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Dynamic analysis of HCV infection and drug resistance using an age-structured multiscale model

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Direct-acting antiviral agents (DAAs) are known to interfere with various intracellular stages of the hepatitis C virus (HCV) life cycle and have demonstrated efficacy in treating HCV infection. However, DAA monotherapy can lead to drug resistance due to mutations. This paper explores the impact of DAA therapy on HCV dynamics using a multiscale age-structured partial differential equation (PDE) model that incorporates intracellular viral RNA replication within infected cells and two strains of viruses representing a drug-sensitive strain and a drug-resistant mutant variant, respectively. We derived an equivalent ordinary differential equation (ODE) model from the PDE model to simplify mathematical analysis and numerical simulations. We studied the dynamics of the two virus strains before treatment and investigated the impact of mutations on the evolution kinetics of drug-sensitive and drug-resistant viruses, as well as the competition between the two strains during treatment. We also explored the role of DAAs in blocking HCV RNA replication and releasing new virus particles from cells. During treatment, mutations do not significantly influence the dynamics of various virus strains; however, they can generate low-level HCV that may be completely inhibited due to their poor fitness. The fitness of the mutant strain compared to the drug-sensitive strain determines which strain dominates the virus population. We also investigated the prevalence and drug resistance evolution of HCV variants during DAA treatment.

Keywords: Hepatitis C virus; multiscale model; drug-resistance; drug-sensitive; RNA.

Mathematics Subject Classification 2020: 35Q92, 92D30

1. Introduction

Despite significant advances in medical treatment and prevention, many people worldwide still suffer from infectious diseases. Direct-acting antiviral agents (DAAs)

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that have been developed to treat hepatitis C virus (HCV) infection target the different stages of the viral lifecycle and have significantly increased the sustained virological response (SVR) rate in infected individuals [15]. The multi-drug therapies have greatly improved the clinical outcomes of HCV, and accurate quantification with mathematical models can further optimize their antiviral effects. Ordinary differential equations (ODEs) were often used to establish mathematical models for quantitative data analysis of antiviral activity [21, 26]. To more accurately describe and quantify the different antiviral effects of antiviral drugs, some researchers have proposed multiscale age-structured models with the age of cell infection, which have been used to study the in-host dynamics of a few viral infections [4, 5, 14], particularly for analyzing HCV clinical datasets under the treatment with DAAs [17, 23].

Multiscale models can describe the kinetics of intercellular and intracellular processes, including intracellular viral replication, which may uncover the mechanisms of action of antiviral drugs [19, 27]. These models can accurately quantify antiviral effects from clinical datasets from patients under treatment [11, 20]. For example, Guedj *et al.* developed an age-structured multiscale HCV infection model that accounts for intracellular HCV RNA replication and degradation [4]. The original PDE model for multi-drug HCV treatment, with some mathematical and biological assumptions, provides an approximate solution for clinical data fitting [24]. The age-structured model is typically expressed using partial differential equations (PDEs), which makes it challenging to carry out mathematical and numerical analysis due to potential convergence issues in numerical simulations [4, 23, 24]. Using the method of "model aggregation", which has been well-established in theoretical biology [1, 8, 9], mathematically identical ODEs can be derived from the original PDE model [11, 12].

In this paper, we will develop a multiscale age-structured model encompassing two virus strains and transform it into an equivalent ODE model without requiring additional assumptions. We will analyze the resulting differential equation model and show the local stability of all potential steady states. Furthermore, we will investigate the competition between drug-sensitive and drug-resistant viruses during treatment, as well as the impact of mutations on variant evolution. Comprehensive mathematical analysis of the multiscale model described by PDEs has been challenging due to the mathematical complexity inherent in conducting stability analyses of PDEs with two strains. By deriving an equivalent system from the PDE model, we can expedite the mathematical analysis of the multiscale model through the transformed ODE model and its modified version. These transformations circumvent tedious calculations and may prove valuable for data analysis purposes.

2. Models and Analysis

The basic viral dynamic model was enhanced by incorporating the dynamics of HCV RNA replication in infected cells, resulting in the development of a novel multiscale mathematical model with the age structure of infection. The new model

provides a more accurate description of various antiviral effects throughout the viral life cycle, with a few similar models previously proposed and studied for data analysis as evidenced by previous works such as [5, 23, 24]. We will extend this multiscale model by including another strain of mutants to study the emergence of drug resistance under the treatment of HCV with DAAs [4]. The multiscale model is given by

$$\begin{cases} \dfrac{dT(t)}{dt} = s - \beta V(t)T(t) - dT(t), \\ \dfrac{\partial i(a,t)}{\partial a} + \dfrac{\partial i(a,t)}{\partial t} = -\delta i(a,t), \\ i(0,t) = \beta V(t)T(t), \quad i(a,0) = i_0(a), \\ \dfrac{\partial R(a,t)}{\partial a} + \dfrac{\partial R(a,t)}{\partial t} = \alpha - (\mu+\rho)R(a,t), \\ R(0,t) = 1, \quad R(a,0) = R_0(a), \\ \dfrac{dV(t)}{dt} = \displaystyle\int_0^\infty \rho R(a,t)i(a,t)da - cV(t). \end{cases} \tag{2.1}$$

Here, a represents the age of infection, which is the time elapsed since the virus enters the target cell. The state variables $T(t)$ and $V(t)$ represent the density of target cells and free viral particles, respectively, at time t. $i(a,t)$ denotes the density distribution of infected cells with an infected age of a at time t. Similarly, $R(a,t)$ represents the distribution of intracellular viral RNA in an infected cell with the age of infection a at time t.

It is assumed that target cells are produced at a rate of s, infected with the virus at a rate of β, and die naturally at a rate of d. Infected cells die at a rate of δ, and viruses are cleared at a rate of c. The parameters α and μ represent the production and degradation rate of intracellular viral RNA, respectively. Furthermore, it is assumed that viral RNA is assembled with viral proteins and secreted from infected cells in the form of viral particles at the rate ρ.

2.1. *A multiscale PDE model of HCV drug resistance*

The low fidelity of the HCV RNA polymerase is responsible for the high mutation rate of the HCV RNA genome, which is a significant factor in the development and emergence of resistant viruses. This can lead to incomplete virus inhibition and increase the risk of disease progression [2]. Inspired by this, we propose a new multiscale age-structured model comprising two strains to investigate the prevalence of HCV strains before treatment and the quasispecies dynamics during treatment. The mechanism of DAAs indicates that the gene site encoding a nonstructural protein in HCV RNA targeted by DAAs is the primary mutation site of drug-resistant strains. Taking into account the error-prone nature of HCV polymerase, it is assumed that hepatocytes infected with drug-sensitive viruses will produce both

drug-sensitive and mutation variants of the virus (assuming that a single mutation will lead to a certain level of resistance). This is described by the following equation:

$$\begin{cases} \dfrac{dT(t)}{dt} = s - dT(t) - \beta_s V_s(t)T(t) - \beta_r V_r(t)T(t), \\ \dfrac{\partial i_s(a,t)}{\partial a} + \dfrac{\partial i_s(a,t)}{\partial t} = -\delta i_s(a,t), \\ \dfrac{\partial i_r(a,t)}{\partial a} + \dfrac{\partial i_r(a,t)}{\partial t} = -\delta i_r(a,t), \\ \dfrac{\partial R_s(a,t)}{\partial a} + \dfrac{\partial R_s(a,t)}{\partial t} = (1-m)\alpha_s - (\mu+\rho)R_s(a,t), \\ \dfrac{\partial R_r(a,t)}{\partial a} + \dfrac{\partial R_r(a,t)}{\partial t} = m\alpha_s - (\mu+\rho)R_r(a,t), \\ \dfrac{\partial \widetilde{R_r}(a,t)}{\partial a} + \dfrac{\partial \widetilde{R_r}(a,t)}{\partial t} = \alpha_r - (\mu+\rho)\widetilde{R_r}(a,t), \\ \dfrac{dV_s(t)}{dt} = \displaystyle\int_0^\infty \rho R_s(a,t) i_s(a,t) da - cV_s(t), \\ \dfrac{dV_r(t)}{dt} = \displaystyle\int_0^\infty \rho R_r(a,t) i_s(a,t) da + \int_0^\infty \rho \widetilde{R_r}(a,t) i_r(a,t) da - cV_r(t), \end{cases} \tag{2.2}$$

with the following initial conditions

$$T(0) = T_0, \quad V_s(0) = V_{s0}, \quad V_r(0) = V_{r0}, \quad i_s(a,0) = i_{s_0}(a), \quad i_r(a,0) = i_{r_0}(a),$$
$$R_s(a,0) = R_{s_0}(a), \quad R_r(a,0) = R_{r_0}(a), \quad \widetilde{R_r}(a,0) = \widetilde{R_{r_0}}(a),$$

and boundary conditions

$$i_s(0,t) = \beta_s V_s(t)T(t), \quad i_r(0,t) = \beta_r V_r(t)T(t), \quad R_s(0,t) = 1,$$
$$R_r(0,t) = 0, \quad \widetilde{R_r}(0,t) = 1,$$

where $T(t)$, $V_s(t)$, and $V_r(t)$ represent the density of target cells, drug-sensitive and drug-resistant virus at time t, respectively. These viruses invade the target cells $T(t)$ at rates $\beta_s V_s(t)T(t)$ and $\beta_r V_r(t)T(t)$ to produce infectious infected cells. The variables $i_s(a,t)$ and $i_r(a,t)$ represent the age distribution of drug-sensitive and drug-resistant infected cells at time t. Similarly, $R_s(a,t)$ and $\widetilde{R_r}(a,t)$ represent the age and time distribution of drug-sensitive and drug-resistant intracellular viral RNA. In comparison, $R_r(a,t)$ is the age and temporal distribution of resistant viral RNA produced by mutations from drug-sensitive infected cells. $i_{s_0}(a)$ and $i_{r_0}(a)$ are the initial distributions of drug-sensitive and drug-resistant infected cells, respectively. $R_{s_0}(a)$, $R_{r_0}(a)$, and $\widetilde{R_{r_0}}(a)$ represent the initial distributions of drug-sensitive and drug-resistant intracellular viral RNA. With a single mutation, we assume that the probability of drug-resistant viral RNA produced by replication errors in viral RNA

in drug-sensitive infected hepatocytes is m. The probability of each nucleotide mutation being replicated is approximately between 10^{-4} and 10^{-5}. The parameters α_s and α_r represent the production rates of drug-sensitive and drug-resistant intracellular viral RNA, respectively. $\int_0^\infty \rho R_s(a,t)i_s(a,t)da$ represents the production rate of drug-sensitive virus, where drug-sensitive intracellular viral RNA is assembled with viral proteins and secreted viral particles from drug-sensitive infected cells at the rate ρ. Furthermore, it is assumed that drug-resistant produced by mutations and drug-resistant intracellular viral RNA are assembled with viral proteins at rates $\int_0^\infty \rho R_r(a,t)i_s(a,t)da$ and $\int_0^\infty \rho\widetilde{R_r}(a,t)i_r(a,t)da$ to secrete from drug-sensitive and drug-resistant infected cells in the form of viral particles, respectively. Other parameters have the same biological meaning as in model (2.1).

2.2. *Transformation of the multiscale PDE to ODE model*

The total number of drug-sensitive and drug-resistant infected cells is calculated as $I_s(t) = \int_0^\infty i_s(a,t)da$ and $I_r(t) = \int_0^\infty i_r(a,t)da$, respectively, by integrating over the cell infection age a. Similarly, the total amount of intracellular viral RNA accumulated in both types of infected cells is given by

$$\begin{aligned} P_s(t) &= \int_0^\infty R_s(a,t)i_s(a,t)da, \\ P_r(t) &= \int_0^\infty R_r(a,t)i_s(a,t)da + \int_0^\infty \widetilde{R_r}(a,t)i_r(a,t)da. \end{aligned}$$

The initial values are calculated by the integrals as follows:

$$\begin{aligned} &I_s(0) = \int_0^\infty i_{s0}(a)da, \quad I_r(0) = \int_0^\infty i_{r0}(a)da, \quad P_s(0) = \int_0^\infty R_{s0}(a)i_{s0}(a)da, \\ &P_r(0) = \int_0^\infty R_{r0}(a)i_{s0}(a)da + \int_0^\infty \widetilde{R_{r0}}(a)i_{r0}(a)da. \end{aligned}$$

Next, the partial derivative of $I_s(t)$ and $I_r(t)$ is integrated over the age to obtain the following differential equations:

$$\begin{aligned} \frac{dI_s(t)}{dt} &= \int_0^\infty \frac{\partial i_s(a,t)}{\partial t}da = \int_0^\infty \left\{-\frac{\partial i_s(a,t)}{\partial a} - \delta i_s(a,t)\right\}da \\ &= -[i_s(a,t)]_0^\infty - \delta I_s(t), \\ \frac{dI_r(t)}{dt} &= \int_0^\infty \frac{\partial i_r(a,t)}{\partial t} = \int_0^\infty \left\{-\frac{\partial i_r(a,t)}{\partial a} - \delta i_r(a,t)\right\}da \\ &= -[i_r(a,t)]_0^\infty - \delta I_r(t). \end{aligned} \tag{2.3}$$

Noting that $\lim_{a\to\infty} i_s(a,t) = 0$ and $\lim_{a\to\infty} i_r(a,t) = 0$, and from the boundary conditions of system (2.2), i.e. $i_s(0,t) = \beta_s V_s(t)T(t)$ and $i_r(0,t) = \beta_r V_r(t)T(t)$, we

rewrite (2.3) and obtain

$$\frac{dI_s(t)}{dt} = \beta_s V_s(t)T(t) - \delta I_s(t),$$
$$\frac{dI_r(t)}{dt} = \beta_r V_r(t)T(t) - \delta I_r(t).$$

Similarly, $P_s(t)$ is given by

$$\begin{aligned}
\frac{dP_s(t)}{dt} &= \int_0^\infty \frac{\partial(R_s(a,t)i_s(a,t))}{\partial t}da \\
&= \int_0^\infty \left\{\frac{\partial R_s(a,t)}{\partial t}i_s(a,t) + R_s(a,t)\frac{\partial i_s(a,t)}{\partial t}\right\}da \\
&= \int_0^\infty \left\{\left(-\frac{\partial R_s(a,t)}{\partial a} + (1-m)\alpha_s - (\mu+\rho)R_s(a,t)\right)i_s(a,t)\right. \\
&\quad \left. + R_s(a,t)\left(-\frac{\partial i_s(a,t)}{\partial a} - \delta i_s(a,t)\right)\right\}da \\
&= -\int_0^\infty \frac{\partial(R_s(a,t)i_s(a,t))}{\partial a}da + (1-m)\alpha_s\int_0^\infty i_s(a,t)da - (\mu+\rho+\delta) \\
&\quad \times \int_0^\infty R_s(a,t)i_s(a,t)da \\
&= -[R_s(a,t)i_s(a,t)]_0^\infty + (1-m)\alpha_s I_s(t) - (\mu+\rho+\delta)P_s(t).
\end{aligned}$$

Recall that $\lim_{a\to\infty} i_s(a,t) = 0$ and $\lim_{a\to\infty} R_s(a,t) = (1-m)\alpha_s/(\mu+\rho)$. We have $\lim_{a\to\infty} R_s(a,t)i_s(a,t) = 0$ and $R_s(0,t)i_s(0,t) = \beta_s V_s(t)T(t)$. Therefore, we have the following ODE:

$$\frac{dP_s(t)}{dt} = \beta_s V_s(t)T(t) + (1-m)\alpha_s I_s(t) - (\mu+\rho+\delta)P_s(t).$$

From $\lim_{a\to\infty} i_r(a,t) = 0$, $\lim_{a\to\infty} R_r(a,t) = m\alpha_s/(\mu+\rho)$ and $\lim_{a\to\infty}\widetilde{R_r}(a,t) = \alpha_r/(\mu+\rho)$, we have that $\lim_{a\to\infty} R_r(a,t)i_s(a,t) = 0$, $\lim_{a\to\infty}\widetilde{R_r}(a,t)i_r(a,t) = 0$ and $\widetilde{R_r}(0,t)i_r(0,t) = \beta_r V_r(t)T(t)$. Finally, we see that

$$\begin{aligned}
\frac{dP_r(t)}{dt} &= \int_0^\infty \frac{\partial(R_r(a,t)i_s(a,t))}{\partial t}da + \int_0^\infty \frac{\partial(\widetilde{R_r}(a,t)i_r(a,t))}{\partial t}da \\
&= \int_0^\infty \left\{\frac{\partial R_r(a,t)}{\partial t}i_s(a,t) + \frac{\partial i_s(a,t)}{\partial t}R_r(a,t)\right\}da \\
&\quad + \int_0^\infty \left\{\frac{\partial\widetilde{R_r}(a,t)}{\partial t}i_r(a,t) + \frac{\partial i_r(a,t)}{\partial t}\widetilde{R_r}(a,t)\right\}da \\
&= \int_0^\infty \left\{\left(-\frac{\partial R_r(a,t)}{\partial a} + m\alpha_s - (\mu+\rho)R_r(a,t)\right)i_s(a,t)\right.
\end{aligned}$$

$$
\begin{aligned}
&+\left(-\frac{\partial i_s(a,t)}{\partial a}-\delta i_s(a,t)\right)R_r(a,t)\Big\}da\\
&+\int_0^\infty\Big\{\left(-\frac{\partial \widetilde{R_r}(a,t)}{\partial a}+\alpha_r-(\mu+\rho)\widetilde{R_r}(a,t)\right)i_r(a,t)\\
&+\left(-\frac{\partial i_r(a,t)}{\partial a}-\delta i_r(a,t)\right)\widetilde{R_r}(a,t)\Big\}da\\
=&-\int_0^\infty\frac{\partial(R_r(a,t)i_s(a,t))}{\partial a}da+m\alpha_s\int_0^\infty i_s(a,t)da-(\mu+\rho+\delta)\\
&\times\int_0^\infty R_r(a,t)i_s(a,t)da-\int_0^\infty\frac{\partial(\widetilde{R_r}(a,t)i_r(a,t))}{\partial a}da\\
&+\alpha_r\int_0^\infty i_r(a,t)da-(\mu+\rho+\delta)\int_0^\infty\widetilde{R_r}(a,t)i_r(a,t)da\\
=&-[R_r(a,t)i_s(a,t)]_0^\infty-[\widetilde{R_r}(a,t)i_r(a,t)]_0^\infty+m\alpha_sI_s(t)+\alpha_rI_r(t)\\
&-(\mu+\rho+\delta)P_r(t)\\
=&\beta_rV_r(t)T(t)+m\alpha_sI_s(t)+\alpha_rI_r(t)-(\mu+\rho+\delta)P_r(t).
\end{aligned}
$$

According to the above derivation, the multiscale two-strain PDE system (2.2) is transformed into the ODE system as follows:

$$
\begin{cases}
\dfrac{dT(t)}{dt}=s-dT(t)-\beta_sV_s(t)T(t)-\beta_rV_r(t)T(t),\\
\dfrac{dI_s(t)}{dt}=\beta_sV_s(t)T(t)-\delta I_s(t),\\
\dfrac{dI_r(t)}{dt}=\beta_rV_r(t)T(t)-\delta I_r(t),\\
\dfrac{dP_s(t)}{dt}=\beta_sV_s(t)T(t)+(1-m)\alpha_sI_s(t)-(\mu+\rho+\delta)P_s(t),\\
\dfrac{dP_r(t)}{dt}=\beta_rV_r(t)T(t)+m\alpha_sI_s(t)+\alpha_rI_r(t)-(\mu+\rho+\delta)P_r(t),\\
\dfrac{dV_s(t)}{dt}=\rho P_s(t)-cV_s(t),\\
\dfrac{dV_r(t)}{dt}=\rho P_r(t)-cV_r(t).
\end{cases}
\tag{2.4}
$$

Since our formulation does not make additional assumptions, the original PDE model and our transformed ODE model are mathematically equivalent.

Next, we show that the solution of system (2.4) with the initial conditions is non-negative and ultimately bounded.

Theorem 2.1. *Let* $(T(t), I_s(t), I_r(t), P_s(t), P_r(t), V_s(t), V_r(t))$ *be the solution of system* (2.4) *with the initial conditions in* $\mathbb{R}^7_+$. $T(t), I_s(t), I_r(t), P_s(t), P_r(t), V_s(t)$ *and* $V_r(t)$ *are all positive and ultimately bounded for* $t > 0$.

Proof. First, we consider that $T(t) > 0$ for all $t > 0$. Suppose that there exists a $t_1 > 0$ such that $T(t_1) = 0$ and $T(t) > 0$ for $t \in [0, t_1)$. Thus, $\dot{T}(t_1) \leq 0$. It follows from the first equation of system (2.4) that $\dot{T}(t_1) = s > 0$, which is a contradiction. This proves the claim.

Second, according to the method of variation of constant, equations of system (2.4) except the first are given

$$
\begin{aligned}
I_s(t) &= I_s(0)e^{-\delta t} + \int_0^t \beta_s V_s(\xi)T(\xi)e^{-\delta(t-\xi)}d\xi,\\
I_r(t) &= I_r(0)e^{-\delta t} + \int_0^t \beta_r V_r(\xi)T(\xi)e^{-\delta(t-\xi)}d\xi,\\
P_s(t) &= P_s(0)e^{-(\mu+\rho+\delta)t} + \int_0^t (\beta_s V_s(\xi)T(\xi) + (1-m)\alpha_s I_s(\xi))e^{-(\mu+\rho+\delta)(t-\xi)}d\xi,\\
P_r(t) &= P_r(0)e^{-(\mu+\rho+\delta)t} + \int_0^t (\beta_r V_r(\xi)T(\xi) + m\alpha_s I_s(\xi)\\
&\quad + \alpha_r I_r(\xi))e^{-(\mu+\rho+\delta)(t-\xi)}d\xi,\\
V_s(t) &= V_s(0)e^{-ct} + \int_0^t \rho P_s(\xi)e^{-c(t-\xi)}d\xi,\\
V_r(t) &= V_r(0)e^{-ct} + \int_0^t \rho P_r(\xi)e^{-c(t-\xi)}d\xi,
\end{aligned}
$$

which implies that $I_i(t) > 0, P_i(t) > 0, V_i(t) > 0$ $(i = s, r)$ for a small $t > 0$ with the initial values $I_s(0) = \int_0^\infty i_{s0}(a)da > 0$, $I_r(0) = \int_0^\infty i_{r0}(a)da > 0$, $P_s(0) = \int_0^\infty R_{s0}(a)i_{s0}(a)da > 0$, $P_r(0) = \int_0^\infty R_{r0}(a)i_{s0}(a)da + \int_0^\infty \widetilde{R_{r0}}(a)i_{r0}(a)da > 0$.

Next, we claim that $I_i(t) > 0$, $P_i(t) > 0$, $V_i(t) > 0$ $(i = s, r)$ for all $t > 0$. Assume that there exists a $t_2 > 0$ such that $\min\{I_s(t_2), P_s(t_2), V_s(t_2)\} = 0$.

(i) If $I_s(t_2) = 0, I_s(t) > 0$ for $t \in [0, t_2)$ and $P_s(t), V_s(t) > 0, t \in [0, t_2]$, then we have

$$
I_s(t_2) = I_s(0)e^{-\delta t_2} + \int_0^{t_2} \beta_s V_s(\xi)T(\xi)e^{-\delta(t_2-\xi)}d\xi > 0,
$$

this is a contradiction. Then $I_s(t) > 0$, for all $t > 0$.

(a) If $P_s(t_2) = 0, P_s(t) > 0$ for $t \in [0, t_2)$ and $V_s(t) > 0, t \in [0, t_2]$, then we have

$$P_s(t_2) = P_s(0)e^{-(\mu+\rho+\delta)t_2} + \int_0^{t_2} (\beta_s V_s(\xi)T(\xi) + (1-m)\alpha_s I_s(\xi))e^{-(\mu+\rho+\delta)(t_2-\xi)}d\xi > 0,$$

this is a contradiction. Then $P_s(t) > 0$, for all $t > 0$, and

$$V_s(t) = V_s(0)e^{-ct} + \int_0^t \rho P_s(\xi)e^{-c(t-\xi)}d\xi > 0, t > 0.$$

Thus, we get $P_s(t), V_s(t) > 0$, for all $t > 0$.

(b) If $V_s(t_2) = 0, V_s(t) > 0$ for $t \in [0, t_2)$ and $P_s(t) > 0, t \in [0, t_2]$, then we have

$$V_s(t_2) = V_s(0)e^{-ct_2} + \int_0^{t_2} \rho P_s(\xi)e^{-c(t_2-\xi)}d\xi > 0,$$

this is a contradiction. Then $V_s(t) > 0$, for all $t > 0$, and

$$P_s(t) = P_s(0)e^{-(\mu+\rho+\delta)t} + \int_0^t (\beta_s V_s(\xi)T(\xi) + (1-m)\alpha_s I_s(\xi))e^{-(\mu+\rho+\delta)(t-\xi)}d\xi > 0, \quad t > 0.$$

Thus, we get $P_s(t), V_s(t) > 0$, for all $t > 0$.

This proves that $I_s(t), P_s(t), V_s(t) > 0$ for all $t > 0$.

(ii) If $I_s(t_2) = 0$, $P_s(t_2) = 0$, $I_s(t), P_s(t) > 0$ for $t \in [0, t_2)$ and $V_s(t) > 0, t \in [0, t_2]$, then we get

$$I_s(t_2) = I_s(0)e^{-\delta t_2} + \int_0^{t_2} \beta_s V_s(\xi)T(\xi)e^{-\delta(t_2-\xi)}d\xi > 0,$$

and

$$P_s(t_2) = P_s(0)e^{-(\mu+\rho+\delta)t_2} + \int_0^{t_2} (\beta_s V_s(\xi)T(\xi) + (1-m)\alpha_s I_s(\xi))e^{-(\mu+\rho+\delta)(t_2-\xi)}d\xi > 0,$$

this is a contradiction. Then $I_s(t) > 0, P_s(t) > 0$, for all $t > 0$, and

$$V_s(t) = V_s(0)e^{-ct} + \int_0^t \rho P_s(\xi)e^{-c(t-\xi)}d\xi > 0, \quad t > 0.$$

Therefore, $I_s(t), P_s(t), V_s(t) > 0$ for all $t > 0$.

Similarly, we can prove that $I_s(t_2) = 0, V_s(t_2) = 0$, $I_s(t) > 0, V_s(t) > 0$ for $t \in [0, t_2)$, $P_s(t) > 0$, $t \in [0, t_2]$ and $P_s(t_2) = 0, V_s(t_2) = 0$, $P_s(t) > 0, V_s(t) > 0$ for $t \in [0, t_2)$, $I_s(t) > 0$, $t \in [0, t_2]$ are impossible using the same method. Thus, $I_s(t), P_s(t), V_s(t) > 0$ for all $t > 0$.

(iii) If $I_s(t_2) = 0$, $P_s(t_2) = 0$, $V_s(t_2) = 0$, $I_s(t), P_s(t), V_s(t) > 0$ for $t \in [0, t_2)$, then we obtain

$$I_s(t_2) = I_s(0)e^{-\delta t_2} + \int_0^{t_2} \beta_s V_s(\xi)T(\xi)e^{-\delta(t_2-\xi)}d\xi > 0,$$

$$P_s(t_2) = P_s(0)e^{-(\mu+\rho+\delta)t_2} + \int_0^{t_2} (\beta_s V_s(\xi)T(\xi) + (1-m)\alpha_s I_s(\xi))e^{-(\mu+\rho+\delta)(t_2-\xi)}d\xi > 0,$$

and

$$V_s(t_2) = V_s(0)e^{-ct_2} + \int_0^{t_2} \rho P_s(\xi)e^{-c(t_2-\xi)}d\xi > 0.$$

Therefore, $I_s(t), P_s(t), V_s(t) > 0$ for all $t > 0$.

To sum up, we can prove that $I_s(t), P_s(t), V_s(t) > 0$ for all $t > 0$. Similarly, we can prove that $I_r(t), P_r(t), V_r(t) > 0$ for all $t > 0$. This completes the positivity of the solution.

Next, we prove the boundedness of the solution of system (2.4) with the initial conditions. Denote

$$H_1(t) = T(t) + I_s(t) + I_r(t).$$

We get

$$\frac{dH_1(t)}{dt} = s - dT(t) - \delta(I_s(t) + I_r(t)) \leq s - \sigma_1 H_1(t),$$

where $\sigma_1 = \min\{d, \delta\}$. This implies that $\limsup_{t\to\infty} H_1(t) \leq \frac{s}{\sigma_1}$.

Let

$$H_2(t) = T(t) + P_s(t) + P_r(t) + V_s(t) + V_r(t).$$

We have

$$\frac{dH_2(t)}{dt} = s + \alpha_s I_s(t) + \alpha_r I_r(t) - dT(t) - (\mu+\delta)(P_s(t) + P_r(t)) - c(V_s(t) + V_r(t)) \leq s + M - \sigma_2 H_2(t),$$

where $\sigma_2 = \min\{d, \mu+\delta, c\}$ and $\alpha_s I_s(t) + \alpha_r I_r(t) \leq M$. Therefore, $\limsup_{t\to\infty} H_2(t) \leq \frac{s+M}{\sigma_2}$. In summary, the set

$$\Omega = \left\{ (T(t), I_s(t), I_r(t), P_s(t), P_r(t), V_s(t), V_r(t)) \in \mathbb{R}_+^7 : \ T(t) + I_s(t) + I_r(t) \leq \frac{s}{\sigma_1}, \right.$$
$$\left. T(t) + P_s(t) + P_r(t) + V_s(t) + V_r(t) \leq \frac{s+M}{\sigma_2} \right\}$$

is a positive invariant of the system (2.4). □

2.3. *Basic reproduction numbers and steady states of transformed ODEs*

System (2.4) with the initial conditions has a unique infection-free steady state $P_0 = (T_0, 0, 0, 0, 0, 0, 0)$, where $T_0 = s/d$. Applying the general theory of the basic reproduction number [7], we can obtain

$$R_1 = \frac{\beta_r s\rho}{dc(\mu+\rho+\delta)} + \frac{\beta_r s\rho\alpha_r}{dc\delta(\mu+\rho+\delta)} = R_r^0 + R_r^1,$$
$$R_2 = \frac{\beta_s s\rho}{dc(\mu+\rho+\delta)} + \frac{(1-m)\beta_s s\rho\alpha_s}{dc\delta(\mu+\rho+\delta)} = R_s^0 + R_s^1.$$

Note that R_1 and R_2 are each composed of two renewal processes. R_r^0 (R_s^0) represents the reproduction number of infected cells mediated by viral RNA carrying drug-resistant (drug-sensitive) strains, which enter the host cell as virions. R_r^1 represents the replication of intracellular drug-resistant viral RNA, while R_s^1 represents the replication of intracellular drug-sensitive viral RNA. Our ODE model explicitly accounts for the life cycle of extracellular and intracellular viral RNA, with viral production being driven by viral RNA.

As many ODE models have shown, the basic reproduction number can determine the dynamics of mathematical models, particularly in relation to the existence and stability of steady states. In this section, we study the existence of steady states in the transformed ODEs.

Theorem 2.2. *For the system (2.4) with the initial conditions, we have the following existence results for the steady state.*

(i) *If $R_1 < 1$ and $R_2 < 1$, then there is only the infection-free steady state P_0.*
(ii) *If $R_1 > 1$, then besides P_0, there is also a unique boundary steady state $P_1 = (T_1, 0, I_{r1}, 0, P_{r1}, 0, V_{r1})$, where $T_1 = \frac{s}{dR_1}$, $I_{r1} = \frac{s}{\delta}(1 - \frac{1}{R_1})$, $P_{r1} = \frac{dc}{\beta_r\rho}(R_1 - 1)$ and $V_{r1} = \frac{d}{\beta_r}(R_1 - 1)$.*
(iii) *If $R_2 > 1$ and $R_2 > R_1$, then in addition to P_0, there is also a unique coexistence steady state $P_2 = (T_2, \frac{\beta_s V_{s2} T_2}{\delta}, \frac{\beta_r V_{r2} T_2}{\delta}, \frac{cV_{s2}}{\rho}, \frac{cV_{r2}}{\rho}, V_{s2}, V_{r2})$, where*

$$T_2 = \frac{s}{dR_2}, \quad V_{r2} = \frac{dmR_s^1(R_2-1)}{(1-m)\beta_s\left(\frac{m\beta_r}{(1-m)\beta_s}R_s^1 + R_2 - R_1\right)},$$
$$V_{s2} = \frac{(1-m)(R_2-R_1)}{mR_s^1}V_{r2} = \frac{d(R_2-1)(R_2-R_1)}{\beta_s\left(\frac{m\beta_r}{(1-m)\beta_s}R_s^1 + R_2 - R_1\right)}.$$

2.4. *Local stability analysis of transformed ODEs*

In order to study the stability of the steady states, we linearize the system (2.4) and obtain the following characteristic equation at an arbitrary steady state $P =$

$(T, I_s, I_r, P_s, P_r, V_s, V_r)$

$$\begin{vmatrix} \lambda+d+\beta_s V_s+\beta_r V_r & 0 & 0 & 0 & 0 & \beta_s T & \beta_r T \\ -\beta_s V_s & \lambda+\delta & 0 & 0 & 0 & -\beta_s T & 0 \\ -\beta_r V_r & 0 & \lambda+\delta & 0 & 0 & 0 & -\beta_r T \\ -\beta_s V_s & -(1-m)\alpha_s & 0 & \lambda+\mu+\rho+\delta & 0 & -\beta_s T & 0 \\ -\beta_r V_r & -m\alpha_s & -\alpha_r & 0 & \lambda+\mu+\rho+\delta & 0 & -\beta_r T \\ 0 & 0 & 0 & -\rho & 0 & \lambda+c & 0 \\ 0 & 0 & 0 & 0 & -\rho & 0 & \lambda+c \end{vmatrix} = 0, \tag{2.5}$$

where λ is the eigenvalue.

By studying the three steady-state characteristic equations (2.5) of Theorem 2.2, we have the following stability results.

Theorem 2.3. *The infection-free steady state P_0 of system (2.4) is locally asymptotically stable when $R_1 < 1$ and $R_2 < 1$, and unstable when $R_1 > 1$ or $R_2 > 1$.*

Proof. Taking the linearization of system (2.4) at the point $P = P_0$, we obtain the characteristic equation

$$(\lambda+d)(\lambda+\delta)^2(\lambda+\mu+\rho+\delta)^2(\lambda+c)^2 g_1(\lambda)g_2(\lambda) = 0.$$

Clearly, the stability of P_0 is determined by the following equation:

$$g_1(\lambda) = 1 - \frac{\beta_r \rho T_0}{(\lambda+\mu+\rho+\delta)(\lambda+c)} - \frac{\beta_r \rho T_0 \alpha_r}{(\lambda+\delta)(\lambda+\mu+\rho+\delta)(\lambda+c)} = 0, \tag{2.6}$$

or

$$g_2(\lambda) = 1 - \frac{\beta_s \rho T_0}{(\lambda+\mu+\rho+\delta)(\lambda+c)} - \frac{\beta_s \rho T_0 (1-m)\alpha_s}{(\lambda+\delta)(\lambda+\mu+\rho+\delta)(\lambda+c)} = 0. \tag{2.7}$$

Next, we will study the stability of the steady state by directly comparing the modulus of the characteristic equation. Our first claim is that if the eigenvalue $\lambda = x + iy$ is a solution of Eqs. (2.6) and (2.7), then the real part $x < 0$ when $R_1 < 1$ and $R_2 < 1$. Otherwise, assuming that $x > 0$, we have

$$\begin{aligned} 1 &= \left| \frac{\beta_r \rho T_0}{(\lambda+\mu+\rho+\delta)(\lambda+c)} + \frac{\beta_r \rho T_0 \alpha_r}{(\lambda+\delta)(\lambda+\mu+\rho+\delta)(\lambda+c)} \right| \\ &\le \frac{\beta_r \rho T_0}{c(\mu+\rho+\delta)} + \frac{\beta_r \rho T_0 \alpha_r}{\delta c(\mu+\rho+\delta)} \\ &= R_1, \end{aligned}$$

and

$$\begin{aligned} 1 &= \left| \frac{\beta_s \rho T_0}{(\lambda+\mu+\rho+\delta)(\lambda+c)} + \frac{\beta_s \rho T_0 (1-m)\alpha_s}{(\lambda+\delta)(\lambda+\mu+\rho+\delta)(\lambda+c)} \right| \\ &\le \frac{\beta_s \rho T_0}{c(\mu+\rho+\delta)} + \frac{\beta_s \rho T_0 (1-m)\alpha_s}{\delta c(\mu+\rho+\delta)} \\ &= R_2, \end{aligned}$$

which leads to a contradiction with $R_1 < 1$ and $R_2 < 1$. Therefore, all the characteristic roots of Eqs. (2.6) and (2.7) have negative real parts. This proves the claim and hence P_0 is locally asymptotically stable when $R_1 < 1$ and $R_2 < 1$.

Second, we assume $R_1 > 1$ or $R_2 > 1$. According to the characteristic equations (2.6) and (2.7), it is clear that $g_1(0) = 1 - R_1 < 0$ and $\lim_{\lambda\to+\infty} g_1(\lambda) = 1 > 0$. Similarly, we have $g_2(0) = 1 - R_2 < 0$ and $\lim_{\lambda\to+\infty} g_2(\lambda) = 1 > 0$ when $R_2 > 1$. Thus, there is at least one positive real root such that $g_1(\lambda) = 0$ or $g_2(\lambda) = 0$, which implies that the infection-free steady-state P_0 is unstable when $R_1 > 1$ or $R_2 > 1$. This completes the proof. □

Theorem 2.4. *The boundary steady state P_1 of system (2.4) exists if and only if $R_1 > 1$. It is locally asymptotically stable when $R_1 > 1$ and $R_1 > R_2$ and unstable when $R_1 < R_2$.*

Proof. We substitute the boundary steady state P_1 into the Jacobian matrix and simplify it to the following equation:

$$\begin{aligned}(\lambda+\delta)(\lambda+\mu+\rho+\delta)(\lambda+c)\Bigg[1-\frac{\beta_s\rho T_1}{(\lambda+\mu+\rho+\delta)(\lambda+c)}\\ -\frac{\beta_s\rho T_1(1-m)\alpha_s}{(\lambda+\delta)(\lambda+\mu+\rho+\delta)(\lambda+c)}\Bigg]\cdot[(\lambda+d+\beta_r V_{r1})(\lambda+\delta)(\lambda+\mu+\rho+\delta)\\ \times(\lambda+c)-(\lambda+d)(\lambda+\delta)\beta_r\rho T_1-(\lambda+d)\beta_r\rho T_1\alpha_r]=0.\end{aligned}$$

The stability of P_1 is determined by the equation

$$g_3(\lambda) = 1 - \frac{\beta_s\rho T_1}{(\lambda+\mu+\rho+\delta)(\lambda+c)} - \frac{\beta_s\rho T_1(1-m)\alpha_s}{(\lambda+\delta)(\lambda+\mu+\rho+\delta)(\lambda+c)} = 0, \quad (2.8)$$

or

$$1 + \frac{\beta_r V_{r1}}{\lambda+d} = \frac{\beta_r\rho T_1}{(\lambda+\mu+\rho+\delta)(\lambda+c)} + \frac{\beta_r\rho T_1\alpha_r}{(\lambda+\delta)(\lambda+\mu+\rho+\delta)(\lambda+c)}. \quad (2.9)$$

Next, we prove that all characteristic roots of Eq. (2.8) have negative real parts when $R_1 > R_2$. Using the same method as in Theorem 2.3, we get

$$\begin{aligned}1 &= \left|\frac{\beta_s\rho T_1}{(\lambda+\mu+\rho+\delta)(\lambda+c)} + \frac{\beta_s\rho T_1(1-m)\alpha_s}{(\lambda+\delta)(\lambda+\mu+\rho+\delta)(\lambda+c)}\right|\\ &\le \frac{\beta_s\rho T_1}{c(\mu+\rho+\delta)} + \frac{\beta_s\rho T_1(1-m)\alpha_s}{\delta c(\mu+\rho+\delta)}\\ &= \frac{R_2}{R_1},\end{aligned}$$

which is a contradiction with $R_1 > R_2$. This proves the claim.

Suppose $R_1 < R_2$. We have that $g_3(0) = 1 - \frac{R_2}{R_1} < 0$ and $\lim_{\lambda\to+\infty} g_3(\lambda) = 1 > 0$. Therefore, there is at least one positive real root for $g_3(\lambda) = 0$. This implies that the boundary steady state P_1 is unstable when $R_1 < R_2$.

Similarly, all the solutions of Eq. (2.9) have negative real parts whenever P_1 exists, i.e. $R_1 > 1$. Assuming that λ has a non-negative real part, we obtain

$$\begin{aligned}\left|1+\frac{\beta_r V_{r1}}{\lambda+d}\right| &= \left|\frac{\beta_r \rho T_1}{(\lambda+\mu+\rho+\delta)(\lambda+c)} + \frac{\beta_r \rho T_1 \alpha_r}{(\lambda+\delta)(\lambda+\mu+\rho+\delta)(\lambda+c)}\right| \\ &\leq \frac{\beta_r \rho T_1}{c(\mu+\rho+\delta)} + \frac{\beta_r \rho T_1 \alpha_r}{\delta c(\mu+\rho+\delta)} \\ &= 1. \end{aligned} \tag{2.10}$$

On the other hand, the modulus of the left-hand side of Eq. (2.9) satisfies $|1 + \frac{\beta_r V_{r1}}{\lambda+d}| = |1 + \frac{d(R_1-1)}{\lambda+d}| > 1$. This leads to a contradiction with (2.10). Therefore, all roots of the characteristic equation have negative real parts and the boundary steady state P_1 is locally asymptotically stable when $R_1 > 1$ and $R_1 > R_2$. This proves the claim. □

2.5. *Global stability analysis of transformed ODEs*

In this section, we study the global stability of the transformed ODEs. Because of the difficulty of constructing a Lyapunov function for the full model, we assume that the mutation rate m is equal to zero to obtain the global stability of the system presented in Eq. (2.4). We consider the function $g(x) = x - 1 - \ln(x)$ for $x > 0$, and observe that $g(x)$ is greater than or equal to zero for all positive x, and $g(x)$ is equal to zero if and only if x is equal to one.

Theorem 2.5. *The infection-free steady state P_0 of system (2.4) is globally asymptotically stable when $R_1 < 1$ and $R_2 < 1$.*

Theorem 2.6. *The boundary steady state P_1 of system (2.4) is globally asymptotically stable when $R_1 > R_2$.*

Theorem 2.7. *The coexistence steady state P_2 of system (2.4) is globally asymptotically stable when $R_2 > 1$ and $R_2 > R_1$.*

The proofs of these theorems are given in Appendices A–C, respectively.

3. Effect of Drug Therapy on Viral Strain's Competition

The multiple serine proteases of HCV play an important role in viral polyproteinization, and protease inhibitors (a class of DAAs) have been developed to block this step in the viral life cycle [16]. They have been shown to significantly reduce plasma viral load in infected individuals [18, 25]. The dynamics of intracellular viral RNA are determined by RNA production and loss due to viral RNA degradation and assembly/secretion into plasma. DAAs therapy inhibits intracellular viral RNA production, blocks viral assembly/secretion into plasma, and promotes RNA degradation.

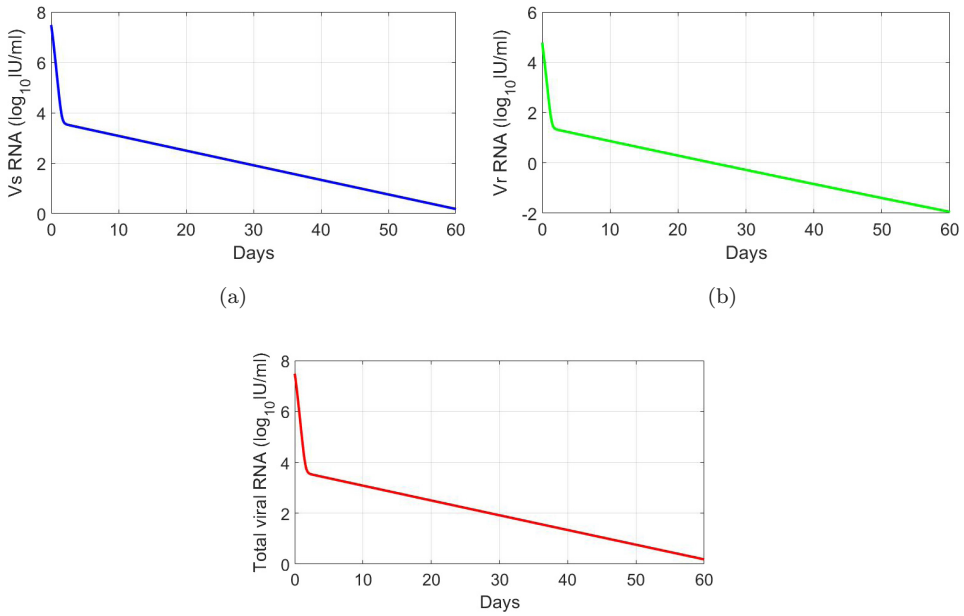


Fig. 1. Dynamics of the drug-sensitive and drug-resistant viruses when mutations produce low levels of resistance during treatment. Both strains are suppressed and $\frac{R_1}{R_2} = 0.95, \alpha_r = 38, \varepsilon_r = 0.99, \eta_r = 0.6$. The other parameters are shown in Table 1.

It is assumed that ε_s and ε_r are drug efficacies that inhibit the production of viral RNA by drug-sensitive and drug-resistant infected cells, respectively, where $0 \leq \varepsilon_s, \varepsilon_r \leq 1$. η_s and η_r are drug effectiveness blocking the assembly/secretion of drug-sensitive and drug-resistant viral RNA in plasma in infected cells, respectively, where $0 \leq \eta_s, \eta_r \leq 1$. The parameter k measures the promotion of the RNA degradation rate of the two strains and $k \geq 1$. It is further assumed that the drug-sensitive and drug-resistant viruses coexist in a stable state at the beginning of treatment, and the multiscale model under treatment can be described by the following system:

$$\begin{cases} \dfrac{dT(t)}{dt} = s - dT(t) - \beta_s V_s(t)T(t) - \beta_r V_r(t)T(t), \\ \dfrac{dI_s(t)}{dt} = \beta_s V_s(t)T(t) - \delta I_s(t), \\ \dfrac{dI_r(t)}{dt} = \beta_r V_r(t)T(t) - \delta I_r(t), \\ \dfrac{dP_s(t)}{dt} = \beta_s V_s(t)T(t) + (1-m)(1-\varepsilon_s)\alpha_s I_s(t) \\ \qquad\qquad - (k\mu + (1-\eta_s)\rho + \delta)P_s(t), \end{cases}$$

$$\begin{cases} \dfrac{dP_r(t)}{dt} = \beta_r V_r(t)T(t) + m(1-\varepsilon_s)\alpha_s I_s(t) + (1-\varepsilon_r)\alpha_r I_r(t) \\ \qquad\qquad - (k\mu + (1-\eta_r)\rho + \delta)P_r(t), \\ \dfrac{dV_s(t)}{dt} = (1-\eta_s)\rho P_s(t) - cV_s(t), \\ \dfrac{dV_r(t)}{dt} = (1-\eta_r)\rho P_r(t) - cV_r(t). \end{cases} \tag{3.1}$$

The basic reproduction numbers for the two strains during treatment are given as follows:

$$R_1' = \frac{(1-\eta_r)\beta_r s\rho}{dc(k\mu + (1-\eta_r)\rho + \delta)} + \frac{(1-\varepsilon_r)(1-\eta_r)\beta_r s\rho\alpha_r}{dc\delta(k\mu + (1-\eta_r)\rho + \delta)},$$
$$R_2' = \frac{(1-\eta_s)\beta_s s\rho}{dc(k\mu + (1-\eta_s)\rho + \delta)} + \frac{(1-\varepsilon_s)(1-\eta_s)(1-m)\beta_s s\rho\alpha_s}{dc\delta(k\mu + (1-\eta_s)\rho + \delta)}.$$

Before treatment, two virus strains coexisted and reached a stable steady state, but the level of drug-resistant virus is very low ($R_2 > 1$). During DAAs therapy, the drug-sensitive virus was effectively inhibited ($R_2' = 0.54 < 1$, Fig. 1(a)), and if the mutation resulted in only low levels of resistance ($\varepsilon_r = 0.99, \eta_r = 0.6$), the resistant virus was also inhibited ($R_1' = 0.18 < 1$, Fig. 1(b)), leading to a decrease in the total viral load (Fig. 1(c)). However, if the mutation resulted in a high level of resistance ($\varepsilon_r = 0.1, \eta_r = 0.1$), R_1' exceeded 1 and the drug-resistant virus initially declined and then rebounded under DAAs treatment, eventually dominating the viral population (Fig. 2(c)). The drug-sensitive virus was still effectively inhibited in this scenario (Fig. 2(a)).

4. The Effect of Mutations on Strain's Evolution During Treatment

In this section, we introduce the backward mutation in model (3.1). Specifically, we consider the scenario where drug-resistant infected cells can also mutate under drug treatment to produce drug-sensitive viral RNA. We will investigate the effect of these mutations on the kinetics of the two virus strains during treatment. We make the simplifying assumption that the rate of backward mutation is equal to the rate of forward mutation.

$$\begin{cases} \dfrac{dT(t)}{dt} = s - dT(t) - \beta_s V_s(t)T(t) - \beta_r V_r(t)T(t), \\ \dfrac{dI_s(t)}{dt} = \beta_s V_s(t)T(t) - \delta I_s(t), \end{cases}$$

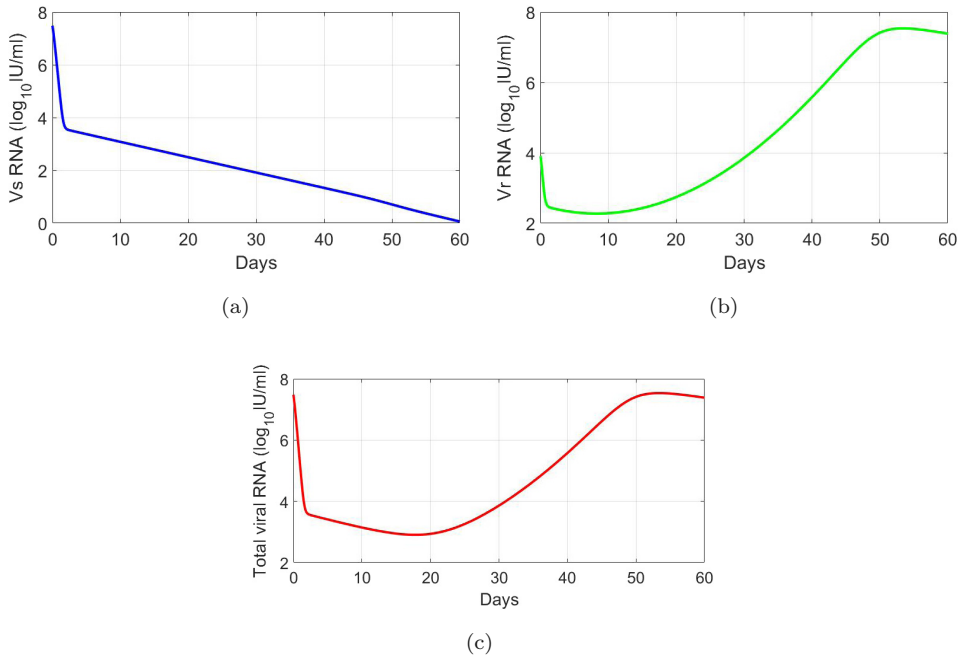


Fig. 2. During treatment, when mutations produced a high level of resistance, there was a competition between drug-sensitive and drug-resistant viruses. As a result, the drug-sensitive viruses were inhibited, and the drug-resistant viruses appeared and eventually dominated the virus population. This led to an overall rebound in the virus level, which stabilized at a certain value. In this simulation, we have $\frac{R_1}{R_2} = 0.63$, $\alpha_r = 25, \varepsilon_r = 0.1, \eta_r = 0.1$, with the other parameters shown in Table 1.

$$\begin{cases} \dfrac{dI_r(t)}{dt} = \beta_r V_r(t)T(t) - \delta I_r(t), \\ \dfrac{dP_s(t)}{dt} = \beta_s V_s(t)T(t) + (1-m)(1-\varepsilon_s)\alpha_s I_s(t) + m(1-\varepsilon_r)\alpha_r I_r(t) \\ \qquad\qquad - (k\mu + (1-\eta_s)\rho + \delta)P_s(t), \\ \dfrac{dP_r(t)}{dt} = \beta_r V_r(t)T(t) + m(1-\varepsilon_s)\alpha_s I_s(t) + (1-m)(1-\varepsilon_r)\alpha_r I_r(t) \\ \qquad\qquad - (k\mu + (1-\eta_r)\rho + \delta)P_r(t), \\ \dfrac{dV_s(t)}{dt} = (1-\eta_s)\rho P_s(t) - cV_s(t), \\ \dfrac{dV_r(t)}{dt} = (1-\eta_r)\rho P_r(t) - cV_r(t). \end{cases} \tag{4.1}$$

Before treatment, the model has an infection-free steady state $E_0 = (s/d, 0, 0, 0, 0, 0, 0)$ and a coexisting steady state $E^* = (T^*, I_s^*, I_r^*, P_s^*, P_r^*, V_s^*, V_r^*)$.

Table 1. Values of fixed parameters in numerical simulation.

Parameter	Unit	Values	References
s	cells mL^{-1}day^{-1}	7.5×10^5	[11]
d	day^{-1}	0.01	[11]
β_s	mL day^{-1}virions^{-1}	10^{-7}	[23]
β_r	mL day^{-1}virions^{-1}	10^{-8}	[23]
δ	day^{-1}	0.14	[23]
ρ	day^{-1}	8.18	[23]
k	1	4.94	[23]
μ	day^{-1}	1	[23]
c	day^{-1}	6.2	[11]
m	per copied nucleotide	10^{-4}	[22]
ε_s	1	0.9997	[23]
η_s	1	0.56	[23]
α_s	virions cell^{-1}day^{-1}	40	[23]

From I_s, P_s and V_s, we obtain

$$m\alpha_r\rho\beta_r V_r^* T^* = [(\mu+\rho+\delta)c\delta - (\delta+(1-m)\alpha_s)\beta_s T^*\rho]V_s^*, \tag{4.2}$$

where T^*, V_r^* and V_s^* represent the steady state of uninfected target cells, drug-resistant and drug-sensitive viruses, respectively. Similarly, from the I_r, P_r and V_r equations, we have

$$m\alpha_s\rho\beta_s V_s^* T^* = [(\mu+\rho+\delta)c\delta - (\delta+(1-m)\alpha_r)\beta_r T^*\rho]V_r^*. \tag{4.3}$$

Therefore, the two strains coexist if the following conditions are satisfied:

$$\frac{[\delta+(1-m)\alpha_s]\beta_s T^*\rho}{(\mu+\rho+\delta)c\delta} < 1, \tag{4.4}$$

and

$$\frac{[\delta+(1-m)\alpha_r]\beta_r T^*\rho}{(\mu+\rho+\delta)c\delta} < 1. \tag{4.5}$$

Based on (4.2) and (4.3), we can find the equation for the steady state of uninfected target cells:

$$\begin{aligned}&\{[\delta+(1-m)\alpha_s][\delta+(1-m)\alpha_r] - m^2\alpha_s\alpha_r\}\beta_s\beta_r\rho^2 T^{*2}\\ &\quad - \{[\delta+(1-m)\alpha_s]\beta_s + [\delta+(1-m)\alpha_r]\beta_r\}(\mu+\rho+\delta)c\delta\rho T^*\\ &\quad + (\mu+\rho+\delta)^2 c^2\delta^2 = 0.\end{aligned}$$

Two solutions of the above equation can be obtained by using the quadratic formula:

$$T_{1,2}^* = \frac{B \pm \sqrt{(B^2-4A)}}{2\rho A}(\mu+\rho+\delta)c\delta, \tag{4.6}$$

where $A = \{[\delta+(1-m)\alpha_s][\delta+(1-m)\alpha_r]-m^2\alpha_s\alpha_r\}\beta_s\beta_r$, $B = \{[\delta+(1-m)\alpha_s]\beta_s + [\delta+(1-m)\alpha_r]\beta_r\}$. Ignoring m, we get two approximate solutions

$$T_1^* \approx \frac{(\mu+\rho+\delta)c\delta}{\beta_r\rho(\delta+\alpha_r)}, \quad \text{when (4.6) takes the positive sign,}$$

$$T_2^* \approx \frac{(\mu+\rho+\delta)c\delta}{\beta_s\rho(\delta+\alpha_s)}, \quad \text{when (4.6) takes the negative sign.}$$

We know that only T_2^* exists if the existing condition of coexistence is satisfied. Therefore, according to model (4.1) and T_2^*, the coexistence steady state E^* is calculated as follows:

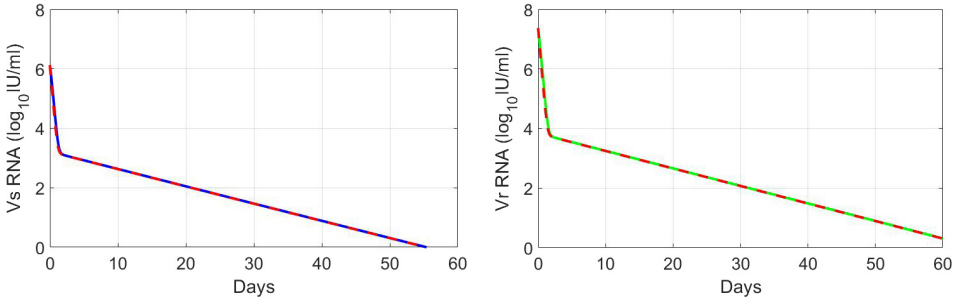
$$I_s^* = \frac{s-dT_2^*-\delta I_r^*}{\delta}, \quad I_r^* = \frac{(s-dT_2^*)\beta_r[(\delta+\alpha_s)\beta_s\rho T_2^* - (\mu+\rho+\delta)c\delta]}{c\delta^2(\mu+\rho+\delta)(\beta_s-\beta_r)+\rho\delta\beta_s\beta_r T_2^*(\alpha_s-\alpha_r)},$$

$$P_s^* = \frac{cV_s^*}{\rho}, \quad P_r^* = \frac{cV_r^*}{\rho}, \quad V_s^* = \frac{\delta I_s^*}{\beta_s T_2^*}, \; V_r^* = \frac{\delta I_r^*}{\beta_r T_2^*}.$$

We compared the viral kinetics of model (4.1) after drug administration with the no-mutation (i.e. $m = 0$) model that ignores both forward and backward mutations, assuming that both drug-sensitive and drug-resistant viruses were at baseline levels before treatment. Figure 3 shows the viral kinetics of the drug-resistant virus when the drug efficacy that inhibits RNA during treatment was large ($\varepsilon_r = 0.999, \eta_r = 0.6$). The blue and green solid lines in Figs. 3(a) and 3(b) almost overlap with the red dashed lines, respectively, suggesting that the kinetic effects of the mutation on drug-sensitive and drug-resistant strains are negligible. Even when the mutation confers a high degree of resistance, the contribution of the mutation to the level of drug-resistant virus variants remains small (Fig. 4(b)). However, in this case, the drug-sensitive virus can be maintained at a low level by backward mutation rather than being completely suppressed (Fig. 4(a)). These phenomena can be explained by the on-treatment reproduction numbers $\varepsilon_s = 0.9997$ and $\eta_s = 0.56$ because $m(1-\varepsilon_s)$ is small under effective treatment for the drug-sensitive virus. Therefore, the contribution of drug-sensitive infectious cell mutations to the generation of resistant viral RNA is negligible. In summary, mutations play a minor role in the evolution of drug resistance during treatment but they generate pre-existing mutants that can be selected and grow under therapy.

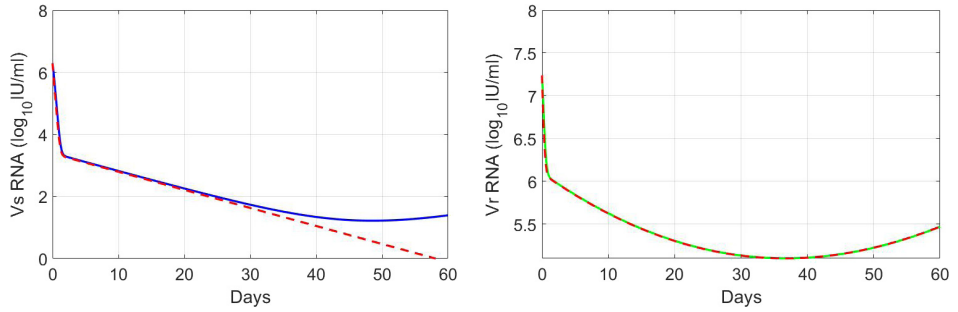
5. Conclusion and Discussion

Mathematical modeling can significantly enhance our understanding of disease transmission and the intricate biological phenomena involving numerous variables and parameters across different time scales. Various mathematical models have been employed to evaluate how key parameters can prevent and mitigate the spread of epidemics [1, 8–10]. Simplifying a detailed structured model into a more manage-



(a) (b)

Fig. 3. (Color online) During treatment, assuming that the mutation had low resistance ($\frac{R_1}{R_2} = 0.75$), we examined the effect of the mutations on the evolution of the two strains using the (4.1) model with and without mutations ($m = 10^{-4}$ and $m = 0$, respectively). The blue and green solid lines represent the trends of the drug-sensitive and drug-resistant viruses in the presence of mutations, respectively, while the red dashed line represents the model without mutations. The almost overlapping solid and dashed lines in (a) and (b) indicate that when the mutation produced a low level of resistance, it had a negligible effect on the evolution of both strains. The parameters used were $\alpha_r = 30$, $\varepsilon_r = 0.999, \eta_r = 0.6$, and the other parameters are listed in Table 1.



(a) (b)

Fig. 4. (Color online) During treatment, assuming a high level of resistance of the mutant ($\frac{R_1}{R_2} = 0.25$), we studied the effect of mutations on the evolution of both strains: the blue and green solid lines show the trend of susceptibility and resistance viruses, respectively, in the presence of mutations in model (4.1) ($m = 10^{-4}$), while the red dashed line represents the model with no mutations in (4.1) ($m = 0$). In (a), it can be seen that drug-sensitive viruses are maintained at low levels by mutations rather than being completely suppressed. In (b), it is indicated that the evolution of drug-resistant viruses is not greatly facilitated, and the emergence and rebound of drug-resistant viruses dominate the viral populations. Here we chose $\alpha_r = 10, \varepsilon_r = 0.4, \eta_r = 0.4$ and other parameters were from Table 1.

able one, without losing essential information for data analysis, is a cornerstone of theoretical biology known as "model aggregation". The aggregate model introduced here is simpler than the original model and more tractable both analytically and numerically. Consequently, this model aggregation aids in analyzing model dynamics and facilitates comparison with experimental data.

In this paper, we developed a multiscale PDE model incorporating two strains of HCV infection. By transforming the age-structured PDE model (2.2) into an ODE model (2.4) without additional assumptions, we derived a mathematically identical model that is more suitable for parameter estimation from clinical datasets. This transformed ODE model (2.4) is advantageous for numerical calculations, as numerical simulations of PDEs can have convergence issues and are computationally intensive. It is also noted that our transformed ODE model (2.4) describes the total number of intracellular drug-sensitive and drug-resistant viral RNAs (i.e. $P_s(t)$ and $P_r(t)$ in model (2.4)), while the original PDE model describes the number of intracellular drug-sensitive and drug-resistant viral RNAs within an infected cell (i.e. $R_s(t)$, $R_r(t)$, and $\widetilde{R_r}(t)$ in (2.2)).

Starting with a model that includes two virus strains (drug-sensitive and drug-resistant), we analyzed the coexistence of the two strains before treatment, noting that resistant viruses comprised a minor fraction of the virus population. We defined the basic reproduction numbers R_1 and R_2 for the ODE model, providing a threshold for the system's mathematical structure. Our analysis clarified the stability dynamics of the interaction between the two virus strains before treatment. Additionally, we studied the impact of reverse or backward mutations on the evolution kinetics of drug-sensitive and drug-resistant viruses, as well as the competition between the two strains during treatment.

Our findings indicate that the effect of backward mutations on mutation frequency before treatment is negligible. Since DAAs are highly effective against drug-sensitive viruses, forward and backward mutations do not significantly influence the evolution of resistant viruses. However, when drug-resistant viruses dominate the viral population, backward mutations can sustain very low levels of drug-sensitive viruses. Therefore, treatment-emergent mutations do not substantially affect the kinetics of HCV variation. During treatment, drug-sensitive viruses are typically inhibited by effective agents, while the inhibition of drug-resistant viruses depends on the mutants' relative fitness and drug efficacy.

While DAAs are currently effective in treating HCV infection, therapeutic failure can occur in some patients with HCV harboring resistance-associated substitutions, especially in the Nonstructuralon protein 5A (NS5A) region of the HCV genome [3]. Transformed ODE models are beneficial for accurately quantifying antiviral effects from clinical datasets. Our method may be particularly useful for studying cell culture experiments, where frequent samples of various kinetic measurements are available in a simpler environment than *in vivo* infections. Our mathematical model can be fully parameterized with *in vitro* data to quantify the kinetics of viral infection.

In summary, the ODE model derived from the multiscale age-structured PDE model simplifies data analysis without involving heavy computations. This approach provides a practical tool for researchers and clinicians to better understand HCV dynamics and the implications of drug resistance, ultimately aiding in the development of more effective therapeutic strategies.

Acknowledgment

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Appendix A. Global Stability of the Infection-Free Steady State

We define the following Lyapunov function:

$$\begin{aligned} &L_0(t)\\ &:= \frac{\rho}{\delta(\mu+\rho+\delta)}T_0 g\left(\frac{T}{T_0}\right) + \frac{\rho\alpha_s}{\delta(\mu+\rho+\delta)(\delta+\alpha_s)}I_s + \frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}I_r\\ &\quad + \frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_s)}P_s + \frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}P_r + \frac{1}{\delta+\alpha_s}V_s + \frac{1}{\delta+\alpha_r}V_r. \end{aligned}$$

Taking the time derivative of $L_0(T, I_s, I_r, P_s, P_r, V_s, V_r)$ along the solution of (2.4) with $m = 0$, we obtain

$$\begin{aligned} &\frac{dL_0(t)}{dt}\\ &= \frac{\rho}{\delta(\mu+\rho+\delta)}\left(1-\frac{T_0}{T}\right)(s - dT - \beta_s V_s T - \beta_r V_r T) + \frac{\rho\alpha_s}{\delta(\mu+\rho+\delta)(\delta+\alpha_s)}\\ &\quad \times(\beta_s V_s T - \delta I_s) + \frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}(\beta_r V_r T - \delta I_r)\\ &\quad + \frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_s)}(\beta_s V_s T + \alpha_s I_s - (\mu+\rho+\delta)P_s)\\ &\quad + \frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}(\beta_r V_r T + \alpha_r I_r - (\mu+\rho+\delta)P_r) + \frac{1}{\delta+\alpha_s}\\ &\quad \times(\rho P_s - cV_s) + \frac{1}{\delta+\alpha_r}(\rho P_r - cV_r). \end{aligned}$$

Substituting $s = dT_0$ into the above formula, we get

$$\begin{aligned} &\frac{dL_0(t)}{dt}\\ &= \frac{\rho}{\delta(\mu+\rho+\delta)}dT_0\left(2-\frac{T}{T_0}-\frac{T_0}{T}\right) - \frac{\rho}{\delta(\mu+\rho+\delta)}\left(1-\frac{T_0}{T}\right)\beta_s V_s T\\ &\quad - \frac{\rho}{\delta(\mu+\rho+\delta)}\left(1-\frac{T_0}{T}\right)\beta_r V_r T + \frac{\rho\alpha_s}{\delta(\mu+\rho+\delta)(\delta+\alpha_s)}(\beta_s V_s T - \delta I_s)\\ &\quad + \frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}(\beta_r V_r T - \delta I_r) + \frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_s)}(\beta_s V_s T + \alpha_s I_s\\ &\quad - (\mu+\rho+\delta)P_s) + \frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}(\beta_r V_r T + \alpha_r I_r - (\mu+\rho+\delta)P_r) \end{aligned}$$

$$
\begin{aligned}
&+\frac{1}{\delta+\alpha_s}(\rho P_s - cV_s)+\frac{1}{\delta+\alpha_r}(\rho P_r - cV_r)\\
&=\frac{\rho}{\delta(\mu+\rho+\delta)}dT_0\left(-g\left(\frac{T_0}{T}\right)-g\left(\frac{T}{T_0}\right)\right)+\frac{\rho}{\delta(\mu+\rho+\delta)}\beta_s V_s T_0\\
&+\frac{\rho}{\delta(\mu+\rho+\delta)}\beta_r V_r T_0-\frac{c}{\delta+\alpha_s}V_s-\frac{c}{\delta+\alpha_r}V_r\\
&=\frac{\rho}{\delta(\mu+\rho+\delta)}dT_0\left(-g\left(\frac{T_0}{T}\right)-g\left(\frac{T}{T_0}\right)\right)+\frac{c}{\delta+\alpha_r}\left(\frac{\beta_r\rho T_0(\delta+\alpha_r)}{c\delta(\mu+\rho+\delta)}-1\right)\\
&\times V_r+\frac{c}{\delta+\alpha_s}\left(\frac{\beta_s\rho T_0(\delta+\alpha_s)}{c\delta(\mu+\rho+\delta)}-1\right)V_s\\
&=\frac{\rho}{\delta(\mu+\rho+\delta)}dT_0\left(-g\left(\frac{T_0}{T}\right)-g\left(\frac{T}{T_0}\right)\right)+\frac{c}{\delta+\alpha_r}(R_1-1)V_r\\
&+\frac{c}{\delta+\alpha_s}(R_2-1)V_s.
\end{aligned}
$$

Therefore, we show that $\frac{dL_0(t)}{dt}\leq 0$ when $R_1<1$ and $R_2<1$. Assume that M_0 is the largest invariant set $\{(T,I_s,I_r,P_s,P_r,V_s,V_r)|\frac{dL_0}{dt}\}=0$. We further have $\frac{dL_0}{dt}=0$ if and only if $T=T_0, I_s=0, I_r=0, P_s=0, P_r=0, V_s=0$, and $V_r=0$. Obviously, $M_0=\{P_0\}$. Therefore, by applying LaSalle's Invariance Principle (see [13, Theorem 3.4.7] or [6]), P_0 is globally asymptotically stable if $R_1<1$ and $R_2<1$.

Appendix B. Global Stability of the Boundary Steady State

We define the following Lyapunov function:

$$
\begin{aligned}
L_1(t):=&\frac{\rho}{\delta(\mu+\rho+\delta)}T_1 g\left(\frac{T}{T_1}\right)+\frac{\rho\alpha_s}{\delta(\mu+\rho+\delta)(\delta+\alpha_s)}I_s\\
&+\frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}I_{r1}g\left(\frac{I_r}{I_{r1}}\right)+\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_s)}P_s\\
&+\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}P_{r1}g\left(\frac{P_r}{P_{r1}}\right)+\frac{1}{\delta+\alpha_s}V_s+\frac{1}{\delta+\alpha_r}V_{r1}g\left(\frac{V_r}{V_{r1}}\right).
\end{aligned}
$$

Taking the time derivative of $L_1(t)$ along the solution of (2.4) with $m=0$, we obtain

$$
\begin{aligned}
&\frac{dL_1(t)}{dt}\\
&=\frac{\rho}{\delta(\mu+\rho+\delta)}\left(1-\frac{T_1}{T}\right)(s-dT-\beta_s V_s T-\beta_r V_r T)+\frac{\rho\alpha_s}{\delta(\mu+\rho+\delta)(\delta+\alpha_s)}\\
&\times(\beta_s V_s T-\delta I_s)+\frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\left(1-\frac{I_{r1}}{I_r}\right)(\beta_r V_r T-\delta I_r)
\end{aligned}
$$

$$+\frac{1}{\delta+\alpha_r}\left(1-\frac{V_{r1}}{V_r}\right)(\rho P_r - cV_r)+\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\left(1-\frac{P_{r1}}{P_r}\right)$$
$$\times(\beta_r V_r T+\alpha_r I_r-(\mu+\rho+\delta)P_r)+\frac{1}{\delta+\alpha_s}(\rho P_s - cV_s)$$
$$+\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_s)}(\beta_s V_s T+\alpha_s I_s-(\mu+\rho+\delta)P_s).$$

Substituting $s = dT_1 + \beta_r V_{r1} T_1$ into the above first term, we get

$$\frac{\rho}{\delta(\mu+\rho+\delta)}\left(1-\frac{T_1}{T}\right)(s-dT-\beta_s V_s T-\beta_r V_r T)$$
$$=\frac{\rho}{\delta(\mu+\rho+\delta)}\left(1-\frac{T_1}{T}\right)(dT_1+\beta_r V_{r1}T_1-dT-\beta_s V_s T-\beta_r V_r T)$$
$$=\frac{\rho}{\delta(\mu+\rho+\delta)}\left\{dT_1\left(2-\frac{T_1}{T}-\frac{T}{T_1}\right)+\left(1-\frac{T_1}{T}\right)\right.$$
$$\left.\times(\beta_r V_{r1}T_1-\beta_s V_s T-\beta_r V_r T)\right\}$$
$$=\frac{\rho}{\delta(\mu+\rho+\delta)}\left\{dT_1\left(2-\frac{T_1}{T}-\frac{T}{T_1}\right)+\beta_r V_{r1}T_1\left(1-\frac{T_1}{T}-\frac{V_r T}{V_{r1}T_1}+\frac{V_r}{V_{r1}}\right)\right\}$$
$$-\frac{\rho}{\delta(\mu+\rho+\delta)}\left(1-\frac{T_1}{T}\right)\beta_s V_s T$$
$$=\frac{\rho}{\delta(\mu+\rho+\delta)}dT_1\left(2-\frac{T_1}{T}-\frac{T}{T_1}\right)+\frac{\rho}{\delta(\mu+\rho+\delta)}\beta_r V_{r1}T_1\left[-g\left(\frac{T_1}{T}\right)\right.$$
$$\left.-g\left(\frac{TV_r}{T_1V_{r1}}\right)+g\left(\frac{V_r}{V_{r1}}\right)\right]-\frac{\rho}{\delta(\mu+\rho+\delta)}\left(1-\frac{T_1}{T}\right)\beta_s V_s T.$$

Using the steady state equations $\delta I_{r1} = \beta_r V_{r1} T_1$, $(\mu+\rho+\delta)P_{r1} = \beta_r V_{r1} T_1 + \alpha_r I_{r1}$ and $\rho P_{r1} = cV_{r1}$, the third, fourth and fifth items are rewritten as follows:

$$\frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\left(1-\frac{I_{r1}}{I_r}\right)(\beta_r V_r T-\delta I_r)$$
$$=\frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\left(1-\frac{I_{r1}}{I_r}\right)\left(\beta_r V_r T-\delta I_{r1}\frac{I_r}{I_{r1}}\right)$$
$$=\frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r1}T_1\left(1-\frac{I_{r1}}{I_r}\right)\left(\frac{TV_r}{T_1V_{r1}}-\frac{I_r}{I_{r1}}\right)$$
$$=\frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r1}T_1\left(\frac{TV_r}{T_1V_{r1}}-\frac{I_r}{I_{r1}}-\frac{I_{r1}TV_r}{I_rT_1V_{r1}}+1\right)$$
$$=\frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r1}T_1\left[g\left(\frac{TV_r}{T_1V_{r1}}\right)-g\left(\frac{I_r}{I_{r1}}\right)-g\left(\frac{I_{r1}TV_r}{I_rT_1V_{r1}}\right)\right],$$

$$\begin{aligned}
&\frac{1}{\delta+\alpha_r}\left(1-\frac{V_{r1}}{V_r}\right)(\rho P_r - cV_r)\\
&\quad=\frac{1}{\delta+\alpha_r}\left(1-\frac{V_{r1}}{V_r}\right)\left(\frac{\rho}{\mu+\rho+\delta}\frac{P_r}{P_{r1}}(\mu+\rho+\delta)P_{r1}-\frac{\rho}{\mu+\rho+\delta}\right.\\
&\qquad\left.\times\frac{V_r}{V_{r1}}(\mu+\rho+\delta)P_{r1}\right)\\
&\quad=\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}(\beta_r V_{r1}T_1+\alpha_r I_{r1})\left(1-\frac{V_{r1}}{V_r}\right)\left(\frac{P_r}{P_{r1}}-\frac{V_r}{V_{r1}}\right)\\
&\quad=\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}(\beta_r V_{r1}T_1+\frac{\alpha_r}{\delta}\delta I_{r1})\left(\frac{P_r}{P_{r1}}-\frac{V_r}{V_{r1}}-\frac{V_{r1}P_r}{V_rP_{r1}}+1\right)\\
&\quad=\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r1}T_1\left[g\left(\frac{P_r}{P_{r1}}\right)-g\left(\frac{V_r}{V_{r1}}\right)-g\left(\frac{V_{r1}P_r}{V_rP_{r1}}\right)\right]\\
&\qquad+\frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r1}T_1\left[g\left(\frac{P_r}{P_{r1}}\right)-g\left(\frac{V_r}{V_{r1}}\right)-g\left(\frac{V_{r1}P_r}{V_rP_{r1}}\right)\right]
\end{aligned}$$

and

$$\begin{aligned}
&\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\left(1-\frac{P_{r1}}{P_r}\right)(\beta_r V_rT+\alpha_r I_r-(\mu+\rho+\delta)P_r)\\
&\quad=\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\left(1-\frac{P_{r1}}{P_r}\right)\left[\beta_r V_rT+\alpha_r I_r-(\mu+\rho+\delta)P_{r1}\frac{P_r}{P_{r1}}\right]\\
&\quad=\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\left(1-\frac{P_{r1}}{P_r}\right)\left[\beta_r V_rT+\alpha_r I_r-(\beta_r V_{r1}T_1+\alpha_r I_{r1})\frac{P_r}{P_{r1}}\right]\\
&\quad=\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\left(1-\frac{P_{r1}}{P_r}\right)\left[\beta_r V_rT+\frac{\alpha_r}{\delta}\delta I_{r1}\frac{I_r}{I_{r1}}\right.\\
&\qquad\left.-\left(\beta_r V_{r1}T_1+\frac{\alpha_r}{\delta}\delta I_{r1}\right)\frac{P_r}{P_{r1}}\right]\\
&\quad=\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\left(1-\frac{P_{r1}}{P_r}\right)\beta_r V_{r1}T_1\\
&\qquad\times\left[\left(\frac{V_rT}{V_{r1}T_1}-\frac{P_r}{P_{r1}}\right)+\frac{\alpha_r}{\delta}\left(\frac{I_r}{I_{r1}}-\frac{P_r}{P_{r1}}\right)\right]\\
&\quad=\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r1}T_1\left(\frac{V_rT}{V_{r1}T_1}-\frac{P_r}{P_{r1}}-\frac{P_{r1}V_rT}{P_rV_{r1}T_1}+1\right)\\
&\qquad+\frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r1}T_1\left(\frac{I_r}{I_{r1}}-\frac{P_r}{P_{r1}}-\frac{P_{r1}I_r}{P_rI_{r1}}+1\right)
\end{aligned}$$

$$= \frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r1}T_1\left[g\left(\frac{V_rT}{V_{r1}T_1}\right) - g\left(\frac{P_r}{P_{r1}}\right) - g\left(\frac{P_{r1}V_rT}{P_rV_{r1}T_1}\right)\right]$$
$$+ \frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r1}T_1\left[g\left(\frac{I_r}{I_{r1}}\right) - g\left(\frac{P_r}{P_{r1}}\right) - g\left(\frac{P_{r1}I_r}{P_rI_{r1}}\right)\right].$$

The above items are added and simplified to obtain

$$\frac{dL_1(t)}{dt}$$
$$= \frac{\rho}{\delta(\mu+\rho+\delta)}dT_1\left(2 - \frac{T_1}{T} - \frac{T}{T_1}\right) + \frac{c}{\delta+\alpha_s}\left(\frac{R_2}{R_1} - 1\right)V_s$$
$$+ \frac{\rho}{\delta(\mu+\rho+\delta)}\beta_r V_{r1}T_1\left[-g\left(\frac{T_1}{T}\right) - g\left(\frac{TV_r}{T_1V_{r1}}\right) + g\left(\frac{V_r}{V_{r1}}\right)\right]$$
$$+ \frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r1}T_1\left[g\left(\frac{TV_r}{T_1V_{r1}}\right) - g\left(\frac{I_{r1}TV_r}{I_rT_1V_{r1}}\right) - g\left(\frac{V_r}{V_{r1}}\right)\right.$$
$$\left. - g\left(\frac{V_{r1}P_r}{V_rP_{r1}}\right) - g\left(\frac{P_{r1}I_r}{P_rI_{r1}}\right)\right] + \frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r1}T_1$$
$$\times\left[g\left(\frac{TV_r}{T_1V_{r1}}\right) - g\left(\frac{P_{r1}TV_r}{P_rT_1V_{r1}}\right) - g\left(\frac{V_r}{V_{r1}}\right) - g\left(\frac{V_{r1}P_r}{V_rP_{r1}}\right)\right]$$
$$= \frac{\rho}{\delta(\mu+\rho+\delta)}dT_1\left(2 - \frac{T_1}{T} - \frac{T}{T_1}\right) + \frac{c}{\delta+\alpha_s}\left(\frac{R_2}{R_1} - 1\right)V_s$$
$$- \frac{\rho}{\delta(\mu+\rho+\delta)}\beta_r V_{r1}T_1\left[g\left(\frac{T_1}{T}\right) + g\left(\frac{V_{r1}P_r}{V_rP_{r1}}\right)\right]$$
$$- \frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r1}T_1\left[g\left(\frac{I_{r1}TV_r}{I_rT_1V_{r1}}\right) + g\left(\frac{P_{r1}I_r}{P_rI_{r1}}\right)\right]$$
$$- \frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r1}T_1 g\left(\frac{P_{r1}TV_r}{P_rT_1V_{r1}}\right).$$

In view of $2 - \frac{T_1}{T} - \frac{T}{T_1} \leq 0$, we have $\frac{dL_1(t)}{dt} \leq 0$ when $R_1 > R_2$. Moreover, if $\frac{dL_1(t)}{dt} = 0$, then $T = T_1, I_r = I_{r1}, P_r = P_{r1}$, and $V_r = V_{r1}$, which implies that M_r is the largest invariant set $\{(T, I_s, I_r, P_s, P_r, V_s, V_r)|\frac{dL_1(t)}{dt}\} = 0$. It is clear that $M_r = \{P_1\}$. Therefore, by applying Lyapunov–LaSalle's Invariance Principle, P_1 is globally asymptotically stable if $R_1 > R_2$.

Appendix C. Global Stability of the Coexistence Steady State

Similar to Theorem 2.6, we define a Lyapunov function

$$L_2(t) := L_{21}(t) + L_{22}(t) + L_{23}(t) + L_{24}(t),$$

where

$$L_{21}(t) = \frac{\rho}{\delta(\mu+\rho+\delta)}T_2 g\left(\frac{T}{T_2}\right),$$

$$L_{22}(t) = \frac{\rho\alpha_s}{\delta(\mu+\rho+\delta)(\delta+\alpha_s)}I_{s2}g\left(\frac{I_s}{I_{s2}}\right) + \frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}I_{r2}g\left(\frac{I_r}{I_{r2}}\right),$$

$$L_{23}(t) = \frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_s)}P_{s2}g\left(\frac{P_s}{P_{s2}}\right) + \frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}P_{r2}g\left(\frac{P_r}{P_{r2}}\right),$$

$$L_{24}(t) = \frac{1}{\delta+\alpha_s}V_{s2}g\left(\frac{V_s}{V_{s2}}\right) + \frac{1}{\delta+\alpha_r}V_{r2}g\left(\frac{V_r}{V_{r2}}\right).$$

We calculate the time derivative of $L_2(t)$ along the solutions of (2.4) with $m = 0$. Combined with $s = dT_2 + \beta_s V_{s2}T_2 + \beta_r V_{r2}T_2$ and the above construction method, we obtain the following reduction:

$$\begin{aligned}\frac{dL_{21}(t)}{dt} &= \frac{\rho}{\delta(\mu+\rho+\delta)}\left(1-\frac{T_2}{T}\right)(s - dT - \beta_s V_s T - \beta_r V_r T)\\ &= \frac{\rho}{\delta(\mu+\rho+\delta)}dT_2\left(2-\frac{T_2}{T}-\frac{T}{T_2}\right)\\ &\quad + \frac{\rho}{\delta(\mu+\rho+\delta)}\beta_r V_{r2}T_2\left[-g\left(\frac{T_2}{T}\right) - g\left(\frac{TV_r}{T_2V_{r2}}\right) + g\left(\frac{V_r}{V_{r2}}\right)\right]\\ &\quad + \frac{\rho}{\delta(\mu+\rho+\delta)}\beta_s V_{s2}T_2\left[-g\left(\frac{T_2}{T}\right) - g\left(\frac{TV_s}{T_2V_{s2}}\right) + g\left(\frac{V_s}{V_{s2}}\right)\right].\end{aligned}$$

Note that

$$\delta I_{i2} = \beta_i V_{i2}T_2,\ \ \rho P_{i2} = cV_{i2},\ \ (\mu+\rho+\delta)P_{i2} = \beta_i V_{i2}T_2 + \alpha_i I_{i2},\quad i = s, r.$$

We have

$$\begin{aligned}&\frac{dL_{22}(t)}{dt}\\ &\quad= \frac{\rho\alpha_s}{\delta(\mu+\rho+\delta)(\delta+\alpha_s)}\left(1-\frac{I_{s2}}{I_s}\right)(\beta_s V_s T - \delta I_s)\\ &\qquad + \frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\left(1-\frac{I_{r2}}{I_r}\right)(\beta_r V_r T - \delta I_r)\\ &\quad= \frac{\rho\alpha_s}{\delta(\mu+\rho+\delta)(\delta+\alpha_s)}\beta_s V_{s2}T_2\left[g\left(\frac{TV_s}{T_2V_{s2}}\right) - g\left(\frac{I_s}{I_{s2}}\right) - g\left(\frac{I_{s2}TV_s}{I_sT_2V_{s2}}\right)\right]\\ &\qquad + \frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r2}T_2\left[g\left(\frac{TV_r}{T_2V_{r2}}\right) - g\left(\frac{I_r}{I_{r2}}\right) - g\left(\frac{I_{r2}TV_r}{I_rT_2V_{r2}}\right)\right].\end{aligned}$$

Similarly,

$$\begin{aligned}
&\frac{dL_{23}(t)}{dt}\\
&\quad=\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\left(1-\frac{P_{r2}}{P_r}\right)(\beta_r V_r T+\alpha_r I_r-(\mu+\rho+\delta)P_r)\\
&\qquad+\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_s)}\left(1-\frac{P_{s2}}{P_s}\right)(\beta_s V_s T+\alpha_s I_s-(\mu+\rho+\delta)P_s)\\
&\quad=\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r2}T_2\left[g\left(\frac{V_r T}{V_{r2}T_2}\right)-g\left(\frac{P_r}{P_{r2}}\right)-g\left(\frac{P_{r2}V_r T}{P_r V_{r2}T_2}\right)\right]\\
&\qquad+\frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r2}T_2\left[g\left(\frac{I_r}{I_{r2}}\right)-g\left(\frac{P_r}{P_{r2}}\right)-g\left(\frac{P_{r2}I_r}{P_r I_{r2}}\right)\right]\\
&\qquad+\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_s)}\beta_s V_{s2}T_2\left[g\left(\frac{V_s T}{V_{s2}T_2}\right)-g\left(\frac{P_s}{P_{s2}}\right)-g\left(\frac{P_{s2}V_s T}{P_s V_{s2}T_2}\right)\right]\\
&\qquad+\frac{\rho\alpha_s}{\delta(\mu+\rho+\delta)(\delta+\alpha_s)}\beta_s V_{s2}T_2\left[g\left(\frac{I_s}{I_{s2}}\right)-g\left(\frac{P_s}{P_{s2}}\right)-g\left(\frac{P_{s2}I_s}{P_s I_{s2}}\right)\right],
\end{aligned}$$

and

$$\begin{aligned}
&\frac{dL_{24}(t)}{dt}\\
&\quad=\frac{1}{\delta+\alpha_s}\left(1-\frac{V_{s2}}{V_s}\right)(\rho P_s-cV_s)+\frac{1}{\delta+\alpha_r}\left(1-\frac{V_{r2}}{V_r}\right)(\rho P_r-cV_r)\\
&\quad=\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_s)}\beta_s V_{s2}T_2\left[g\left(\frac{P_s}{P_{s2}}\right)-g\left(\frac{V_s}{V_{s2}}\right)-g\left(\frac{V_{s2}P_s}{V_s P_{s2}}\right)\right]\\
&\qquad+\frac{\rho\alpha_s}{\delta(\mu+\rho+\delta)(\delta+\alpha_s)}\beta_s V_{s2}T_2\left[g\left(\frac{P_s}{P_{s2}}\right)-g\left(\frac{V_s}{V_{s2}}\right)-g\left(\frac{V_{s2}P_s}{V_s P_{s2}}\right)\right]\\
&\qquad+\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r2}T_2\left[g\left(\frac{P_r}{P_{r2}}\right)-g\left(\frac{V_r}{V_{r2}}\right)-g\left(\frac{V_{r2}P_r}{V_r P_{r2}}\right)\right]\\
&\qquad+\frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_r V_{r2}T_2\left[g\left(\frac{P_r}{P_{r2}}\right)-g\left(\frac{V_r}{V_{r2}}\right)-g\left(\frac{V_{r2}P_r}{V_r P_{r2}}\right)\right].
\end{aligned}$$

We sum and rearrange the above expressions to get

$$\begin{aligned}
&\frac{dL_2(t)}{dt}\\
&\quad=\frac{dL_{21}(t)}{dt}+\frac{dL_{22}(t)}{dt}+\frac{dL_{23}(t)}{dt}+\frac{dL_{24}(t)}{dt}\\
&\quad=\frac{\rho}{\delta(\mu+\rho+\delta)}dT_2\left(2-\frac{T_2}{T}-\frac{T}{T_2}\right)-\frac{\rho}{\delta(\mu+\rho+\delta)}\beta_r V_{r2}T_2\left[g\left(\frac{T_2}{T}\right)\right.
\end{aligned}$$

$$+g\left(\frac{V_{r2}P_r}{V_rP_{r2}}\right)\Bigg]-\frac{\rho\alpha_r}{\delta(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_rV_{r2}T_2\left[g\left(\frac{I_{r2}TV_r}{I_rT_2V_{r2}}\right)\right.$$

$$\left.+g\left(\frac{P_{r2}I_r}{P_rI_{r2}}\right)\right]-\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_r)}\beta_rV_{r2}T_2g\left(\frac{P_{r2}TV_r}{P_rT_2V_{r2}}\right)$$

$$-\frac{\rho}{\delta(\mu+\rho+\delta)}\beta_sV_{s2}T_2\left[g\left(\frac{T_2}{T}\right)+g\left(\frac{V_{s2}P_s}{V_sP_{s2}}\right)\right]$$

$$-\frac{\rho\alpha_s}{\delta(\mu+\rho+\delta)(\delta+\alpha_s)}\beta_sV_{s2}T_2\left[g\left(\frac{I_{s2}TV_s}{I_sT_2V_{s2}}\right)+g\left(\frac{P_{s2}I_s}{P_sI_{s2}}\right)\right]$$

$$-\frac{\rho}{(\mu+\rho+\delta)(\delta+\alpha_s)}\beta_sV_{s2}T_2g\left(\frac{P_{s2}TV_s}{P_sT_2V_{s2}}\right).$$

Therefore, we have $\frac{dL_2(t)}{dt}\leq 0$. Moreover, $\frac{dL_2(t)}{dt}=0$ holds if and only if $T=T_2$, $I_s=I_{s2}$, $I_r=I_{r2}$, $P_s=P_{s2}$, $P_r=P_{r2}$, $V_s=V_{s2}$, and $V_r=V_{r2}$. The largest invariant subset of $\frac{dL_2(t)}{dt}=0$ is $\{P_2\}$. Thus, $\frac{dL_2(t)}{dt}=0$ only if the solution is in Ω. By LaSalle's Invariance Principle, the coexistence steady state P_2 is the global asymptotic stability in Ω whenever it exists.

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