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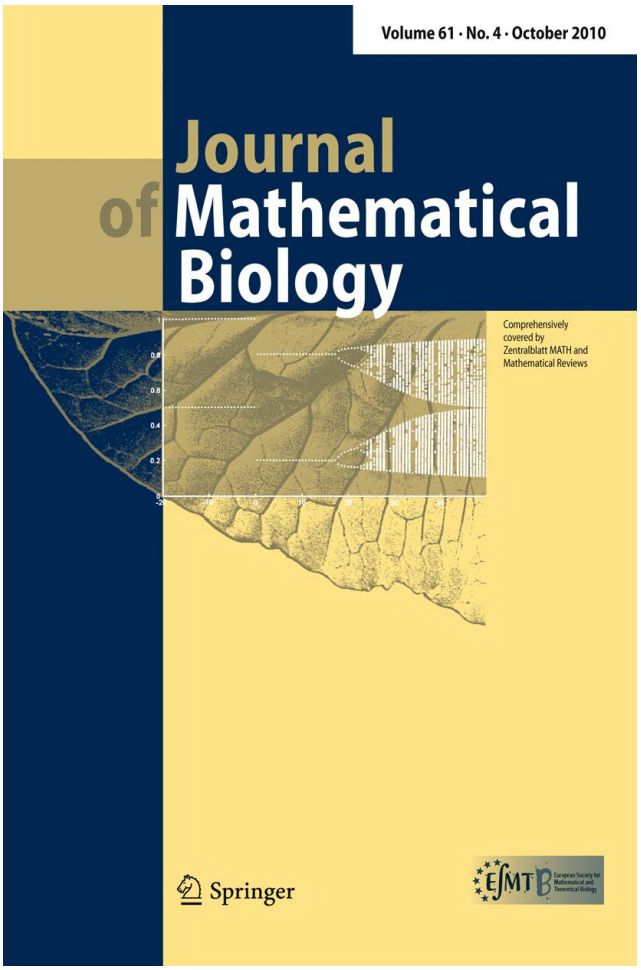

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Senescence and antibiotic resistance in an age-structured population model

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Abstract Different theories have been proposed to understand the growing problem of antibiotic resistance of microbial populations. Here we investigate a model that is based on the hypothesis that senescence is a possible explanation for the existence of so-called persister cells which are resistant to antibiotic treatment. We study a chemostat model with a microbial population which is age-structured and show that if the growth rates of cells in different age classes are sufficiently close to a scalar multiple of a common growth rate, then the population will globally stabilize at a coexistence steady state. This steady state persists under an antibiotic treatment if the level of antibiotics is below a certain threshold; if the level exceeds this threshold, the washout state becomes a globally attracting equilibrium.

Keywords Senescence · Antibiotic resistance · Age structure · Asymmetric division · Coexistence

Mathematics Subject Classification (2000) 34D05 · 34D23 · 92D15 · 92D25 · 93D05

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1 Introduction

Antibiotic resistance is a worldwide problem with severe impact on national health care systems (Levy and Marshall 2004; Spellberg et al. 2008). It has become clear in recent years, that various pathogens are displaying an increased rate of resistance (Siegel et al. 2007). Since fewer new antibiotics have been developed meanwhile, there is a general sense that the spread of resistance should be halted, or at least slowed down. This will only be possible if we develop a deeper understanding of the causes of antibiotic resistance. A significant amount of work is currently being done by both experimentalists and theoreticians, and mathematical modeling has an integral role in this effort. Recent review articles summarize this activity (Consortium REX 2007; Grundmann and Hellriegel 2006; Levin 2001; Temime et al. 2008).

Most of the studies have used deterministic differential equations models, with compartments that represent colonized or un-colonized patient populations and contaminated and uncontaminated health-care worker populations, although some studies use individual-based models (D'Agata et al. 2007). The efficacy of antimicrobial treatments and other interventions that focus on reducing the transmission of antimicrobial-resistant bacteria between patients and health care workers have been evaluated (D'Agata et al. 2007, 2008, 2009; Lipsitch and Levin 1997). However, in the majority of these contributions the emphasis is on public health prevention measures and schedules of antimicrobial application, rather than on the ecology of the pathogen. The pathogens operate in complex environments where they compete with genetically and phenotypically similar, but distinct, individuals. The application of the antimicrobial agent may contribute to selection pressure that may lead to the establishment of the resistant strain in a patient and a hospital (Albrich et al. 2004; McGowan 1983).

In D'Agata et al. (2009) the competition between two methicillin-resistant strains of *Staphylococcus aureus* (MRSA) has been modeled. One strain, community acquired CA-MRSA was assumed to have higher growth rate than the hospital acquired strain HA-MRSA. In agreement with the principle of competitive exclusion (Hardin 1960; Smith and Waltman 1995), the model (D'Agata et al. 2009) predicts that CA-MRSA will eventually dominate and outcompete HA-MRSA.

Naive extrapolation of the principle of competitive exclusion could lead us to a very uncomfortable conclusion that more resistant strains (which have higher growth rate in the presence of antimicrobial agents) will inevitably outcompete the less resistant strains. Despite the wide acceptance of the competitive exclusion principle, more recent work has shown that there are significant exceptions to its validity. These include spatial heterogeneity of the environment, temporal variations in resource availability, cross-feeding between competitors and antagonistic interactions between competitors (Lenas and Pavlou 1995; Korona et al. 1994; Turner et al. 1996; Vance 1985).

Further confirmation that the competition between different strains can play a significant role in development of antimicrobial resistance comes from the following intriguing experiment (Bigger 1944). When microbial populations are subject to antibiotic treatment, an initial rapid decline of the cells is observed. Nevertheless, a small fraction of the cells survives the attack. These cells are often referred to as *persister cells* (a notion which should not be confused with the dynamical systems concept of

persistence). When the antibiotic is removed, and the remaining cells are re-grown with fresh nutrient, one might expect that the restored population would consist of resistant cells only, and thus that the second application of the same antibiotic would not affect this population. Surprisingly however, this is not what happens. Instead, the response of these cells is the same as that of the initial population: Most cells are killed rapidly, while a small population of persisters survives. This phenomenon has long been known, dating back at least to the work of Bigger in Bigger (1944), now more than six decades ago, yet a satisfactory explanation of what its causes are, has not been found so far. Persisters have often been considered to be a phenotype, capable of evading the detrimental effect of antibiotics. Although the biological details are not well understood, cells are believed to switch between the persistent and non-persistent state, depending on environmental conditions (Lewis 2001, 2007; Keren et al. 2004; Balaban et al. 2005; Kussell et al. 2005). This idea has been investigated using mathematical models in Kussell and Leibler (2005), Cogan (2006, 2007), Wiuff and Anderson (2007), Imran and Smith (2006) and De Leenheer and Cogan (2009). Here we investigate the hypothesis that persister formation is attributed to senescence, in the sense that persister cells are those that have undergone many division cycles. It is known that asymmetric division leads to the degradation of parts of the cellular machinery (Stewart et al. 2005; Lindner et al. 2008), which in turn leads to a lower growth rate and to a reduced intake and metabolic processing of the antibiotic, which may be the underlying cause of persistence. There is some experimental evidence that persister cells are slowly growing cells (see, e.g. Balaban et al. 2005). A Partial Differential Equation model for senescence has been described in both chemostat and biofilm settings (Klapper et al. 2007; Ayati and Klapper 2007).

In this paper we will investigate the hypothesis that one of the contributing factors to the development of antibiotic resistance is the stable co-existence of the senescent and normal cells in the pathogen population. Our main result states that such stable co-existence is possible when different age classes compete for the same limiting resource.

We formulate and analyze a general chemostat model expressed in terms of a system of Ordinary Differential Equations. The population consists of a mix of cells of various ages, measured in terms of how many cell divisions they have undergone. We assume that the division is asymmetric. When a cell divides, one cell remains in the youngest age class while the other moves to the next older age class. The oldest age class does not grow or divide. We will show that the population stabilizes at a globally stable coexistence steady state, provided that the growth rate functions of cells in the different age classes do not

We extend our analysis to coexistence in the presence of antibiotics, where we assume that the growth rates in all classes is a decreasing function of the level of the antibiotics. We show that if the level of the antibiotics is smaller than a certain threshold, the stable coexistence equilibrium persists, while if it is larger than this threshold, the washout equilibrium is a globally attracting steady state.

The stable coexistence of all age classes may explain the experimental observation that the persister cells do not give rise to resistant progeny. If any cells survive an antibiotic assault, they would give rise to a new generation of cells and this population will in time converge to the same globally asymptotically stable steady state that contains

all age classes. This new population will be vulnerable to the antibiotic attack to the same extent as before.

Our model does not take into account other sources of antibiotic resistance like horizontal gene transfer of plasmids that confer resistance (Davies 1994; Ochman et al. 2000; Martinez 2008). While horizontal gene transfer also contributes to the antibiotic resistance problem, the plasmid that conveys the resistance is usually heritable. Therefore the progeny of resistant cells are also antibiotic resistant, contrary to the particular experiment described above. Our model shows that the stable coexistence of multiple age classes may be responsible for the experimental observation that the new population remains vulnerable to the antibiotic.

Our results can be interpreted more broadly in the context of competition between genetically and phenotypically closely related pathogens. Since our model predicts a stable coexistence of such strains (provided that the assumptions of our model are met), the intervention against the establishment of resistant strains may be directed towards shifting the coexistence balance in the direction of more benign strains at the expense of the resistant strains. This ecological approach to the management of nosocomial diseases is similar to HIV virus infection management; the goal is not an eradication of the virus, but keeping the viral load in the organism at manageable levels (Layden et al. 2003; Perelson and Nelson 1999).

The paper is organized as follows. We describe the model and our main mathematical result in the next Section. In Sect. 3, we prove some general properties of our model, including an extinction and uniform persistence property. In Sect. 4, we first specialize our model to the case where the growth rates of cells in various age classes are proportional to a common growth rate (with lower proportionality factor for cells in older age classes), and we obtain a global stability result for this case. Finally, we use a global perturbation result to prove global stability for the case when the growth rates are perturbed slightly. In Sect. 5, we state and prove the results that are relevant to the application of antibiotics. An important technical result is stated and proved in the Appendix.

2 Model formulation and main result

We let $S(t)$ denote the concentration of the growth-limiting nutrient, and $x_i(t)$ denote the cell concentration in the ith senescent class. We consider the following senescence model:

$$\dot{x}_1 = \mu_2(S,a)x_2 + \mu_3(S,a)x_3 + \cdots + \mu_n(S,a)x_n - Dx_1, \tag{1}$$

$$\dot{x}_i = \mu_{i-1}(S,a)x_{i-1} - \mu_i(S,a)x_i - Dx_i, \quad i = 2,3,\ldots,n, \tag{2}$$

$$\dot{x}_{n+1} = \mu_n(S,a)x_n - Dx_{n+1}, \tag{3}$$

$$\dot{S} = D(S^0 - S) - \sum_{i=1}^{n} y_i \mu_i(S,a)x_i. \tag{4}$$

Here, D represents the dilution rate, S^0 is the nutrient feed concentration, $\mu_i(S,a)$ is the division rate of cells in the ith senescent class, and $y_i > 0$, $i = 1,2,\ldots,n$

are the reciprocal yield coefficients balancing the growth and nutrient consumption of dividing cells. The parameter a is introduced to study the effect of antibiotic treatment which we address in Sect. 5 of this paper. Until then, we will simply drop the dependence of $\mu_i(S, a)$ on a and write $\mu_i(S)$.

We note that in this particular model, a dividing cell of the ith senescent class ($1 \le i \le n$) produces one daughter cell in the first senescent class and one daughter cell in the $(i+1)$st senescent class. Although unnecessary from the mathematical viewpoint, it is biologically feasible to assume that the proliferative capacity of cells diminishes as they become more senescent, that is,

$$\mu_1(S) \ge \mu_2(S) \ge \cdots \ge \mu_n(S) > \mu_{n+1}(S) \equiv 0.$$

Specifically, in our model, the $(n+1)$st senescent class consists of fully senescent cells that neither divide nor consume the nutrient. Regarding the division rates $\mu_i(S)$, we assume that the functions $\mu_i(S)$ are smooth, with $\mu_i'(S) > 0$ for all $S > 0$ and all i, and $\mu_i(0) = 0$ for all i.

For mathematical convenience, we scale the time in the units of D and scale the state variables in the units of S^0 as follows

$$\tau = Dt, \quad S = S^0\tilde{S}, \quad x_i = \frac{S^0\tilde{x}_i}{\omega}, \quad \tilde{\mu}_i(\tilde{S}) = \frac{1}{D}\mu_i(S^0\tilde{S}), \quad \tilde{y}_i = \omega y_i.$$

After dropping the tildes, we obtain the dimensionless (rescaled) system

$$\begin{aligned}
\dot{x}_1 &= \mu_2(S)x_2 + \mu_3(S)x_3 + \cdots + \mu_n(S)x_n - x_1,\\
\dot{x}_i &= \mu_{i-1}(S)x_{i-1} - \mu_i(S)x_i - x_i, \quad i = 2, 3, \ldots, n,\\
\dot{x}_{n+1} &= \mu_n(S)x_n - x_{n+1},\\
\dot{S} &= 1 - S - \sum_{i=1}^{n} y_i\mu_i(S)x_i,
\end{aligned}$$

where we may assume that $y_i > 1,\ i = 1, 2, \ldots, n$ by choosing a sufficiently large $\omega > 0$. For notational ease, we also introduce the following vector notation for our system:

$$\dot{X} = [M(S) - I_{n+1} + T(S)]X \tag{5}$$

$$\dot{S} = 1 - S - y^T M(S)X, \tag{6}$$

where $X^T = (x_1, x_2, \ldots, x_n, x_{n+1})$, $y^T = (y_1, y_2, \ldots, y_n, 0)$,

$$M(S) = \begin{pmatrix}
\mu_1(S) & 0 & 0 & \ldots & 0 & 0\\
0 & \mu_2(S) & 0 & \ldots & 0 & 0\\
0 & 0 & \mu_3(S) & \ldots & 0 & 0\\
\vdots & \vdots & \ddots & \ddots & \vdots & \vdots\\
0 & 0 & \ldots & \ldots & \mu_n(S) & 0\\
0 & 0 & \ldots & \ldots & 0 & 0
\end{pmatrix}$$

and

$$T(S) = \begin{pmatrix} -\mu_1(S) & +\mu_2(S) & +\mu_3(S) & \dots & +\mu_n(S) & 0 \\ +\mu_1(S) & -2\mu_2(S) & 0 & \dots & 0 & 0 \\ 0 & +\mu_2(S) & -2\mu_3(S) & \dots & \vdots & \vdots \\ 0 & 0 & +\mu_3(S) & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & -2\mu_n(S) & 0 \\ 0 & 0 & \dots & 0 & +\mu_n(S) & 0 \end{pmatrix}.$$

The terms $M(S)X$ and $T(S)X$ represent the net growth rate of cells and the aging effects of cell division, respectively.

The main mathematical result of this paper establishes the global stability of the positive equilibrium of the model in the case where the division rates are nearly proportional to each other.

Theorem 1 *Let $\mu_0(S)$ be smooth, zero at zero with $\mu_0'(S) > 0$ for $S > 0$. Suppose that*

$$\alpha_1 \geq \alpha_2 \geq \cdots \alpha_n > \alpha_{n+1} \equiv 0,$$

and let $F_0(S)$ be as defined in (19).

If $F_0(1) > 1$, then there exist $\varepsilon^* > 0$ such that if $\|\mu_i - \alpha_i \mu_0\|_{C^0} < \varepsilon^*$, then system (5)–(6) has a unique positive steady state (X^*, S^*) which is globally asymptotically stable with respect to initial conditions satisfying $x(0) \neq 0$ where $x = (x_1, \dots, x_n)$.

In Sect. 5 we model the effect of antibiotics on the coexistence steady state (X^*, S^*) under the assumption that the growth rates $\mu_i(S, a)$ are decreasing functions of the level of the antibiotics a. We show in Theorem 6 that if the level of antibiotics is smaller than a critical level a^*, then the coexistence steady state (X^*, S^*) is still globally asymptotically stable, while if $a \geq a^*$, then the washout steady state is globally asymptotically stable.

It will sometimes turn out to be convenient to reduce system (5)–(6) to a $(n+1)$-dimensional system. This is possible because x_{n+1} does not appear in any of the equations for S or x_i for $i \leq n$. Hence, we can drop the x_{n+1}-equation and focus on the following lower-dimensional system:

$$\dot{x} = [M_r(S) - I_n + T_r(S)]x, \tag{7}$$
$$\dot{S} = 1 - S - y_r^T M_r(S)x, \tag{8}$$

where $x^T = (x_1, x_2, \dots, x_n)$ and $y_r^T = (y_1, y_2, \dots, y_n)$, and letting $\mu(S) = (\mu_1(S), \mu_2(S), \dots, \mu_n(S))$:

$$M_r(S) = \operatorname{diag}(\mu(S)),$$

and

$$T_r(S) = \begin{pmatrix} -\mu_1(S) & +\mu_2(S) & +\mu_3(S) & \dots & +\mu_{n-1}(S) & +\mu_n(S) \\ +\mu_1(S) & -2\mu_2(S) & 0 & \dots & 0 & 0 \\ 0 & +\mu_2(S) & -2\mu_3(S) & \dots & 0 & 0 \\ 0 & 0 & +\mu_3(S) & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \ddots & \vdots \\ 0 & 0 & \dots & 0 & +\mu_{n-1}(S) & -2\mu_n(S) \end{pmatrix}.$$

3 Some general properties

3.1 Uniform boundedness

Lemma 1 *The solutions of* (5)–(6) *are uniformly bounded. More precisely, there is some* $m^* > 0$ *such that for every solution* $(X(t), S(t))$*, there is a* τ *such that*

$$(X(t), S(t)) \in L, \quad \text{for all } t \geq \tau,$$

where

$$L := \{(X, S) \in \mathbb{R}^{n+2}_+ \mid 1^T X + S \leq m^*\}.$$

Proof Let us consider the evolution of the variable: $m = S + 1^T X$, along an arbitrary solution $(X(t), S(t))$ of (5)–(6). Since $y_r - 1 \geq 0$, there holds that:

$$\dot{m}(t) = 1 - m(t) - [y_r - 1]^T M_r(S(t))x(t) \leq 1 - m(t),$$

and the conclusion follows by letting $m^* = 1.1$. □

3.2 Steady states

Define the following function:

$$F(S) = \mu_1(S) \sum_{j=2}^{n} \prod_{k=2}^{j} \frac{\mu_k(S)}{1 + \mu_k(S)}. \tag{9}$$

Notice that $F(S)$ is the product of the increasing function of S, $\mu_1(S)$, and a sum of terms in which each term is increasing (each term being the product of a finite number of increasing functions). Hence, $F(S)$ is increasing as well. Also note, $F(0) = 0$. We have:

Lemma 2 *The washout state* $(0, 0, \dots, 0, 1)^T$ *is always a steady state of* (5)–(6). *There are no other steady states on the boundary of* $\mathbb{R}^{n+2}_+$.

If $F(1) \leq 1$, then the washout steady state is the only steady state of (5)–(6), and if $F(1) < 1$, then the washout steady state is hyperbolic and locally asymptotically stable.

If $F(1) > 1$, then the washout steady state is unstable, and (5)–(6) has a unique positive steady state (X^*, S^*). Here, S^* is the unique positive number for which $F(S^*) = 1$.

Proof The first assertion is easily checked by a direct computation.

By (8), no steady state can have $S^* = 0$, and so we can assume without loss of generality that $S^* > 0$ from now on.

Let us now focus on finding steady states (X^*, S^*) with (non-negative) $X^* \neq 0$. It suffices to look for steady states (x^*, S^*) of system (7)–(8) because there is a bijective correspondence between steady states of both systems: (x^*, S^*) is a steady state of (7)–(8) if and only if (X^*, S^*) is a steady state of (5)–(6) with $X^* = (x^*, X^*_{n+1})$, where $X^*_{n+1} = \mu_n(S^*)x^*_n$.

For system (7)–(8), steady states (x^*, S^*) are solutions to:

$$x_1 = \sum_{j=2}^{n} \mu_j(S)x_j, \tag{10}$$

$$x_k = \frac{\mu_{k-1}(S)}{1+\mu_k(S)}x_{k-1}, \quad k > 1, \tag{11}$$

$$1 - S = y_r^T M_r(S)x. \tag{12}$$

Since $S > 0$, equations (11) show that if $x_i = 0$ for some i, then $x_j = 0$ for all j, and hence we recover the washout steady state. Consequently, there are no other steady states for (7)–(8) on the boundary of $\mathbb{R}^{n+1}_+$. The same conclusion follows for (5)–(6) and the boundary of $\mathbb{R}^{n+2}_+$. So we assume that $x > 0$ henceforth.

We can recursively solve for each x_k, $k > 1$ in terms of S and x_1:

$$x_k = x_1 \prod_{j=2}^{k-1} \frac{\mu_{j-1}(S)}{1+\mu_j(S)}, \quad k > 1 \tag{13}$$

and substitute in (10), yielding:

$$x_1 = x_1\mu_1(S) \sum_{j=2}^{n} \prod_{k=2}^{j} \frac{\mu_k(S)}{1+\mu_k(S)},$$

and after dividing by $x_1 > 0$ and recalling the definition of $F(S)$ we find that this is equivalent with

$$F(S) = 1.$$

Since F is increasing, and since (12) requires that $S \in (0, 1)$, there is no solution if $F(1) \leq 1$, and a unique solution $S^* \in (0, 1)$ if $F(1) > 1$. All x^*_k, $k > 1$ can be determined (up to the factor x^*_1) by plugging the value of S^* in (13), and the unique value

of x_1^* then follows from (12). Summarizing, we have found the unique positive steady state (x^*, S^*) of (7)–(8) (and consequently the unique positive steady state (X^*, S^*) of (5)–(6)), provided that $F(1) > 1$. If $F(1) \leq 1$ on the other hand, then the washout steady state is the only steady state.

Linearization of (5)–(6) at the washout steady state yields the following Jacobian matrix:

$$\begin{pmatrix} M(1) - I_{n+1} + T(1) & 0 \\ -y^T M(1) & -1 \end{pmatrix},$$

hence the local stability properties follow from the location of the eigenvalues of the matrix $M_r(1) - I_n + T_r(1)$. This matrix can be re-written as follows (we are suppressing the argument 1 of the various growth rate functions μ_i):

$$\begin{aligned} M_r(1) - I_n + T_r(1) &= \begin{pmatrix} 0 & \mu_2 & \mu_3 & \dots & \mu_n \\ \mu_1 & 0 & 0 & \dots & 0 \\ 0 & \mu_2 & 0 & \dots & 0 \\ \vdots & \vdots & \ddots & \ddots & \vdots \\ 0 & 0 & 0 & \dots & \mu_n \end{pmatrix} - \begin{pmatrix} 1 & 0 & 0 & \dots & 0 \\ 0 & (1+\mu_2) & 0 & \dots & 0 \\ 0 & 0 & (1+\mu_3) & \dots & 0 \\ \vdots & \vdots & \vdots & \dots & \vdots \\ 0 & 0 & 0 & \dots & (1+\mu_n) \end{pmatrix} \\ &=: A - D. \end{aligned}$$

It is well-known (van den Driessche and Watmough 2002; Diekmann et al. 1990) that the eigenvalues of $M_r(1) - I_n + T_r(1)$ are in the open left-half plane if the spectral radius of the matrix AD^{-1} is less than 1. Conversely, if the spectral radius of AD^{-1} is larger than 1, then the matrix $M_r(1) - I_n + T(1)$ has an eigenvalue with positive real part. We have that

$$AD^{-1} = \begin{pmatrix} 0 & \frac{\mu_2}{1+\mu_2} & \frac{\mu_3}{1+\mu_3} & \dots & \frac{\mu_n}{1+\mu_n} \\ \mu_1 & 0 & 0 & \dots & 0 \\ 0 & \frac{\mu_2}{1+\mu_2} & 0 & \dots & 0 \\ \vdots & \vdots & \ddots & \ddots & \vdots \\ 0 & 0 & 0 & \dots & \frac{\mu_n}{1+\mu_n} \end{pmatrix}.$$

A straightforward calculation shows that the characteristic polynomial of AD^{-1} is:

$$p(\lambda) = \lambda^n - \mu_1 \left[\sum_{j=2}^{n} \left(\prod_{k=2}^{j} \frac{\mu_k}{1+\mu_k} \right) \lambda^{n-j} \right].$$

If $p(1) < 0$, or equivalently if $F(1) > 1$, then the spectral radius of AD^{-1} is strictly larger than 1, and then the washout steady state is unstable. Conversely, if $p(1) \geq 0$, or equivalently if $F(1) \leq 1$, then the spectral radius of AD^{-1} is not larger than 1. To see this, we argue by contradiction: If the spectral radius of AD^{-1}, which we denote as

λ^*, were larger than 1, the Perron–Frobenius Theorem implies that λ^* is an eigenvalue of AD^{-1}, and therefore $p(\lambda^*) = 0$. But dividing this equation by $(\lambda^*)^n$ yields:

$$1 = \mu_1 \left[\sum_{j=2}^{n} \left(\prod_{k=2}^{j} \frac{\mu_k}{1+\mu_k} (\lambda^*)^{-j} \right) \right]$$
$$< \mu_1 \left[\sum_{j=2}^{n} \left(\prod_{k=2}^{j} \frac{\mu_k}{1+\mu_k} \right) \right],$$

since $\lambda^* > 1$. But this implies that $p(1) < 0$, a contradiction. If $p(1) > 0$, or equivalently if $F(1) < 1$, then the washout steady state is hyperbolic and locally asymptotically stable. □

Remark 1 We can formally define the *basic reproductive number* $\mathcal{R}_0$ of the structured cell population to be the spectral radius of the so-called next generation matrix AD^{-1} (van den Driessche and Watmough 2002; Diekmann et al. 1990). In the proof of Lemma 2, we showed that the basic reproductive number $\mathcal{R}_0$ is the unique positive eigenvalue of the matrix AD^{-1} due to Perron–Frobenius Theorem. Furthermore, we showed that the following three statements are equivalent: (i) $\mathcal{R}_0 > 1$, (ii) $F(1) > 1$, and (iii) system (5)–(6) has a unique positive steady state (X^*, S^*).

3.3 Extinction

System (5)–(6) has the following extinction property.

Theorem 2 *Suppose that* $F(1) < 1$. *Then every solution* $(X(t), S(t))$ *of* (5)–(6) *is such that*

$$\lim_{t\to\infty} (X(t), S(t)) = (0, 0, \ldots, 0, 1),$$

where $(0, 0, \ldots, 0, 1)$ *is the washout steady state.*

Proof Let $(x(t), S(t))$ be any solution of (7)–(8). By defining $B_r(1) = [M_r(1) - I_n + T_r(1)]$, its dominant eigenvalue $\rho := \lambda(B_r(1))$ is negative by the proof of Lemma 2. We observe that $\rho = q - 1 < 0$ where $q < 1$ is the dominant eigenvalue of the matrix $M_r(1) + T_r(1)$. We claim that $q > 0$. To see this, let $v^T = (v_1, v_2, \ldots, v_n) > 0$ be an eigenvector of the matrix $M_r(1) + T_r(1)$ corresponding to q. Then $(1, 1, \ldots, 1)(M_r(1) + T_r(1)) = (\mu_1(1), \mu_2(1), \ldots, \mu_{n-1}(1), 0)$, and multiplying this by v, we find that $q(1, 1, \ldots, 1)v > 0$, which establishes the claim.

Let $w^T = (w_1, w_2, \ldots, w_n) > 0$ be a left eigenvector of the matrix $M_r(1) + T_r(1)$ corresponding to q. Due to the special form of this matrix, we have that

$$\begin{aligned}
\mu_n(1)(w_1 - w_n) &= q w_n, \\
\mu_{n-1}(1)(w_1 - w_{n-1} + w_n) &= q w_{n-1}, \\
&\vdots \\
\mu_2(1)(w_1 - w_2 + w_3) &= q w_2, \\
\mu_1(1) w_2 &= q w_1,
\end{aligned}$$

or equivalently,

$$\begin{aligned}
w_1 - w_n &= \frac{q w_n}{\mu_n(1)} > 0, \\
w_1 - w_{n-1} + w_n &= \frac{q w_{n-1}}{\mu_{n-1}(1)} > 0, \\
&\vdots \\
w_1 - w_2 + w_3 &= \frac{q w_2}{\mu_2(1)} > 0, \\
w_2 &= \frac{q w_1}{\mu_1(1)}.
\end{aligned}$$

It follows that since $\rho < 0$ there is $\delta > 0$ sufficiently small, such that

$$\begin{aligned}
\delta(w_1 - w_n) &< -\frac{\rho}{2} w_n, \\
\delta(w_1 - w_{n-1} + w_n) &< -\frac{\rho}{2} w_{n-1}, \\
&\vdots \\
\delta(w_1 - w_2 + w_3) &< -\frac{\rho}{2} w_2, \\
\delta w_2 &< -\frac{\rho}{2} w_1.
\end{aligned}$$

Finally, let $\varepsilon > 0$ be sufficiently small so that

$$\mu_i(1 + \varepsilon) \leq \mu_i(1) + \delta, \quad \forall i = 1, 2, \ldots, n.$$

Now consider the auxiliary function $V(t) := \sum_i w_i x_i(t)$. Differentiating V along $(x(t), S(t))$, we find that

$$\begin{aligned}
\dot{V} = {} & w_2 \mu_1(S) x_1 + (w_1 - w_2 + w_3) \mu_2(S) x_2 \\
& + \cdots + (w_1 - w_n) \mu_n(S) x_n - \sum_i w_i x_i.
\end{aligned}$$

Note that all combinations of w_i's are strictly positive.

We see that Lemma 1 is still valid if $m^* = 1 + \epsilon$ for all $\epsilon > 0$ by re-examining its proof, and thus for all sufficiently large t, we have that $S(t) < 1 + \varepsilon$. Hence

$$\begin{aligned}\dot{V} \leq\ & w_2\mu_1(1+\varepsilon)x_1 + (w_1 - w_2 + w_3)\mu_2(1+\varepsilon)x_2 \\ & + \cdots + (w_1 - w_n)\mu_n(1+\varepsilon)x_n - \sum_i w_i x_i.\end{aligned}$$

This implies

$$\begin{aligned}\dot{V} \leq\ & w_2(\mu_1(1)+\delta)x_1 + (w_1 - w_2 + w_3)(\mu_2(1)+\delta)x_2 \\ & + \cdots + (w_1 - w_n)(\mu_n(1)+\delta)x_n - \sum_i w_i x_i.\end{aligned}$$

Rearranging the terms, and using the fact that ρ is an eigenvalue of $B_r(1)$, we have

$$\begin{aligned}\dot{V} &\leq \rho \sum_i w_i x_i + \delta w_2 x_1 + \delta(w_1 - w_2 + w_3)x_2 + \cdots + \delta(w_1 - w_n)x_n \\ &\leq \frac{\rho}{2}\sum_i w_i x_i = \frac{\rho}{2}V.\end{aligned}$$

Since $\rho < 0$, $V(t) \to 0$ as $t \to +\infty$. LaSalle's invariance principle implies that $(x(t), S(t))$ converges to the largest invariant set contained in the set $\Omega = \{(x, S) \in \mathbb{R}_+^{n+1} \mid v^T x = 0\} = \{(x, S) \in \mathbb{R}_+^{n+1} \mid x = 0\}$, the non-negative S-axis. Clearly, the largest invariant set contained in Ω is the steady state $\{(0, 0, \ldots, 0, 1)\}$, and therefore $(x(t), S(t))$ converge to this steady state. But then all solutions $(X(t), S(t))$ of (5)–(6) also converge to the washout steady state because $\dot{X}_{n+1} = \mu_n(S(t))x_n(t) - X_{n+1}$, so that $X_{n+1}(t)$ converges to 0 as well. □

3.4 Uniform persistence

System (5)–(6) has the following persistence property.

Theorem 3 *Suppose that $F(1) > 1$. Then there exists $\Delta > 0$ such that*

$$\liminf_{t\to+\infty} 1^T x(t) \geq \Delta$$

for all solutions $(X(t), S(t)) = (x(t), X_{n+1}(t), S(t))$ of the system (5)–(6) with $1^T x(0) > 0$.

Proof The proof is based on the fluctuation method (Hirsch et al. 1985; Thieme 2003), coupled with the results from Thieme (1993) which demonstrate when uniform weak repellors are uniform strong repellors. First we introduce some notation: For a scalar function $f(t)$, $t \in \mathbb{R}_+$, we denote the (extended) real numbers $\limsup_{t\to\infty} f(t)$ and $\liminf_{t\to\infty} f(t)$ as f^∞ and f_∞ respectively. Letting $B_r(1) = [M_r(1) - I_n + T_r(1)]$, its dominant eigenvalue $\lambda(B_r(1))$ is positive by the proof of Lemma 2. Let $\epsilon > 0$ be

small enough so that the dominant eigenvalue of the matrix $\tilde{B}_r = [M_r(1-\epsilon) - I_n + \tilde{T}_r]$ is positive, where

$$\tilde{T}_r = \begin{pmatrix} -\mu_1(1+\epsilon) & +\mu_2(1-\epsilon) & +\mu_3(1-\epsilon) & \dots & +\mu_{n-1}(1-\epsilon) & +\mu_n(1-\epsilon) \\ +\mu_1(1-\epsilon) & -2\mu_2(1+\epsilon) & 0 & \dots & 0 & 0 \\ 0 & +\mu_2(1-\epsilon) & -2\mu_3(1+\epsilon) & \dots & 0 & 0 \\ 0 & 0 & +\mu_3(1-\epsilon) & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \ddots & \vdots \\ 0 & 0 & 0 & \dots & +\mu_{n-1}(1-\epsilon) & -2\mu_n(1+\epsilon) \end{pmatrix}.$$

Such $\epsilon > 0$ exists because $\lambda(B_r(1)) > 0$ and by continuity of eigenvalues.

Assume that the quantity $1^T x$ is not uniformly weakly persistent for (7)–(8). Then there is a solution $(x(t), S(t))$ with $x(0) \neq 0$ such that

$$(1^T x)^\infty \leq \frac{\epsilon}{2\gamma}, \tag{14}$$

where $\gamma := \max_i Y_i^{-1}\mu_i(1)$. Equation (8) implies that $S_\infty \leq 1$. By the "fluctuation method" (Thieme 2003, Proposition A.22) it follows that

$$\begin{aligned} 0 &\geq \liminf_{t\to\infty} \left(1 - S_\infty - y_r^T M_r(S_\infty)x(t)\right) \\ &\geq \liminf_{t\to\infty} \left(1 - S_\infty - y_r^T M_r(1)x(t)\right) \\ &\geq \liminf_{t\to\infty} \left(1 - S_\infty - \gamma(1^T x(t))\right) \\ &\geq 1 - S_\infty - \gamma(1^T x)^\infty \\ &\geq 1 - S_\infty - \frac{\epsilon}{2}, \end{aligned}$$

where we used (14) to establish the last inequality. Therefore $S_\infty \geq 1-\epsilon/2$, and hence $S(t) \geq 1-\epsilon$ for all sufficiently large t. Also, by Lemma 1, we have $S(t) \leq 1+\epsilon$ for all sufficiently large t. Then (7) implies that for all sufficiently large t:

$$\dot{x}(t) \geq \tilde{B}_r x(t).$$

Since $\lambda(\tilde{B}_r) > 0$, all solutions of $\dot{z} = \tilde{B}_r z$ with $z(0) \neq 0$ and $z(0) \geq 0$ diverge as $t \to \infty$, and thus by a standard comparison argument for monotone system (Smith and Waltman 1995) the same is true for $x(t)$. This contradicts boundedness of $x(t)$, see Lemma 1.

We have established that $1^T x$ is uniformly weakly persistent, or using the terminology of Thieme (1993), that $\mathcal{X}_2 := \{(x, S) \in \mathbb{R}_+^{n+1} \mid 1^T x = 0\}$ is a uniform weak repellor for $\mathcal{X}_1 := \{(x, S) \in \mathbb{R}_+^{n+1} \mid 1^T x > 0\}$. Using Lemma 1, it now follows from Theorem 1.4 of Thieme (1993), that $\mathcal{X}_2$ is in fact a uniform strong repellor for $\mathcal{X}_1$. Since $\dot{X}_{n+1} = \mu_n(S(t))x_n(t) - X_{n+1}$, and using the fact that $S(t) \geq 1-\epsilon$ for all

sufficiently large t, the same is then true for solutions $(X(t), S(t))$ of (5)–(6) with $1^T x(0) > 0$. This concludes the proof. □

4 Global stability results

4.1 Local and global stability when all μ_i are proportional to μ_0

Specializing (5)–(6) and (7)–(8) to the case where $\mu_i(S) = \alpha_i \mu_0(S)$, with

$$\alpha_1 \geq \alpha_2 \geq \cdots \alpha_n > \alpha_{n+1} \equiv 0,$$

yields the following simplified equations:

$$\dot{X} = B(S)X, \tag{15}$$
$$\dot{S} = 1 - S - \mu_0(S)(y^T \tilde{M} X), \tag{16}$$

where $X^T = (x, X_{n+1})$, and

$$B(S) = \mu_0(S) \begin{pmatrix} \alpha_1 & 0 & \dots & 0 & 0 \\ 0 & \alpha_2 & \dots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \dots & \alpha_n & 0 \\ 0 & 0 & \dots & 0 & 0 \end{pmatrix} - I_{n+1} + \mu_0(S) \begin{pmatrix} -\alpha_1 & \alpha_2 & \alpha_3 & \dots & \alpha_n & 0 \\ \alpha_1 & -2\alpha_2 & 0 & \dots & 0 & 0 \\ 0 & \alpha_2 & -2\alpha_3 & \dots & 0 & 0 \\ 0 & 0 & \alpha_3 & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \dots & \alpha_n & 0 \end{pmatrix}$$
$$=: \mu_0(S)\tilde{M} - I_{n+1} + \mu_0(S)\tilde{T}$$

and

$$\dot{x} = B_r(S)x, \tag{17}$$
$$\dot{S} = 1 - S - \mu_0(S)(y_r^T \tilde{M}_r x), \tag{18}$$

respectively, where $B_r(S) = \mu_0(S)\tilde{M}_r + \mu_0(S)\tilde{T}_r - I_n$,

$$\tilde{M}_r = \begin{pmatrix} \alpha_1 & 0 & \dots & 0 \\ 0 & \alpha_2 & \dots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \dots & \alpha_n \end{pmatrix}, \quad \tilde{T}_r = \begin{pmatrix} -\alpha_1 & \alpha_2 & \alpha_3 & \dots & \alpha_n \\ \alpha_1 & -2\alpha_2 & 0 & \dots & 0 \\ 0 & \alpha_2 & -2\alpha_3 & \dots & 0 \\ 0 & 0 & \alpha_3 & \dots & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \dots & \alpha_{n-1} & -2\alpha_n \end{pmatrix}.$$

Recalling (9), we define

$$F_0(S) = \alpha_1 \mu_0(S) \sum_{j=2}^{n} \prod_{k=2}^{j} \frac{\alpha_k \mu_0(S)}{1 + \alpha_k \mu_0(S)}. \tag{19}$$

Then assuming that $F_0(1) > 1$, it follows from Lemma 2 that (15)–(16) and (17)–(18) have a unique positive steady state (X_0^*, S_0^*) and (x_0^*, S_0^*) respectively. We show that these are hyperbolic and locally asymptotically stable.

Lemma 3 *Let $\mu_0(S)$ be smooth, zero at zero, with $\mu_0'(S) > 0$ for $S > 0$. If $F_0(1) > 1$, then the steady state (X_0^*, S_0^*) is hyperbolic and locally asymptotically stable for* (15)–(16).

Proof In this particular case, the positive steady state (X_0^*, S_0^*) satisfies:

$$B(S_0^*) X_0^* = 0, \tag{20}$$

$$\mu_0(S_0^*)(y^T \tilde{M} X_0^*) = 1 - S_0^*. \tag{21}$$

The first equation implies that 0 is an eigenvalue of $B(S_0^*)$, but since $B(S_0^*)$ is reducible, it is not immediately clear that it is the dominant eigenvalue, nor that it is simple. To see that this is indeed the case, notice that the triangular structure of $B(S_0^*)$ implies that its spectrum $\sigma(B(S_0^*))$ is given by:

$$\sigma(B(S_0^*)) = \sigma(B_r(S_0^*)) \cup \{-1\},$$

and that (20) is equivalent to:

$$B_r(S_0^*) x_0^* = 0 \quad \text{and} \quad (X_0^*)_{n+1} = \mu_0(S_0^*)(x_0^*)_n,$$

where $X_0^* = (x_0^*, (X_0^*)_{n+1})$. The matrix $B_r(S_0^*)$ is quasi-monotone and irreducible, and since x_0^* is a positive vector, it follows that 0 is the real, simple and dominant eigenvalue of $B_r(S_0^*)$. Consequently, 0 is also a simple and dominant eigenvalue of matrix $B(S_0^*)$.

Linearization of (15)–(16) at (X_0^*, S_0^*) yields the following block-matrix:

$$\begin{pmatrix} B(S_0^*) & \mu_0'(S_0^*)(\tilde{M} + \tilde{T}) X_0^* \\ -\mu_0(S_0^*) y^T \tilde{M} & -1 - \mu_0'(S_0^*)(y^T \tilde{M} X_0^*) \end{pmatrix} = \begin{pmatrix} B(S_0^*) & \frac{\mu_0'(S_0^*)}{\mu_0(S_0^*)} X_0^* \\ -\mu_0(S_0^*) y^T \tilde{M} & -1 - \frac{\mu_0'(S_0^*)}{\mu_0(S_0^*)}(1 - S_0^*) \end{pmatrix},$$

where we used (20) and (21). Now, we decompose the matrix as follows:

$$A + kbc^T := \begin{pmatrix} B(S_0^*) & 0 \\ m u_0(S_0^*) y^T \tilde{M} & -1 \end{pmatrix} + \frac{\mu_0'(S_0^*)}{\mu_0(S_0^*)} \begin{pmatrix} X_0^* \\ -(1 - S_0^*) \end{pmatrix} \begin{pmatrix} 0 & 0 & \cdots & 0 & 1 \end{pmatrix}.$$

The spectrum of the $(n+2)\times(n+2)$ matrix A is given by

$$\sigma(A) = \sigma(B(S_0^*)) \cup \{-1\},$$

and thus all eigenvalues of A have negative real parts, except for the simple eigenvalue at 0. Also notice that the parameter

$$k = \frac{\mu_0'(S_0^*)}{\mu_0(S_0^*)}$$

is positive. We will show that for all $k > 0$, the eigenvalues of $A + kbc^T$ have negative real parts. To see this, we perform a similarity transformation as follows. Let

$$P = \begin{pmatrix} b & v_1 & v_2 & \cdots & v_{n+1} \end{pmatrix},$$

where $v_1, \ldots, v_{n+1}$ are chosen so that

$$\text{span}\{b\} \oplus \text{span}\{v_1, v_2, \ldots, v_{n+1}\} = \mathbb{R}^{n+2}.$$

Then using (20)–(21) it follows that:

$$P^{-1}AP + kP^{-1}bc^TP = \begin{pmatrix} 0 & * \\ 0 & \tilde{A} \end{pmatrix} + k \begin{pmatrix} -(1-S_0^*) & * \\ 0 & 0 \end{pmatrix},$$

where the $*$'s do not matter for our purposes, and the eigenvalues of the $(n+1)\times(n+1)$ matrix $\tilde{A}$ are -1 and n eigenvalues that belong to the open left-half plane (Indeed, this follows from similarity which implies that $\sigma(A) = \{0\} \cup \sigma(\tilde{A})$, and since $\sigma(A) = \sigma(B(S_0^*)) \cup \{-1\}$; moreover, we have already shown that $B(S_0^*)$ has a simple dominant eigenvalue 0. It follows that the spectrum of $A + kbc^T$ includes $-k(1-S_0^*)$, which is negative because $k > 0$ and $1 - S_0^* > 0$ by (21), and the eigenvalues of $\tilde{A}$, all of which have negative real part. This concludes the proof. □

Theorem 4 *Let $\mu_0(S)$ be smooth, zero at zero, with $\mu_0'(S) > 0$ for $S > 0$. If $F_0(1) > 1$, then the positive steady state (X_0^*, S_0^*) is globally asymptotically stable for (15)–(16) with respect to solutions $(X(t), S(t))$ satisfying $1^T x(0) > 0$.*

Proof The local asymptotic stability follows from Lemma 3. Let us first show that the positive equilibrium (x_0^*, S_0^*) of (17)–(18) is globally asymptotically stable with respect to solutions $(x(t), S(t))$ satisfying $1^T x(0) > 0$. This follows from Theorem 7 in the Appendix because system (17)–(18) fits the framework of model (30)–(31)

with the following choice of the quasi-positive and irreducible matrix Q:

$$Q = \begin{pmatrix} 0 & \alpha_2 & \alpha_3 & \dots & \alpha_{n-1} & \alpha_n \\ \alpha_1 & -\alpha_2 & 0 & \dots & 0 & 0 \\ 0 & \alpha_2 & -\alpha_3 & \dots & 0 & 0 \\ 0 & 0 & \alpha_3 & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \ddots & \vdots \\ 0 & 0 & 0 & \dots & \alpha_{n-1} & -\alpha_n \end{pmatrix}. \tag{22}$$

By the cascade structure of system (15)–(16) (the cascade consists of system (17)–(18) which drives system $\dot{X}_{n+1} = -X_{n+1} + \mu_0(S(t))x_n(t)$ via $x_n(t)$ and $S(t)$) it follows that the positive equilibrium (X_0^*, S_0^*) of (15)–(16) is globally asymptotically stable for solutions $(X(t), S(t))$ satisfying $1^T x(t) > 0$. □

4.2 A persistence property, uniform in model parameters

Before we can prove the main result we need to establish the following persistence result.

Theorem 5 *Let $\mu_0(S)$ be smooth, with $\mu_0(0) = 0$, $\mu_0'(S) > 0$ for $S > 0$, and suppose that $F_0(1) > 1$. Then there exist $\varepsilon > 0$, $\Delta > 0$, and a forward invariant set $K \subset \{(X, S) | X, S \geq 0,\ 1^T x \geq \Delta\}$, such that for any solution $(X(t), S(t))$ of the system (5)–(6) with $\|\mu_i - \alpha_i \mu_0\|_{C^0} < \varepsilon$ and $1^T x(t) > 0$, there exists a $\tau > 0$ such that $(x(t), S(t)) \in K$ for all $t > \tau$.*

Proof By the cascade structure of (5)–(6) it suffices to prove the result for solutions of system (7)–(8). For notational convenience, we first re-write the relevant part of the unperturbed system (17)–(18) as

$$\dot{x} = [\mu_0(S)Q - I_n]\, x, \tag{23}$$
$$\dot{S} = 1 - S - \mu_0(S) y_r^T \tilde{M}_r x, \tag{24}$$

where Q is defined in (22), and the corresponding perturbed system (7)–(8) as

$$\dot{x} = [\mu_0(S)Q + R_r(S) - I_n]\, x, \tag{25}$$
$$\dot{S} = 1 - S - \mu_0(S) y_r^T \tilde{M}_r x - y_r^T D_r(S) x, \tag{26}$$

where we introduced the matrices

$$R_r(S) := M_r(S) + T_r(S) - \mu_0(S)Q, \quad D_r(S) := M_r(S) - \mu_0(S)\tilde{M}_r.$$

The matrix Q is quasi-positive and irreducible with principal eigenvalue $r_1 > 0$ and the principal left eigenvector $w^T > 0$. By scaling w if necessary, we may assume that the vector $r_1 w^T - y_r^T \tilde{M}_r$ is nonnegative. In addition, we claim that the inequality $F_0(1) > 1$ implies that $r_1 \mu_0(1) > 1$. To see this, we note that by Lemma 2 there

exists a positive steady state (x_0^*, S_0^*) for (23)–(24). In particular, there is $x_0^* > 0$ and $S_0^* \in (0, 1)$ such that

$$\mu_0(S_0^*)Qx_0^* = x_0^*,$$

and thus

$$r_1 = \frac{1}{\mu_0(S_0^*)}.$$

Since $S_0^* < 1$ the monotonicity of $\mu_0(S)$ implies that $r_1\mu_0(1) > 1$, which establishes our claim.

We proceed by obtaining several auxiliary estimates. We let $|\cdot|$ denote the Euclidean norm on $\mathbb{R}^n$ and let $\|\cdot\|$ denote the induced matrix norm. Observe that for any non-negative vector $x \in \mathbb{R}^n$,

$$1^T x \geq |x| \Rightarrow w^T x \geq w_{\min}(1^T x) \geq w_{\min}|x| \Rightarrow |x| \leq \frac{1}{w_{\min}} w^T x.$$

Hence,

$$|w||x| \leq \frac{|w|}{w_{\min}} w^T x,$$

which in turn implies that

$$|w^T R_r(S)x| \leq \|R_r(S)\||w||x| \leq \|R_r(S)\|\frac{|w|}{w_{\min}} w^T x.$$

Similarly, we find that

$$|y_r^T D_r(S)x| \leq \|D_r(S)\|\frac{|y_r|}{y_{r,\min}} y_r^T x \leq \|D_r(S)\|\frac{|y_r|y_{r,\max}}{y_{r,\min}w_{\min}} w^T x.$$

Here, $w_{\min}$ (respectively $w_{\max}$) denote the smallest (respectively the largest) component of the vector w.

The continuity of $\mu_0(S)$ and the inequality $r_1\mu_0(1) > 1$ imply that there exists a sufficiently small $\delta > 0$ such that

$$r_1\mu_0\left(\frac{1}{1+4\delta}\right) - 1 - \delta > 0. \tag{27}$$

Now we choose $\varepsilon > 0$ sufficiently small, so that the inequalities

$$\|R_r(S)\|\frac{|w|}{w_{\min}} < \delta, \quad \|D_r(S)\|\frac{|y_r|y_{r,\max}}{y_{r,\min}w_{\min}} < \delta$$

hold as long as $\|\mu_i - \alpha_i\mu_0\|_{C^0} < \varepsilon$.

Introducing $m(t) := S(t) + w^T x(t)$, we find that for (25)–(26)

$$\dot{m} = 1 - S - w^T x + \mu_0(S)(r_1 w^T - y_r^T \tilde{M}_r)x - y_r^T D_r(S)x + w^T R_r(S)x.$$

Since $r_1 w^T - y^T \tilde{M}_r \geq 0$, we obtain the inequality

$$\dot{m} \geq 1 - S - w^T x - |y_r^T D_r(S)x| - |w^T R_r(S)x| \geq 1 - S - w^T x - 2\delta w^T x,$$

hence

$$\dot{m} \geq 1 - (1 + 2\delta)S - (1 + 2\delta)w^T x = 1 - (1 + 2\delta)m.$$

The latter inequality implies that all solutions of the perturbed system (25)–(26) eventually enter the forward invariant set

$$K_0 := \left\{ (x, S) | x \geq 0,\ S \geq 0,\ m \geq \frac{1}{1 + 4\delta} \right\}.$$

Since all solutions of (25)–(26) satisfy

$$w^T \dot{x} = (r_1 \mu_0(S) - 1)w^T x + w^T R_r(S)x,$$

the monotonicity of $\mu_0(S)$ implies that in K_0, the following inequality holds:

$$w^T \dot{x} \geq \left(r_1 \mu_0 \left(\frac{1}{1 + 4\delta} - w^T x \right) - 1 - \delta \right) w^T x.$$

By the monotonicity of $\mu_0(S)$ and the inequality (27), there exists $\Delta_0 > 0$ such that

$$r_1 \mu_0 \left(\frac{1}{1 + 4\delta} - z \right) - 1 - \delta > \Delta_0 > 0, \quad \forall z \in (0, \Delta_0),$$

hence all solutions of (25)–(26) with $w^T x > 0$ in K_0 eventually enter the forward invariant set

$$K := \{(x, S) \in K_0 | w^T x \geq \Delta_0\}.$$

The claim of the Theorem follows by observing that $1^T x > 0$ implies $w^T x > 0$, and that $w^T x \geq \Delta_0$ implies $1^T x \geq \frac{\Delta_0}{w_{\max}} := \Delta > 0$. □

4.3 Proof of Theorem 1

The proof is an application of Theorem 2.2 in Smith and Waltman (1999).

First notice that if $\mu_i(S) = \alpha_i \mu_0(S)$ for all $i = 1, 2, \ldots, n$, then system (5)–(6) has a unique positive steady state (X_0^*, S_0^*) by Lemma 2 which is hyperbolic and locally

asymptotically stable by Lemma 3. Moreover, all solutions with $x(0) \neq 0$ converge to (X_0^*, S_0^*) by Theorem 4.

By Theorem 5 and Lemma 1, there exist $\varepsilon > 0$ and $\Delta > 0$ such that whenever $\|\mu_i - \alpha_i \mu_0\|_{C^0} < \varepsilon$, all solutions of (5)–(6) with $x(0) \neq 0$ eventually enter the compact invariant set

$$D = K \cap L.$$

The conclusion now follows immediately from Theorem 2.2 in Smith and Waltman (1999).

5 Application of antibiotics

In a continuous culture, the application of antibiotics is a complex process involving the pharmaco-dynamic and pharmaco-kinetic processes. For purposes of this paper, we will only study the primary effect of the antibiotic treatment which lowers the growth/division rate of all cells. The cells in different senescence classes may be affected differently. Specifically, we introduce a parameter $a \in \mathbb{R}^+$ that models the strength of the antibiotic treatment. The resulting growth rates of cells in the ith senescent class are given by $\mu_i(S, a)$, where $\mu_i : \mathbb{R}^+ \times \mathbb{R}^+ \to \mathbb{R}^+$ are such that for all $(S, a) \in \mathbb{R}^+ \times \mathbb{R}^+$

$$\mu_i(0, a) = 0, \quad \frac{\partial \mu_i}{\partial S}(S, a) > 0, \quad \frac{\partial \mu_i}{\partial a}(S, a) < 0. \tag{28}$$

We also define the auxiliary function

$$G(S, a) := \mu_1(S, a) \sum_{j=2}^{n} \prod_{k=2}^{j} \frac{\mu_k(S, a)}{1 + \mu_k(S, a)}. \tag{29}$$

We assume that $a = 0$ corresponds to the situation where no antibiotic is applied to the population, and thus we have that $\mu_i(S, 0) = \mu_i(S)$ for all i, and $G(S, 0) = F(S)$, where $\mu_i(S)$ and $F(S)$ are the functions that were defined in the preceding sections.

The response of the microbial population to antibiotic treatment is described in the following Theorem.

Theorem 6 *Model (1)–(4) has the following properties.*

1. *There exists a unique extended real number $a^* \in [0, +\infty]$ such that for all $a \geq a^*$ the only equilibrium of (1)–(4) is the washout steady state. For $a < a^*$, (1)–(4) admits a unique positive coexistence equilibrium $E(a)$.*
2. *If $a \geq a^*$, then all positive solutions of (1)–(4) converge to the washout steady state.*
3. *For any function $\mu_0(S)$ (smooth, zero at zero with $\mu_0'(S) > 0$) and any collection of nonnegative numbers*

$$\alpha_1 \geq \alpha_2 \geq \cdots \alpha_n > \alpha_{n+1} \equiv 0,$$

such that $F_0(1) > 1$ (*here,* $F_0(S)$ *is as defined in* (19)), *there exists* $\varepsilon^* > 0$ *such that if* $\|\mu_i - \alpha_i \mu_0\|_{C^0} < \varepsilon^*$, *then* $E(a)$ *is a globally asymptotically stable equilibrium which attracts all positive solutions of* (1)–(4).

Proof Statements (2) and (3) follow directly from Theorems 2 and 1, respectively. To prove (1), we observe that the equilibrium value S_a must satisfy $G(S_a, a) = 1$. Due to (28) and (29),

$$\frac{\partial G}{\partial S} = \sum_i \frac{\partial G}{\partial \mu_i}\frac{\partial \mu_i}{\partial S} > 0, \quad \frac{\partial G}{\partial a} = \sum_i \frac{\partial G}{\partial \mu_i}\frac{\partial \mu_i}{\partial a} < 0, \quad \text{since } \frac{\partial G}{\partial \mu_i} > 0.$$

In particular, the function $G(1, a)$ is strictly decreasing on $\mathbb{R}^+$. There exists a unique value a^* such that $G(1, a^*) = 1$. If $G(1, a) > 1$ for all $a \in \mathbb{R}^+$, we define $a^* = +\infty$. For a given $a \in [0, a^*)$, we have that $G(1, a) > 1$ and $G(0, a) = 0$, hence the equation $G(S, a) = 1$ admits a unique solution $S = S_a \in (0, 1)$ for which all x_k^*, $k > 1$ can be determined (up to the factor x_1^*) by plugging the value of S_a in (13), and the unique value of x_1^* then follows from (12). If, on the contrary, $a \geq a^*$, then $G(1, a) \leq 1$, and the equation $G(S, a) = 1$ has no solutions in the interval $(0, 1)$, in which case no positive equilibrium exists. □

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Appendix

Consider

$$\dot{x} = [\mu_0(S)Q - I_n]\, x, \tag{30}$$
$$\dot{S} = 1 - S - \mu_0(S) y_r^T x, \tag{31}$$

where Q is a quasi-positive and irreducible matrix and $y_r^T = (y_1, y_2, \ldots, y_n)$. As before, we assume that μ_0 is smooth, zero at zero and $\mu_0'(S) > 0$ for $S > 0$. Denote the real dominant eigenvalue of Q by r_1 and let v_1 be a corresponding positive eigenvector ($Qv_1 = r_1 v_1$). Also let w_1 be a positive left eigenvector of Q corresponding to r_1 ($w_1^T Q = r_1 w_1^T$). Note that all non-negative solutions of (30)–(31) are bounded. Indeed, letting $V(x, S) = w_1^T x + S$, we have that

$$\frac{dV}{dt} = 1 - V + \mu_0(S)(r_1 w_1 - y_r)^T x.$$

Then $dV/dt \leq 1 - V$ if $r_1 \leq 0$, but also if $r_1 > 0$ (by re-scaling the positive vector w_1 so that $r_1 w_1 - y_r < 0$). This implies boundedness of solutions of (30)–(31).

Consider the auxiliary system

$$\dot{\beta} = Q\beta - (1^T Q\beta)\beta, \quad \beta \in \mathbb{R}^n. \tag{32}$$

Lemma 4 *All non-zero and non-negative solutions of* (32) *satisfy*

$$\lim_{t\to\infty}\beta(t)=\frac{v_1}{1^Tv_1}.$$

Proof Let $y(t)$ be a non-zero and non-negative solution of the linear system $\dot{y}=Qy$, and let the vector $\beta(t)$ be defined via the relation

$$(1^Ty(t))\beta(t)=y(t). \tag{33}$$

Differentiating both sides of this equation, we obtain

$$(1^TQy(t))\beta(t)+(1^Ty(t))\dot{\beta}(t)=\dot{y}(t)=Qy(t).$$

Substituting $(1^Ty(t))\beta(t)=y(t)$, and dividing out the quantity $(1^Ty(t))$, results in

$$\dot{\beta}(t)=Q\beta(t)-(1^TQ\beta(t))\beta(t),$$

hence $\beta(t)$ is a non-zero and non-negative solution of (32). Using the eigenfunction expansion, we can represent $y(t)$ as

$$y(t)=c_1e^{r_1t}v_1+\sum_{j\geq 2}c_je^{r_jt}v_j,$$

where $c_1>0$ and $r_1>\Re(r_j),\ j\geq 2$. By (33) we have

$$e^{-r_1t}(1^Ty(t))\beta(t)=e^{-r_1t}y(t),$$

or equivalently

$$\left(c_1(1^Tv_1)+\sum_{j\geq 2}c_je^{(r_j-r_1)t}(1^Tv_j)\right)\beta(t)=c_1v_1+\sum_{j\geq 2}c_je^{(r_j-r_1)t}v_j.$$

It follows immediately that

$$\lim_{t\to\infty}\beta(t)=\frac{c_1v_1}{c_1(1^Tv_1)}=\frac{v_1}{1^Tv_1}.$$

□

Theorem 7 *Assume that system* (30)–(31) *has a positive equilibrium point* (x_0^*, S_0^*). *Then all solutions* $(x(t), S(t))$ *of* (30)–(31) *with* $1^Tx(t)>0$, *converge to* (x_0^*, S_0^*).

Proof Let the vector $\alpha(t)$ be defined via the relation $(1^Tx(t))\alpha(t)=x(t)$.Differentiating both sides of this equation, we obtain

$$(\mu_0(S(t))(1^TQ)x(t)-(1^Tx(t)))\alpha(t)+(1^Tx(t))\dot{\alpha}_i(t)=\mu_0(S(t))Qx(t)-x(t).$$

After canceling the equal terms $(1^T x(t))\alpha(t) = x(t)$ from both sides and dividing out the common factor $(1^T x(t))$, we obtain the equivalent system

$$\mu_0(S(t))(1^T Q\alpha(t))\alpha(t) + \dot{\alpha}(t) = \mu_0(S(t))Q\alpha(t).$$

Introducing the new time τ so that

$$\frac{d}{d\tau} = \frac{1}{\mu_0(S(t))}\frac{d}{dt},$$

we find that the functions $\alpha_i(\tau)$ satisfy (32). Since there exist m, M such that $0 < m \leq \mu_0(S(t)) \leq M$, it follows that $t \to +\infty$ as $\tau \to +\infty$. Hence by Lemma 4,

$$\lim_{t\to+\infty} \alpha(t) = \lim_{\tau\to+\infty} \alpha(\tau) = \alpha^* > 0.$$

The dynamics of the original system (30)–(31) is asymptotic to the dynamics of the limiting system

$$\dot{z} = (a\mu_0(S) - 1)\, z, \tag{34}$$
$$\dot{S} = 1 - S - b\mu_0(S)z, \tag{35}$$

where $z = 1^T x$, $a = 1^T Q\alpha^* = r_1(1^T\alpha^*) = r_1 > 0$, and $b = y_r^T\alpha^* > 0$. Linearization at the positive equilibrium (z_0^*, S_0^*) of the limiting system (34)–(35) yields:

$$\begin{pmatrix} 0 & a\mu_0'(S_0^*) \\ -b\mu_0(S_0^*) & -1 - b\mu_0'(S_0^*)z_0^* \end{pmatrix},$$

which has negative trace and positive determinant. Thus, (z_0^*, S_0^*) is hyperbolic and asymptotically stable for (34)–(35). Moreover, system (34)–(35) admits the following Lyapunov function on $\{(z, S) \in \mathbb{R}^2_+ | z > 0,\ S > 0\}$:

$$V(z, S) = c\int_{z_0^*}^{z} \frac{s - z_0^*}{s} ds + \int_{S_0^*}^{S} \frac{\mu_0(s) - \mu_0(S_0^*)}{\mu_0(s)} ds,$$

where $c = b/a > 0$.

Indeed,

$$\begin{aligned}
\frac{dV}{dt} &= c\left(\frac{z - z_0^*}{z}\right) z(a\mu_0(S) - 1) + \left(\frac{\mu_0(S) - \mu_0(S_0^*)}{\mu_0(S)}\right)(1 - S - b\mu_0(S)z) \\
&= -cz_0^*(a\mu_0(S) - 1) + \left(\frac{\mu_0(S) - \mu_0(S_0^*)}{\mu_0(S)}\right)(1 - S) + z(-c + \mu_0(S_0^*)b) \\
&= -bz_0^*(\mu_0(S) - \mu_0(S_0^*)) + \left(\frac{\mu_0(S) - \mu_0(S_0^*)}{\mu_0(S)}\right)(1 - S) + 0 \\
&= \left(\frac{\mu_0(S) - \mu_0(S_0^*)}{\mu_0(S)}\right)(1 - S - b\mu_0(S)z_0^*) \leq 0,
\end{aligned}$$

since both factors in the last expression are 0 if and only if $S = S_0^*$, and have opposite signs when $S < S_0^*$ and $S > S_0^*$. By Lasalle's invariance principle, all solutions $(z(t), S(t))$ of (34)–(35) with $z(t) > 0$, converge to the largest invariant set where $S = S_0^*$, which is the equilibrium (z_0^*, S_0^*).

Hence, the positive equilibrium is globally asymptotically stable with respect to solutions $(z(t), S(t))$ for which $z(t) > 0$, whenever it exists. A standard application of the theory of asymptotically autonomous systems (see, e.g. Appendix F in Smith and Waltman 1995) concludes the proof. □

References

Albrich WC, Monnet DL, Harbarth S (2004) Antibiotic selection pressure and resistance in Streptococcus pneumoniae and Streptococcus pyogenes. Emerg Infect Dis 10(3):514–517

Ayati BP, Klapper I (2007) A multiscale model of biofilm as a senescence-structured fluid. SIAM Multi Model Sim 6:347–365

Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S (2005) Bacterial persistence as a phenotypic switch. Science 305:1622–1625

Bigger J (1944) Treatment of staphylococcal infections with penicillin by intermittent sterilisation. The Lancet 244:497–500

Cogan NG (2006) Effects of persister formation on bacterial response dosing. J Theor Biol 238:694–703

Cogan NG (2007) Incorporating toxin hypothesis into a mathematical model of persister formation and dynamics. J Theor Biol 248:340–349

Consortium REX (2007) Structure of the scientific community modelling the evolution of resistance. PLoS ONE 2(12):e1275. doi:10.1371/journal.pone.0001275

Davies J (1994) Inactivation of antibiotics and the dissemination of resistance genes. Science 264:375–382

D'Agata EMC, Magal P, Olivier D, Ruan S, Webb GF (2007) Modeling antibiotic resistance in hospitals: the impact of minimizing treatment duration. J Theor Biol 249:487–499

D'Agata EMC, Dupont-Rouzeyrol M, Magal P, Olivier D, Ruan S (2008) The impact of different antibiotic regimens on the emergence of antimicrobial-resistant bacteria. PLoS ONE 3(12):e4036. doi:10.1371/journal.pone.0004036

D'Agata EMC, Webb GF, Horn MA, Moellering RC Jr, Ruan S (2009) Modeling the invasion of community-acquired methicillin-resistant Staphylococcus aureusi into the hospital setting. Clin Infect Dis 48:274–284

De Leenheer P, Cogan NG (2009) Failure of antibiotic treatment in microbial populations. J Math Biol 59(4):563–579

Diekmann O, Heesterbeek JAP, Metz JAJ (1990) On the definition and the computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. J Math Biol 28:365–382

Grundmann H, Hellriegel B (2006) Mathematical modeling: a tool for hospital infection control. Lancet Infect Dis 6:39–45

Hardin G (1960) Competitive exclusion principle. Science 131:1292–1297

Hirsch MW, Hanisch H, Gabriel J-P (1985) Differential equation models for some parasitic infections: methods for the study of asymptotic behavior. Comm Pure Appl Math 38:733–753

Imran M, Smith HL (2006) The pharmacodynamics of antibiotic treatment. J Comp Math Meth Med 7:229–263

Keren I, Kaldalu N, Spoering A, Wang Y, Lewis K (2004) Persister cells and tolerance to antimicrobials. FEMS Microbiol Lett 230:13–18

Klapper I, Gilbert P, Ayati BP, Dockery J, Stewart PS (2007) Senescence can explain microbial persistence. Microbiology 153:3623–3630

Korona R, Nakatsu CH, Forney LJ, Lenski RE (1994) Evidence for multiple adaptive peaks from populations of bacteria evolving in a structured habitat. Proc Natl Acad Sci USA 91:9037–9041

Kussell E, Leibler S (2005) Phenotypic diversity, population growth, and information in fluctuating environments. Science 309:275–278

Kussell E, Kishony R, Balaban NQ, Leibler S (2005) Bacterial persistence: a model of survival in changing environments. Genetics 169:1807–1814
Layden T, Layden J, Ribeiro R, Perelson A (2003) Mathematical modeling of viral kinetics: a tool to understand and optimize therapy. Clin Liver Dis 7:163–178
Lenas P, Pavlou S (1995) Coexistence of three microbial populations in a chemostat with periodically varying dilution rate. Math Biosci 129:111–142
Levin BR (2001) Minimizing potential resistance: a population dynamics view. Clin Infect Dis 33:S161–S169
Levy S, Marshall B (2004) Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 10:S122–S129
Lewis K (2001) Riddle of biofilm resistance. Antimicrob Agents Chemoth 45:999–1007
Lewis K (2007) Persister cells, dormancy and infectious disease. Nat Rev Micro 5:48–56
Lindner AB, Madden R, Demarez A, Stewart EJ, Taddei F (2008) Asymmetric segregation of protein aggregates is associated with cellular aging and rejuvenation. Proc Natl Acad Sci USA 105:3076–3081
Lipsitch M, Levin B (1997) The population dynamics of antimicrobial chemotherapy. Antimicrob Agents Chemother 41:363–373
Martinez JL (2008) Antibiotics and antibiotic resistance genes in natural environments. Science 321:365–367
McGowan JE (1983) Antimicrobial resistance in hospital organisms and its relation to antibiotic use. Rev Inf Dis 5(6):1033–1048
Ochman H, Lawrence JG, Groisman EA (2000) Lateral gene transfer and the nature of bacterial innovation. Nature 405:299–304
Perelson A, Nelson P (1999) Mathematical analysis of HIV-1 dynamics in vivo. SIAM Rev 41:3–44
Siegel JD, Rhinehart E, Jackson M, Chiarello L (2007) Management of multidrug-resistant organisms in health care settings, 2006. Am J Infect Control 35:S165–S193
Spellberg B, Guidos R, Gilbert D, Bradley J, Boucher HW, Scheld WM, Bartlett JG, Edwards J Jr (2008) The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. Clin Infect Dis 46:155–164
Smith HL, Waltman P (1995) The theory of the chemostat. Cambridge University Press, Cambridge
Smith HL, Waltman P (1999) Perturbation of a globally stable steady state. Proc AMS 127:447–453
Stewart EJ, Madden R, Paul G, Taddei F (2005) Aging and death in an organism that reproduces by morphologically symmetric division. PLoS Comp Biol 3:295–300
Temime L, Hejblum G, Setbon M, Valleron AJ (2008) The rising impact of mathematical modeling in epidemiology: antibiotic resistance research as a case study. Epidemiol Infect 136:289–298
Thieme HR (1993) Persistence under relaxed point-dissipativity (with application to an endemic model). SIAM J Math Anal 24:407–435
Thieme HR (2003) Mathematics in population biology. Princeton University Press, Princeton
Turner PE, Souza V, Lenski RE (1996) Tests of ecological mechanisms promoting the stable coexistence of two bacterial genotypes. Ecology 77:2119–2129
Vance RR (1985) The stable coexistence of two competitors for one resource. Am Nat 126:72–86
van den Driessche P, Watmough J (2002) Reproduction numbers and sub-treshold endemic equilibria for compartmental models of disease transmission. Math Biosci 180:29–48
Wiuff C, Anderson D (2007) Antibiotic treatment in vitro of phenotypically tolerant bacterial populations. J Antimicrob Chemother 59:254–263