) to higher than γ_1 ($\gamma_2 = 0.004$ and $\gamma_2 = 0.005$).

level. We mainly vary the parameter μ ($\mu = 0, 1, 5$) and fix the remaining parameters as in Table 1 (here we set $\gamma_1 = \gamma_2 = 0.001$, because they are not given in Table 1). High level chemotherapy leads to a large production of drug resistant parasites. The more aggressive the chemotherapy is, the more the drug sensitive population will be reduced, which accordingly will promote the competitive release of resistant parasites, leading them to maintain at a high level ultimately. The results are also in line with experimental findings of [4, 30]. In particular, it can be discovered that high level chemotherapy also accelerates the evolution of drug resistance. Both the peak and equilibrium of the number of drug resistant parasites appear much earlier than low level chemotherapy.

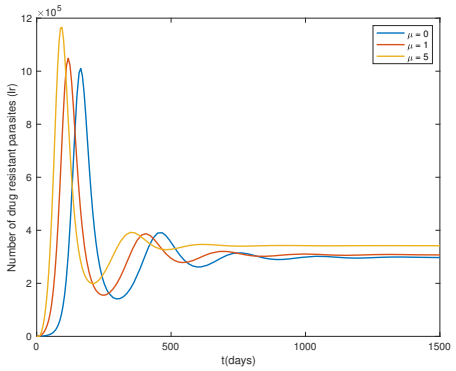


Figure 3. The impact of antimalarial drug on resistant parasites(I_r) without considering the immune response, three levels of drug treatment ($\mu = 0, \mu = 1, \mu = 5$) are examined in this simulation respectively.

3.2. *Within-host model in the presence of immune response*

Now, the role of immune response is added into the mathematical model. Three cases are studied in our paper, including the single strain infection, mixed strains infection (incorporate the competition) and mixed strains infection (incorporate the competition and the immune response). They are set to have same parameters as in Table 1 (here we set $\gamma_1 = \gamma_2 = 0.001$ and $\mu = 0.1$). Figure 4 (a) plots that, in single infections with drug resistant parasites, the number of drug resistant parasites stands at a quite high level. Whereas in mixed strains infection (Figure 4 (b)), the number of resistant populations drops roughly by two orders of magnitude. The influence of both competition and immune response on drug resistance is shown in Figure 4 (c). The result reveals that the number of resistant population downs unceasingly to two orders of magnitude, indicating that the immune response could inhibit the spread of drug resistance to some extent. This is in accord with the experimental result of Ataide in [9] that immunity may play an important role in the emergence and transmission potential of artemisinin-resistant parasites.

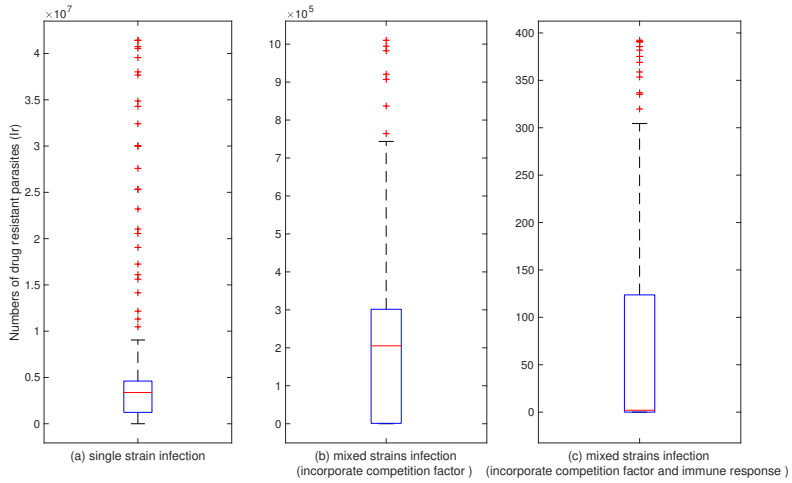
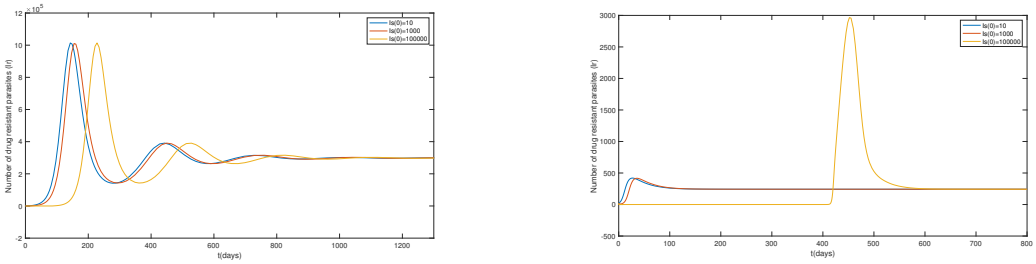


Figure 4. Number of drug resistant parasites in different cases, including single strain infection (a), mixed strains infection(incorporate competition) (b) and mixed strains infection (incorporate competition and immunity response) (c). Box-and-whisker plots show median, interquartile range, and maximum/minimum.

The impact of the ratio of initial drug sensitive and resistant parasites is also examined on the prevalence of drug resistant strains. We take all parameters as in Table 1 (here we set $\gamma_1 = \gamma_2 = 0.001$ and $\mu = 0.1$). Figure 5 (a) presents the effect of different initial values of drug sensitive parasites on drug resistant parasites without considering the immune response. As the initial value of drug sensitive parasites $I_s(0) = 10$, $I_s(0) = 1000$ and $I_s(0) = 100000$, the number of drug resistant parasites peaked on the 140th, 160th and 220th day, respectively. The case $I_s(0) = 100000$ is the latest among the three cases. It can be deduced that a large initial number of drug sensitive parasites could delay the emergence of the peak. This phenomenon may result from the competition between the two parasites, and drug resistant parasites can be suppressed by sensitive ones when they have a large initial number.

If the effect of the immune response is incorporated, Figure 5 (b) shows that the number of drug resistant parasites reduces by around 3 to 4 orders of magnitude. As the initial value of drug sensitive

parasites $I_s(0) = 10$, $I_s(0) = 1000$ and $I_s(0) = 100000$, the number of drug resistant parasites peaked on the 40th, 50th and 460th day, respectively. Compared with Figure 5 (a), the peak of drug resistant parasites comes about 100 days earlier in the case of $I_s(0) = 10$ and $I_s(0) = 1000$. This may be explained by immunosuppression. On this account, parasites would be eliminated in large quantities, especially, only a few parasites could survive when the given initial number of drug sensitive parasites is relatively small. Hence, the number of two types of parasites may remain the same order of magnitude, thereby leading to the failure of competitive suppression on drug resistant parasites. However, the peak of drug resistant parasites in the case of $I_s(0) = 100000$ appears around 240 days later than the same case without considering the immune response. This may be because the initial number of drug sensitive parasites is much larger, hence immune cells are unable to kill these parasites as fast as possible, so that there are enough sensitive parasites left to play a role in suppressing resistant ones. Nevertheless, the values of both the peak and equilibrium of drug resistant parasites remain at low levels since the total cardinal number of two types of parasites is relatively small, which is reduced by immune cells. Wale et al. [29] had an analogous experiment to investigate the intensity of competition between strains of P. chabaudi in the period before they were cleared by the immune system. They discovered that immunity could be responsible for the clearance and the post-peak control of malaria infections, which also explained Figure 5 (b) to some extent.



(a) (b)

Figure 5. The effect of initial value of drug sensitive parasites on drug resistant parasites in different two conditions: (a) in the absence of the immune response, (b) in the presence of the immune response, where given three levels of the initial value of drug sensitive parasites: $I_s(0) = 10, I_s(0) = 1000, I_s(0) = 100000$, and fixed the initial value of drug resistant parasites $I_r(0) = 20$ and the initial value of immune cells $E(0) = 40000000$.

The above analysis has shown that within-host competition and immune mechanisms are able to inhibit the spread of drug resistance. The following step is measuring how the drug treatment level influences the number of drug resistant parasites. Figure 6 illustrates the simulation results with three different levels of antimalarial drug treatment (one of which equals zero, in order to examine the effect from competition alone) combined with three different competitive intensities. Equilibrium values of two parasites gradually fall with the rise of the immunity level in every case. And the number of two parasites moves in a similar trend, but the sensitive type always goes below the resistant one.

Figure 6 (a)-(c) depict the simulation results with no drug use and different levels of competitive intensity. It is observed that resistant parasites take more time to reach equilibrium with the increasingly fierce competition. Under the lowest level of competitive intensity ($\gamma_1 = \gamma_2 = 0.001$) (Figure 6 (a)), the gap of the number between the two parasites is also the lowest. This may indicate that drug sen-

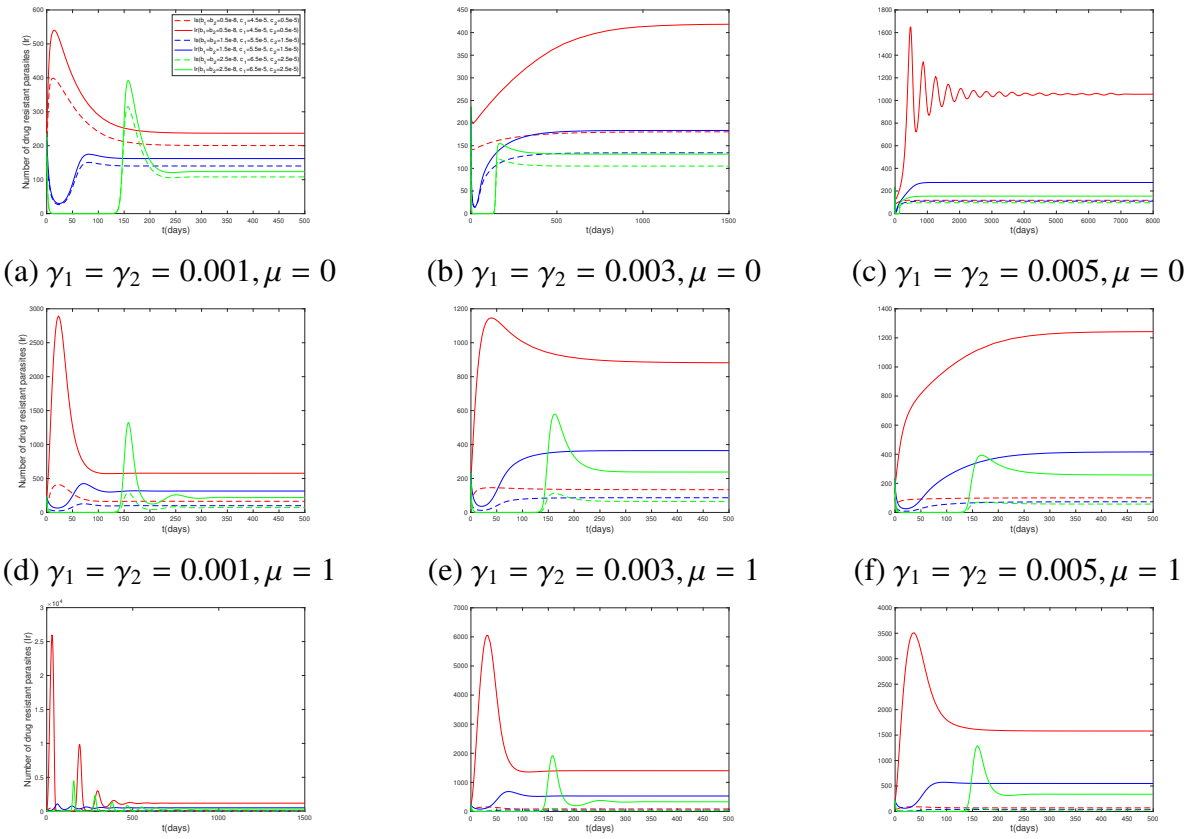
sitive parasites can survive more equally with resistant parasites in a loose competitive environment. The resistant population has not shown its strong aggressiveness yet. However, the gap would be significantly widened once the competition is intensified(Figure 6 (b) (c)), indicating that drug resistant parasites are able to survive in all settings, moreover, the number of drug resistant parasites is larger in a highly fierce competition setting. In addition, the immune response also plays an important role in controlling the number of parasites. The aforementioned gap is narrowed due to the immune effect in each case(Figure 6 (a)-(c)). Especially, in the case of high level competition and low level immune response (Figure 6 (c)), drug resistant parasites exhibit a tendency of decaying oscillation until they reach equilibrium eventually. It also spends the longest time among these three cases. In conclusion, the results demonstrate that in highly immunity settings and competition is light, drug resistant parasites are inclined to remain at a low level. But if the competition is strengthened, the death rate of drug sensitive parasites keeps increasing to leave more ecological space for resistant ones, which may actually ascend their survival rate.

Figure 6 (d)-(i) present simulation results including drug treatment and competition, which mainly yield several observations. First of all, with the increase of drug treatment level, accordingly, drug resistance expands rapidly without exception. This can be observed from Figure 6 (d) (g), Figure 6 (e) (h), and Figure 6 (f) (i), respectively, which provide a clear comparison between low- and high- drug dose. This may arise from the competitive release. Aggressive chemotherapy aims to kill sensitive parasites such that resistant parasites could benefit from this behavior in a resource-limited environment. Secondly, the number of resistant parasites stands at a low level with light treatment and a low level of competitive intensity (Figure 6 (d)). Moreover, the spread of drug resistance is again depressed by enhanced immunity. Thirdly, the case of aggressive drug treatment and a low level of competitive intensity(Figure 6 (g)) describes the process of oscillation attenuation of drug resistant parasites and reach the equilibrium ultimately, which is fairly similar to the case with high level of competitive intensity and zero-dose of antimalarial drug(Figure 6 (c)). Immunity effectively diminishes the value of peak and equilibrium, thereby highlighting its importance. Fourthly, with the ascending level of both competitive intensity and antimalarial drug dose, the gap between the amount of two parasites is getting wide. It is clear that case Figure 6 (i) ($\gamma_1 = \gamma_2 = 0.005, \mu = 5$) presents the widest gap of all cases, particular for a relatively low level of the immune response. Lastly, when the competition is relatively moderate(Figure 6 (e) (h)), the tendency of two parasites varies between the case of high level and low level of competitive intensity(Figure 6 (d) (f) and Figure 6 (g) (i)).

To summarize, the results of these simulations could boil down to a fact that immune response is a real threat to resistant parasites. If equal proportions of infections are treated in low- and high-level of immune response settings, a higher proportion of hosts will be treated in a high-level of immune response settings. Similarly, Ataide et al. also studied the effect of both low- and high- level of immunity on the emergence of drug resistant mutant parasites. Results suggest that low levels of immunity may facilitate the transmission of resistant parasites, and resistant parasites may be better able to persist compared with the case of high immunity [9]. Moreover, the number of drug resistant parasites would be increased by employing antimalarial drug treatment, especially the aggressive regimen, which refers to the accelerated spread of drug resistance in a host accompanying with the elimination of drug sensitive competitors.

Many studies have suggested that oscillations are frequently observed in the immune system [17, 31]. Figure 6 (c) and Figure 6 (g) exactly describe this phenomenon of "decaying oscillation"[31]. On

the basis of the above analysis, if the dissipation of energy could be controlled, the regular periodic oscillation is possible to appear. In the case of Figure 6 (c), when the level of immune response is at a high level, the curve depicts a tendency of decaying oscillation of the drug resistant parasites, but there is no analogous phenomenon when the competitive intensity is much lower(Figure 6 (a) and (b)). Similarly, in terms of the Figure 6 (g), the phenomenon of decaying oscillation shows again when the competitive intensity is light and the level of immune response is high with aggressive drug treatment. Nonetheless, decaying oscillation disappears with the increasing competitive intensity (Figure 6 (h) and (i)).



(a) $\gamma_1 = \gamma_2 = 0.001, \mu = 0$ (b) $\gamma_1 = \gamma_2 = 0.003, \mu = 0$ (c) $\gamma_1 = \gamma_2 = 0.005, \mu = 0$

(d) $\gamma_1 = \gamma_2 = 0.001, \mu = 1$ (e) $\gamma_1 = \gamma_2 = 0.003, \mu = 1$ (f) $\gamma_1 = \gamma_2 = 0.005, \mu = 1$

(g) $\gamma_1 = \gamma_2 = 0.001, \mu = 5$ (h) $\gamma_1 = \gamma_2 = 0.003, \mu = 5$ (i) $\gamma_1 = \gamma_2 = 0.005, \mu = 5$

Figure 6. Number of drug resistant parasites(I_r) and drug sensitive parasites(I_s) in four different levels of immune response with varying level of antimalarial drug treatment (μ) and the competitive intensity(γ_1, γ_2). For all figures, solid lines represent drug resistant parasites, dotted lines represent drug sensitive parasites.

In order to explore the interior equilibrium P_4 of system (2.5), we choose $\gamma_1 = \gamma_2 = 0.01$, $\mu = 0.26408$ and take all other parameters as in Table 1. Then, $P_4 = (4972109.16, 58.46, 1732.77, 16284461.14)$, which is stable by checking the stability conditions (2.29), see Figure 7. Furthermore, using the software Matcont (a Matlab package for numerical bifurcation analysis of ODEs), we also detect that the system (2.5) undergoes a Hopf bifurcation at P_4 as μ passes through a critical value $\mu = 0.20408$. In addition, the transversality condition $\xi'(\mu^*) \neq 0$ holds, the first Lyapunov coefficient equals -8.076492×10^{-3}, indicating a supercritical bifurcation, see Figure 8. For instance, if we set

$\mu = 0.10408$, then P_4 is unstable and a stable periodic solution can be observed, see Figure 9.

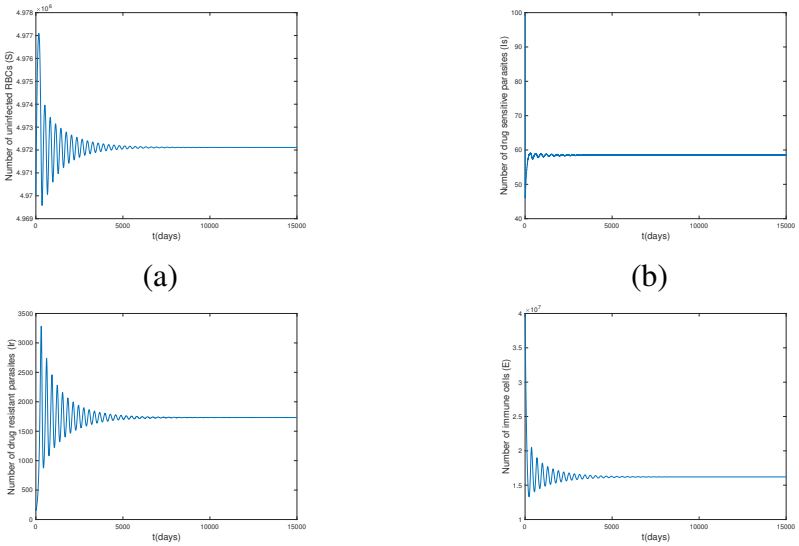


Figure 7. The phase diagram of interior equilibrium P_4=(4972109.16, 58.46, 1732.77, 16284461.14) when parameters $\gamma_1 = \gamma_2 = 0.01, \mu = 0.26408$.

3.3. Sensitivity analysis

The value of sensitivity analysis can be reflected in the natural level of variation in several aspects of the model, including epidemiological and immunological [19]. Generally, global sensitivity analysis is rather complicated in dealing with high-dimensional models from the point of methodology. However, it is still worthwhile to perform for providing further insights on the dynamical infection process of within-host as well as resistance management.

In this paper, a uniform distribution within 20% of nominal values was used for sampling with a base sample size of 2,000 simulations. Sobol' sensitivity analysis was conducted in two cases, with and without considering the immunity of the within-host dynamical system , respectively. Sobol' results are shown in Figure 10 and Figure 11. As for the first case (Figure 10), parameter Λ and d_1 are significant for uninfected RBCs (S). It is clear to figure out that parameters $\alpha, \beta_1, d_2, \gamma_1, \gamma_2$ can be labeled as significant for drug sensitive parasites (I_s). Notice that $\alpha, p, \gamma_1, \gamma_2, d_3$ have a significant influence on drug resistant parasites(I_r).

For another case (Figure 11), the immune effect is added to the dynamical system, therefore, our interest is to observe the variation of the sensitivity of all parameters after the immune response is involved. Parameters $p, d_1, \Lambda, \gamma_1, \gamma_2$ rank the top five in the sensitivity ranking of uninfected RBCs (S). It seems that competition has a relatively significant influence on uninfected RBCs (S) in the current system. Parameters $p, \alpha, \beta_2, c_1, d_4$ can be labeled as significant for drug sensitive parasites (I_s), where the proliferation rate of immunity effectors (c_1) plays an important role after incorporating the immune response. Values of Sobol' index of parameters $p, \gamma_1, \gamma_2, \alpha, b_2$ are much higher than other parameters. Similarly, the removal rate of drug resistant parasites by immune system (b_2) could affect I_r more, indicating that parameters related to immunity act actually on state variables, in particular for I_s and I_r. For the immunity effectors (E), parameters $p, \alpha, \gamma_2, \beta_2, c_2$ have a critical influence.

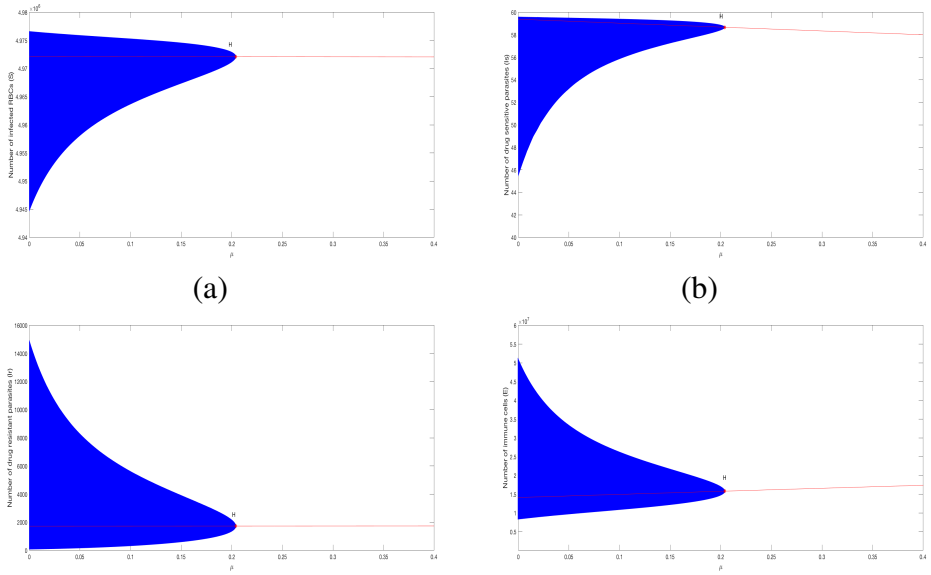


Figure 8. The figure depicts the points of bifurcation for the system parameter μ. The Hopf bifurcation occur at the point $\mu = 0.20408$, which is labeled as "H". The first Lyapunov coefficient equals -8.076492×10^{-3}, which is referred as a supercritical bifurcation. When μ crosses the threshold value, the system(2.5) becomes asymptotically stable. Blue areas represent the periodic solution. And parameter values are specified in the numerical section.

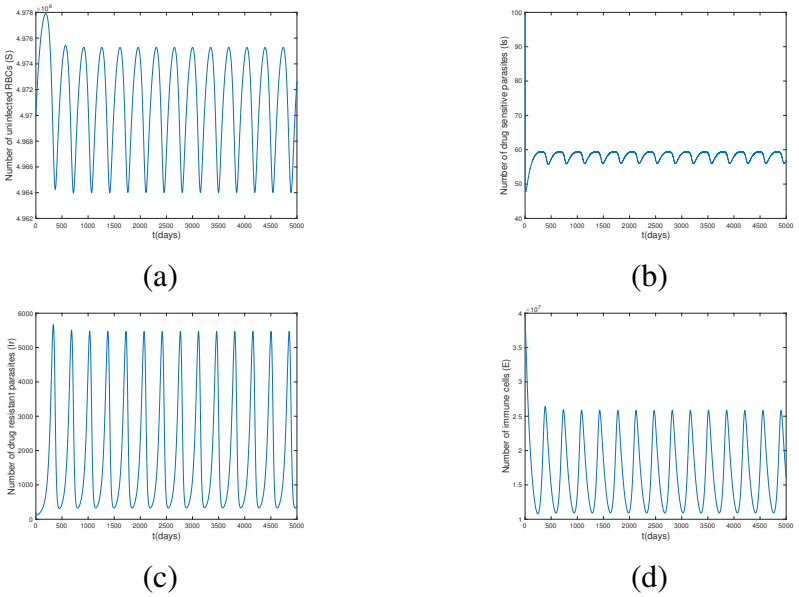


Figure 9. The phase diagram of periodic solution bifurcated from interior equilibrium $P_4 = (4972147.30, 59.02, 1726, 14978311.39)$ when parameters $\gamma_1 = \gamma_2 = 0.01, \mu = 0.10408$.

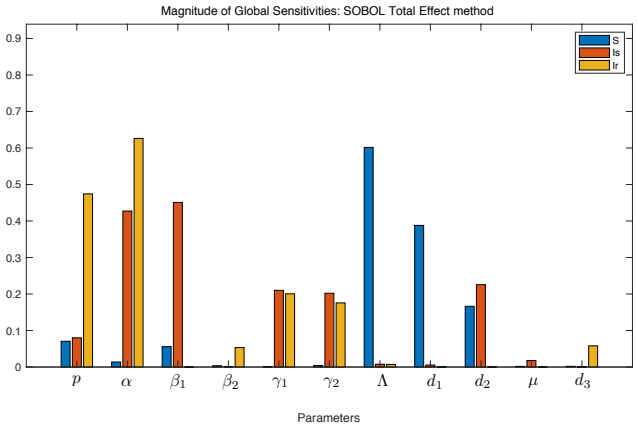


Figure 10. Sobol sensitivity index of all state variables, including infected RBCs(S) (blue), drug sensitive parasites(I_s) (red) and drug resistant parasites(I_r) (yellow) in the absence of immune response.

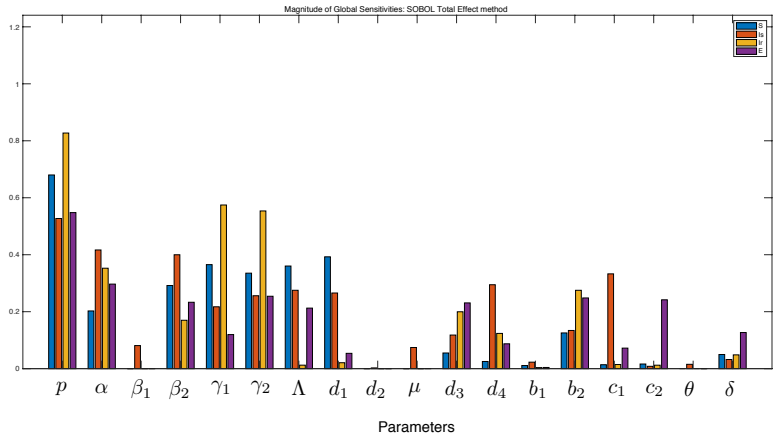


Figure 11. Sobol' sensitivity index of all state variables, including infected RBCs(S) (blue), drug sensitive parasites(I_s) (red) , drug resistant parasites(I_r) (yellow) and immune cells(E) (purple) in the presence of immune response.

4. Disscussion and conclusion

Within-host competition is a real major hurdle in the evolution of drug resistance when the immune response is not considered. Results of the dynamical analysis indicate that, if sensitive parasites achieve a higher ability of competition than resistant ones, such that resistant parasites will be in a competitive disadvantage, and sensitive parasites could suppress their competitors. Wale et al. [29] showed that intensifying competitive interactions between sensitive and resistant parasites by limiting a resource could retard the evolution of drug resistance. Hence, if it is possible to improve the competitive ability of sensitive parasites, it will be a good way to manage drug resistance.

As the immune response is considered in the dynamical system, the interactions between malaria parasites and immune cells can be modeled in analogy to ecological interactions, where two prey species (drug sensitive and resistant parasites) are mediated by a shared predator species (immune

cells) [32]. Figure 5 presents the influence of different initial values of drug sensitive parasites on resistant ones in two cases (with and without immunity). Essentially, it is mainly about the different ratios between the two parasites. Hence, the infection can be divided into different types according to the ratio $\frac{I_r}{I_s}$. If the ratio $\frac{I_r}{I_s}$ is large, the type will be regarded as a host infected with resistant parasites from the very beginning. In contrast, if the ratio $\frac{I_r}{I_s}$ is small, the type will be regarded as resistant mutants of parasites produced directly within a host. The infection with a small $\frac{I_r}{I_s}$ exhibits a delay of drug resistance. This may be due to the competitive inhibition of the replication of resistant parasites by the numerically predominant sensitive parasites. Another interesting fact is detected when the immune effect is involved in this process, which resistant parasites can either be suppressed for a much longer time than before or reach equilibrium rapidly. The inferences are as follows: when the initial value of I_r is fixed and I_s is large enough ($\frac{I_r}{I_s}$ is small), immune cells are insufficient to completely clear parasites as fast as possible, hence the remaining sensitive parasites still can competitive suppress resistant ones, thereby delaying the spread of drug resistance. On the other hand, when I_s is much smaller($\frac{I_r}{I_s}$ is large), immune cells have the ability to eliminate most parasites rapidly to reach equilibrium accordingly.

Simulations with drug treatment show that high dose antimalarial chemotherapy will actually contribute to the spread of drug resistance in both situations. Motivated by studies of [30, 33], we explore the influence of immune response in the treatment process. The results show that the reality of the dynamics of within-host infection is non-linear, even the oscillation occurs in some cases. This mainly results from the role of the immune response, acting in conjunction to produce diverse outcomes, which coincide with the analysis in [12]. From both Figure 8 and 6(c), an interesting fact is observed that the malaria infection exhibits a periodic phenomenon as the drug treatment remains at a lower level and competition stays at a high level. This may illustrate that light drug treatment is ineffective because of the fierce competition between the two parasites. But we also find that resistant parasites still can benefit from the aggressive therapy with a similar tendency of the immunodeficient dynamical system, which is consistent with experimental results of [4, 30]. Hence, it is very necessary to control the drug dose at an appropriate level that not only alleviate the symptoms but also manage the spread of drug resistance effectively [3].

Our models involve a variety of parameters, especially when the immune effect is involved in the system. In order to identify the importance of these parameters, sensitivity analysis is conducted in two models. In particular, we mainly focus on the sensitivity of parameters related to competition, immunity and drug treatment. Results imply that competition remains a critical factor in both cases(with and without immune response) for resistant parasites (I_r), and the immune effect also plays an important role in the immune system. In addition, the significance of drug treatment to drug sensitive parasites (I_s) and drug resistant parasites (I_r) is lower in both cases (with or without immune response), where the sensitivity index of I_s is much higher than I_r, which is obvious and reasonable, because antimalarial drugs have a direct effect on drug sensitive parasites, but pose indirect impact on drug resistant parasites, such as competition.

The within-host dynamical model of malaria infection is extended to consider competition and host immunity. Our goal is mainly to explore the evolution of drug resistance, which is obviously complex due to involving interacting processes, such as immunity, within-host competition and the patterns of drug use. Our model also appropriately embodies the effects of these interacting processes on the spread of resistance to some extent. However, we have to emphasize that this model cannot rule out the possibility, that intraspecific competition, immune-mediated competition and the fitness costs of

resistance may have an influence on the evolution of drug resistance. Hence, it is worth continuously studying these aspects of resistance management.

Acknowledgments

This research is supported by Chinese NSF (grant 11671110) and Heilongjiang NSF (grant LH2019A010).

Conflict of interest

All authors declare that there are no conflicts of interest in this paper.

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Appendix

Coefficients to polynomial Equation (2.24)

$$r_1 = d_1 + d_2 + d_3 + d_4 + \mu + I_{r_2}\beta_2 + I_{s_2}\beta_1 + I_{r_2}\gamma_1 + I_{r_2}\gamma_2 - S_2\alpha\beta_1 - \frac{I_{r_2}c_2}{I_{r_2}\delta + 1} - \frac{I_{s_2}c_1}{I_{s_2}\theta + 1} + S_2\alpha\beta_2(p-1),$$

$$\begin{aligned} r_2 =& \Big(d_3 + I_{r_2}\gamma_2 + S_2\alpha\beta_2(p-1)\Big)(d_2 + \mu + I_{r_2}\gamma_1 - S_2\alpha\beta_1) - \frac{(d_1 + I_{r_2}\beta_2 + I_{s_2}\beta_1)I_{r_2}c_2}{I_{r_2}\delta + 1} - d_4 + \frac{I_{s_2}c_1}{I_{s_2}\theta + 1} \\ &+ \Big(d_3 + I_{r_2}\gamma_2 + S_2\alpha\beta_2(p-1)\Big)(d_1 + I_{r_2}\beta_2 + I_{s_2}\beta_1) - \frac{\Big(d_3 + I_{r_2}\gamma_2 + S_2\alpha\beta_2(p-1)\Big)I_{r_2}c_2}{I_{r_2}\delta + 1} - d_4 + \frac{I_{s_2}c_1}{I_{s_2}\theta + 1} \\ &+ (d_1 + I_{r_2}\beta_2 + I_{s_2}\beta_1)(d_2 + \mu + I_{r_2}\gamma_1 - S_2\alpha\beta_1) - \frac{I_{r_2}c_2}{I_{r_2}\delta + 1} - d_4 + \frac{I_{s_2}c_1}{(I_{s_2}\theta + 1)(d_2 + \mu + I_{r_2}\gamma_1 - S_2\alpha\beta_1)} \\ &- I_{r_2}\gamma_2(I_{s_2}\gamma_1 - S_2\alpha\beta_2 p) + S_2\beta_1\Big(I_{s_2}\alpha\beta_1 + I_{r_2}\alpha\beta_2 p\Big) - S_2\beta_2\Big(I_{r_2}\gamma_2 + I_{r_2}\alpha\beta_2(p-1)\Big), \end{aligned}$$

$$\begin{aligned} r_3 =& \Big(d_3 + I_{r_2}\gamma_2 + S_2\alpha\beta_2(p-1)\Big)(d_1 + I_{r_2}\beta_2 + I_{s_2}\beta_1)(d_2 + \mu + I_{r_2}\gamma_1 - S_2\alpha\beta_1) \\ &- \Big(d_3 + I_{r_2}\gamma_2 + S_2\alpha\beta_2(p-1)\Big)\left(\frac{I_{r_2}c_2}{I_{r_2}\delta + 1} - d_4 + \frac{I_{s_2}c_1}{I_{s_2}\theta + 1}\right)(d_2 + \mu + I_{r_2}\gamma_1 - S_2\alpha\beta_1 + d_1 + I_{r_2}\beta_2 + I_{s_2}\beta_1) \\ &- (d_1 + I_{r_2}\beta_2 + I_{s_2}\beta_1)\left(\frac{I_{r_2}c_2}{I_{r_2}\delta + 1} - d_4 + \frac{I_{s_2}c_1}{I_{s_2}\theta + 1}\right)(d_2 + \mu + I_{r_2}\gamma_1 - S_2\alpha\beta_1) \\ &+ S_2\beta_1(I_{s_2}\gamma_1 - S_2\alpha\beta_2 p)\Big(I_{r_2}\gamma_2 + I_{r_2}\alpha\beta_2(p-1)\Big) - I_{r_2}\gamma_2(I_{s_2}\gamma_1 - S_2\alpha\beta_2 p)(d_1 + I_{r_2}\beta_2 + I_{s_2}\beta_1) \\ &+ \Big(I_{r_2}\gamma_2(I_{s_2}\gamma_1 - S_2\alpha\beta_2 p) - S_2\beta_1(I_{s_2}\alpha\beta_1 + I_{r_2}\alpha\beta_2 p)\Big)\left(\frac{I_{r_2}c_2}{I_{r_2}\delta + 1} - d_4 + \frac{I_{s_2}c_1}{I_{s_2}\theta + 1}\right) \\ &+ S_2\beta_2\Big(I_{r_2}\gamma_2 + I_{r_2}\alpha\beta_2(p-1)\Big)\left(\frac{I_{r_2}c_2}{I_{r_2}\delta + 1} - d_4 + \frac{I_{s_2}c_1}{I_{s_2}\theta + 1} - d_2 - \mu - I_{r_2}\gamma_1 + S_2\alpha\beta_1\right) \\ &+ S_2\beta_1(I_{s_2}\alpha\beta_1 + I_{r_2}\alpha\beta_2 p)\Big(d_3 + I_{r_2}\gamma_2 + S_2\alpha\beta_2(p-1)\Big) - I_{r_2}S_2\beta_2\gamma_2(I_{s_2}\alpha\beta_1 + I_{r_2}\alpha\beta_2 p), \end{aligned}$$

$$\begin{aligned} r_4 =& I_{r_2}\gamma_2(I_{s_2}\gamma_1 - S_2\alpha\beta_2 p)(d_1 + I_{r_2}\beta_2 + I_{s_2}\beta_1)\left(\frac{I_{r_2}c_2}{I_{r_2}\delta + 1} - d_4 + \frac{I_{s_2}c_1}{I_{s_2}\theta + 1}\right) \\ &- \Big(d_3 + I_{r_2}\gamma_2 + S_2\alpha\beta_2(p-1)\Big)(d_1 + I_{r_2}\beta_2 + I_{s_2}\beta_1)\left(\frac{I_{r_2}c_2}{I_{r_2}\delta + 1} - d_4 + \frac{I_{s_2}c_1}{I_{s_2}\theta + 1}\right)(d_2 + \mu + I_{r_2}\gamma_1 - S_2\alpha\beta_1) \\ &+ S_2\beta_2\Big(I_{r_2}\gamma_2 + I_{r_2}\alpha\beta_2(p-1)\Big)\left(\frac{I_{r_2}c_2}{I_{r_2}\delta + 1} - d_4 + \frac{I_{s_2}c_1}{I_{s_2}\theta + 1}\right)(d_2 + \mu + I_{r_2}\gamma_1 - S_2\alpha\beta_1) \\ &- S_2\beta_1(I_{s_2}\alpha\beta_1 + I_{r_2}\alpha\beta_2 p)\Big(d_3 + I_{r_2}\gamma_2 + S_2\alpha\beta_2(p-1)\Big)\left(\frac{I_{r_2}c_2}{I_{r_2}\delta + 1} - d_4 + \frac{I_{s_2}c_1}{I_{s_2}\theta + 1}\right) \\ &- S_2\beta_1(I_{s_2}\gamma_1 - S_2\alpha\beta_2 p)\Big(I_{r_2}\gamma_2 + I_{r_2}\alpha\beta_2(p-1)\Big)\left(\frac{I_{r_2}c_2}{I_{r_2}\delta + 1} - d_4 + \frac{I_{s_2}c_1}{I_{s_2}\theta + 1}\right) \end{aligned}$$

$$+ I_{r_2} S_2 \beta_2 \gamma_2 (I_{s_2} \alpha \beta_1 + I_{r_2} \alpha \beta_2 p) \left(\frac{I_{r_2} c_2}{I_{r_2} \delta + 1} - d_4 + \frac{I_{s_2} c_1}{I_{s_2} \theta + 1} \right).$$

Coefficients to polynomial Equation (2.26)

$$\begin{aligned}
s_1 =& d_1 + d_2 + d_3 + d_4 + \mu + E_3 b_2 + I_{s_3} \beta_1 + I_{s_3} \gamma_2 - S_3 \alpha \beta_1 + \frac{E_3 b_1}{(I_{s_3} \theta + 1)^2} - \frac{I_{s_3} c_1}{I_{s_3} \theta + 1} + S_3 \alpha \beta_2 (p-1), \\
s_2 =& \Big(d_3 + E_3 b_2 + I_{s_3} \gamma_2 + S_3 \alpha \beta_2 (p-1) \Big) \left(d_2 + \mu - S_3 \alpha \beta_1 + \frac{E_3 b_1}{(I_{s_3} \theta + 1)^2} \right) + I_{s_3} S_3 \alpha \beta_1^2 + \frac{E_3 I_{s_3} b_1 c_1}{(I_{s_3} \theta + 1)^3} \\
&+ \left(d_4 - \frac{I_{s_3} c_1}{I_{s_3} \theta + 1} \right) \left(d_3 + E_3 b_2 + I_{s_3} \gamma_2 + S_3 \alpha \beta_2 (p-1) + d_2 + \mu - S_3 \alpha \beta_1 + \frac{E_3 b_1}{(I_{s_3} \theta + 1)^2} + d_1 + I_{s_3} \beta_1 \right) \\
&+ (d_1 + I_{s_3} \beta_1) \left(d_3 + E_3 b_2 + I_{s_3} \gamma_2 + S_3 \alpha \beta_2 (p-1) + d_2 + \mu - S_3 \alpha \beta_1 + \frac{E_3 b_1}{(I_{s_3} \theta + 1)^2} \right) \\
s_3 =& \left(d_4 - \frac{I_{s_3} c_1}{I_{s_3} \theta + 1} \right) (d_1 + I_{s_3} \beta_1) \left(d_2 + \mu - S_3 \alpha \beta_1 + \frac{E_3 b_1}{(I_{s_3} \theta + 1)^2} + d_3 + E_3 b_2 + I_{s_3} \gamma_2 + S_3 \alpha \beta_2 (p-1) \right) \\
&+ \left(d_4 - \frac{I_{s_3} c_1}{(I_{s_3} \theta + 1)} \right) \Big(d_3 + E_3 b_2 + I_{s_3} \gamma_2 + S_3 \alpha \beta_2 (p-1) \Big) \left(d_2 + \mu - S_3 \alpha \beta_1 + \frac{E_3 b_1}{(I_{s_3} \theta + 1)^2} \right) \\
&+ (d_1 + I_{s_3} \beta_1) \Big(d_3 + E_3 b_2 + I_{s_3} \gamma_2 + S_3 \alpha \beta_2 (p-1) \Big) \left(d_2 + \mu - S_3 \alpha \beta_1 + \frac{E_3 b_1}{(I_{s_3} \theta + 1)^2} \right) \\
&+ I_{s_3} S_3 \alpha \beta_1^2 \Big(d_3 + E_3 b_2 + I_{s_3} \gamma_2 + S_3 \alpha \beta_2 (p-1) \Big) + I_{s_3} S_3 \alpha \beta_1^2 \left(d_4 - \frac{I_{s_3} c_1}{I_{s_3} \theta + 1} \right) \\
&+ \frac{E_3 I_{s_3} b_1 c_1}{(I_{s_3} \theta + 1)^3} \Big(d_1 + I_{s_3} \beta_1 + d_3 + E_3 b_2 + I_{s_3} \gamma_2 + S_3 \alpha \beta_2 (p-1) \Big), \\
s_4 =& \left(d_4 - \frac{I_{s_3} c_1}{I_{s_3} \theta + 1} \right) (d_1 + I_{s_3} \beta_1) \Big(d_3 + E_3 b_2 + I_{s_3} \gamma_2 + S_3 \alpha \beta_2 (p-1) \Big) \left(d_2 + \mu - S_3 \alpha \beta_1 + \frac{E_3 b_1}{(I_{s_3} \theta + 1)^2} \right) \\
&+ \left(I_{s_3} S_3 \alpha \beta_1^2 \left(d_4 - \frac{I_{s_3} c_1}{I_{s_3} \theta + 1} \right) + \frac{E_3 I_{s_3} b_1 c_1 (d_1 + I_{s_3} \beta_1)}{(I_{s_3} \theta + 1)^3} \right) \Big(d_3 + E_3 b_2 + I_{s_3} \gamma_2 + S_3 \alpha \beta_2 (p-1) \Big).
\end{aligned}$$

Coefficients to polynomial Equation (2.28)

$$\begin{aligned}
t_1 =& d_1 + d_2 + d_3 + d_4 + \mu + I_{s_4} \beta_1 + I_{r_4} \gamma_1 + I_{r_4} \gamma_2 - S_4 \alpha \beta_1 + \frac{E_4 b_2}{(I_{r_4} \delta + 1)^2} - \frac{I_{r_4} c_2}{I_{r_4} \delta + 1} + \frac{E_4 b_1}{(I_{s_4} \theta + 1)^2} \\
&- \frac{I_{s_4} c_1}{I_{s_4} \theta + 1} + S_4 \alpha \beta_2 (p-1), \\
t_2 =& (d_1 + I_{s_4} \beta_1) \left(d_3 + I_{r_4} \gamma_2 + \frac{E_4 b_2}{(I_{r_4} \delta + 1)^2} + S_4 \alpha \beta_2 (p-1) - \frac{I_{r_4} c_2}{I_{r_4} \delta + 1} - d_4 + \frac{I_{s_4} c_1}{I_{s_4} \theta + 1} \right) \\
&- \left(\frac{I_{r_4} c_2}{I_{r_4} \delta + 1} - d_4 + \frac{I_{s_4} c_1}{I_{s_4} \theta + 1} \right) \left(d_3 + I_{r_4} \gamma_2 + \frac{E_4 b_2}{(I_{r_4} \delta + 1)^2} + S_4 \alpha \beta_2 (p-1) \right)
\end{aligned}$$

$$+\left(d_1+I_{s_4}\beta_1+d_3+I_{r_4}\gamma_2+\frac{E_4b_2}{(I_{r_4}\delta+1)^2}+S_4\alpha\beta_2(p-1)\right)\left(d_2+\mu+I_{r_4}\gamma_1-S_4\alpha\beta_1+\frac{E_4b_1}{(I_{s_4}\theta+1)^2}\right)$$
$$-\left(\frac{I_{r_4}c_2}{I_{r_4}\delta+1}-d_4+\frac{I_{s_4}c_1}{I_{s_4}\theta+1}\right)\left(d_2+\mu+I_{r_4}\gamma_1-S_4\alpha\beta_1+\frac{E_4b_1}{(I_{s_4}\theta+1)^2}\right)+\frac{E_4I_{r_4}b_2c_2}{(I_{r_4}\delta+1)^3}+\frac{E_4I_{s_4}b_1c_1}{(I_{s_4}\theta+1)^3}$$
$$-I_{r_4}\gamma_2(I_{s_4}\gamma_1-S_4\alpha\beta_2p)+S_4\beta_1(I_{s_4}\alpha\beta_1+I_{r_4}\alpha\beta_2p)-S_4\beta_2\Big(I_{r_4}\gamma_2+I_{r_4}\alpha\beta_2(p-1)\Big),$$

$$t_3=(d_1+I_{s_4}\beta_1)\left(d_3+I_{r_4}\gamma_2+\frac{E_4b_2}{(I_{r_4}\delta+1)^2}+S_4\alpha\beta_2(p-1)-\frac{I_{r_4}c_2}{I_{r_4}\delta+1}+d_4-\frac{I_{s_4}c_1}{I_{s_4}\theta+1}\right)\left(d_2+\mu+I_{r_4}\gamma_1\right.$$
$$\left.-S_4\alpha\beta_1+\frac{E_4b_1}{(I_{s_4}\theta+1)^2}\right)-\left(\frac{I_{r_4}c_2}{I_{r_4}\delta+1}-d_4+\frac{I_{s_4}c_1}{I_{s_4}\theta+1}\right)\left(d_3+I_{r_4}\gamma_2+\frac{(E_4b_2)}{(I_{r_4}\delta+1)^2}+S_4\alpha\beta_2(p-1)\right)\left(d_2\right.$$
$$\left.+\mu+I_{r_4}\gamma_1-S_4\alpha\beta_1+\frac{E_4b_1}{(I_{s_4}\theta+1)^2}\right)-(d_1+I_{s_4}\beta_1)\left(\frac{I_{r_4}c_2}{I_{r_4}\delta+1}-d_4+\frac{I_{s_4}c_1}{I_{s_4}\theta+1}\right)\left(d_3+I_{r_4}\gamma_2+\frac{E_4b_2}{(I_{r_4}\delta+1)^2}\right.$$
$$\left.+S_4\alpha\beta_2(p-1)\right)+S_4\beta_1(I_{s_4}\gamma_1-S_4\alpha\beta_2p)\Big(I_{r_4}\gamma_2+I_{r_4}\alpha\beta_2(p-1)\Big)-I_{r_4}\gamma_2(I_{s_4}\gamma_1-S_4\alpha\beta_2p)(d_1+I_{s_4}\beta_1)$$
$$+I_{r_4}\gamma_2(I_{s_4}\gamma_1-S_4\alpha\beta_2p)\left(\frac{I_{r_4}c_2}{(I_{r_4}\delta+1)}-d_4+\frac{I_{s_4}c_1}{I_{s_4}\theta+1}\right)-I_{r_4}S_4\beta_2\gamma_2(I_{s_4}\alpha\beta_1+I_{r_4}\alpha\beta_2p)$$
$$-S_4\beta_2\Big(I_{r_4}\gamma_2+I_{r_4}\alpha\beta_2(p-1)\Big)\left(d_2+\mu+I_{r_4}\gamma_1-S_4\alpha\beta_1+\frac{E_4b_1}{(I_{s_4}\theta+1)^2}+\frac{I_{r_4}c_2}{I_{r_4}\delta+1}-d_4+\frac{I_{s_4}c_1}{I_{s_4}\theta+1}\right)$$
$$+S_4\beta_1(I_{s_4}\alpha\beta_1+I_{r_4}\alpha\beta_2p)\left(d_3+I_{r_4}\gamma_2+\frac{(E_4b_2)}{(I_{r_4}\delta+1)^2}+S_4\alpha\beta_2(p-1)-\frac{I_{r_4}c_2}{I_{r_4}\delta+1}+d_4-\frac{I_{s_4}c_1}{I_{s_4}\theta+1}\right)$$
$$+\frac{E_4I_{r_4}b_2c_2}{(I_{r_4}\delta+1)^3}\left(d_2+\mu+I_{r_4}\gamma_1-S_4\alpha\beta_1+\frac{E_4b_1}{(I_{s_4}\theta+1)^2}+d_1+I_{s_4}\beta_1\right)+\frac{E_4I_{s_4}b_1c_1}{(I_{s_4}\theta+1)^3}\left(d_1+I_{s_4}\beta_1+d_3\right.$$
$$\left.+I_{r_4}\gamma_2+\frac{(E_4b_2)}{(I_{r_4}\delta+1)^2}+S_4\alpha\beta_2(p-1)\right)-\frac{E_4I_{r_4}b_2c_1(I_{s_4}\gamma_1-S_4\alpha\beta_2p)}{(I_{r_4}\delta+1)(I_{s_4}\theta+1)^2}-\frac{E_4I_{r_4}I_{s_4}b_1c_2\gamma_2}{(I_{r_4}\delta+1)^2(I_{s_4}\theta+1)},$$

$$t_4=I_{r_4}\gamma_2(I_{s_4}\gamma_1-S_4\alpha\beta_2p)(d_1+I_{s_4}\beta_1)\left(\frac{I_{r_4}c_2}{I_{r_4}\delta+1}-d_4+\frac{I_{s_4}c_1}{I_{s_4}\theta+1}\right)-(d_1+I_{s_4}\beta_1)\left(\frac{I_{r_4}c_2}{I_{r_4}\delta+1}-d_4+\frac{I_{s_4}c_1}{I_{s_4}\theta+1}\right)\left(d_3\right.$$
$$\left.+I_{r_4}\gamma_2+\frac{E_4b_2}{(I_{r_4}\delta+1)^2}+S_4\alpha\beta_2(p-1)\right)\left(d_2+\mu+I_{r_4}\gamma_1-S_4\alpha\beta_1+\frac{E_4b_1}{(I_{s_4}\theta+1)^2}\right)$$
$$+S_4\beta_2\Big(I_{r_4}\gamma_2+I_{r_4}\alpha\beta_2(p-1)\Big)\left(\frac{I_{r_4}c_2}{I_{r_4}\delta+1}-d_4+\frac{I_{s_4}c_1}{I_{s_4}\theta+1}\right)\left(d_2+\mu+I_{r_4}\gamma_1-S_4\alpha\beta_1+\frac{E_4b_1}{(I_{s_4}\theta+1)^2}\right)$$
$$-S_4\beta_1(I_{s_4}\alpha\beta_1+I_{r_4}\alpha\beta_2p)\left(\frac{I_{r_4}c_2}{I_{r_4}\delta+1}-d_4+\frac{I_{s_4}c_1}{I_{s_4}\theta+1}\right)\left(d_3+I_{r_4}\gamma_2+\frac{E_4b_2}{(I_{r_4}\delta+1)^2}+S_4\alpha\beta_2(p-1)\right)$$
$$-S_4\beta_1(I_{s_4}\gamma_1-S_4\alpha\beta_2p)\Big(I_{r_4}\gamma_2+I_{r_4}\alpha\beta_2(p-1)\Big)\left(\frac{I_{r_4}c_2}{I_{r_4}\delta+1}-d_4+\frac{I_{s_4}c_1}{I_{s_4}\theta+1}\right)+I_{r_4}S_4\beta_2\gamma_2(I_{s_4}\alpha\beta_1$$
$$+I_{r_4}\alpha\beta_2p)\left(\frac{I_{r_4}c_2}{I_{r_4}\delta+1}-d_4+\frac{I_{s_4}c_1}{I_{s_4}\theta+1}\right)+\frac{E_4I_{s_4}b_1c_1}{(I_{s_4}\theta+1)^3}(d_1+I_{s_4}\beta_1)\left(d_3+I_{r_4}\gamma_2+\frac{(E_4b_2)}{(I_{r_4}\delta+1)^2}+S_4\alpha\beta_2(p-1)\right)$$
$$+\frac{E_4I_{r_4}b_2c_2(d_1+I_{s_4}\beta_1)}{(I_{r_4}\delta+1)^3}\left(d_2+\mu+I_{r_4}\gamma_1-S_4\alpha\beta_1+\frac{E_4b_1}{(I_{s_4}\theta+1)^2}\right)-\frac{E_4I_{r_4}b_2c_1(I_{s_4}\gamma_1-S_4\alpha\beta_2p)(d_1+I_{s_4}\beta_1)}{(I_{r_4}\delta+1)(I_{s_4}\theta+1)^2}$$

$$+\frac{E_4 I_{r_4} S_4 b_2 \beta_1 c_2 (I_{s_4}\alpha\beta_1 + I_{r_4}\alpha\beta_2 p)}{(I_{r_4}\delta+1)^3} - \frac{E_4 I_{s_4} S_4 b_1 \beta_2 c_1 \left(I_{r_4}\gamma_2 + I_{r_4}\alpha\beta_2(p-1)\right)}{(I_{s_4}\theta+1)^3} - \frac{E_4 I_{r_4} I_{s_4} b_1 c_2 \gamma_2 (d_1 + I_{s_4}\beta_1)}{(I_{r_4}\delta+1)^2 (I_{s_4}\theta+1)}$$

$$-\frac{E_4 I_{r_4} S_4 b_2 \beta_2 c_1 (I_{s_4}\alpha\beta_1 + I_{r_4}\alpha\beta_2 p)}{(I_{r_4}\delta+1)(I_{s_4}\theta+1)^2} + \frac{E_4 I_{s_4} S_4 b_1 \beta_1 c_2 \left(I_{r_4}\gamma_2 + I_{r_4}\alpha\beta_2(p-1)\right)}{(I_{r_4}\delta+1)^2 (I_{s_4}\theta+1)}.$$