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A kinetic model for horizontal transfer and bacterial antibiotic resistance

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This paper presents a mathematical model for bacterial growth, mutations, horizontal transfer and development of antibiotic resistance. The model is based on the so-called kinetic theory for active particles that is able to capture the main complexity features of the system. Bacterial and immune cells are viewed as active particles whose microscopic state is described by a scalar variable. Particles interact among them and the temporal evolution of the system is described by a generalized distribution function over the microscopic state. The model is derived and tested in a couple of case studies in order to confirm its ability to describe one of the most fundamental problems of modern medicine, namely bacterial resistance to antibiotics.

Keywords: Kinetic theory; active particles; antibiotic resistance; horizontal gene transfer.

Mathematics Subject Classification 2010: 92B05, 92C45, 92C50, 65L99

1. Introduction

The discovery of penicillin by Alexander Fleming in 1928 constituted one of the greatest steps in the development of medicine and biology. It finally made possible to battle against bacterial infections caused by staphylococci and streptococci that had caused thousands of deaths over the years.

However, in the last several years, it has become apparent that bacteria have acquired more and more resistance to antibiotics. This fact was first observed experimentally by Joshua and Esther Lederberg [20], who concluded that resistant bacteria were generated by simple and random genetic variants in the original population

and then naturally selected. It is now known that bacterial genes for drug resistance are carried on plasmids, small DNA molecules that can be transferred from one cell to another [35]. Consequently, the spread of mutations in a bacterial population that confer resistance is much faster than would occur as a result of natural selection only.

Then, the main problem is given by the indiscriminate use of antibiotics that expose groups of bacteria to repeated treatments. Antibiotic resistance occurs when an antibiotic has lost its ability to effectively control or kill bacterial growth; in other words, the bacteria are "resistant" and continue to multiply in the presence of therapeutic levels of antibiotic. This creates a natural selection in which those which are resistant survive. If these bacteria are exposed to chronic treatment, the result is that more and more bacteria are resistant to most antibiotics. Let us stress that antibiotic resistance is a natural phenomenon: when an antibiotic is used, bacteria that are resistant to that antibiotic have a greater chance of survival than those that are susceptible. Susceptible bacteria are killed or inhibited by the antibiotic, resulting in a selective pressure for the survival of resistant strains of bacteria.

Some bacteria are naturally resistant to certain types of antibiotics. However, bacteria may also become resistant in two ways: (1) by a genetic mutation, or (2) by acquiring resistance from another bacterium.

So, on the one hand, bacteria can spread to their offspring the genetic material that will confer on them some resistance to antibiotics. This resistance could be acquired or transferred from other surrounding bacteria. A bacterium divides by binary fission and each daughter cell should be an identical copy of it. However, a mutation might occur during a bacterium replication by an erroneous copy of genetic material, and this mutation can encode a protein that confers resistance to a given antibiotic. In terms of the genetic basis of resistance acquisition, this is known as chromosomal resistance.

As stated by Nowak [31], the term mutation is used to refer to any genetic modification such as point mutations, insertions, deletions, chromosome rearrangements, mitotic recombination, or loss or gain of whole chromosomes or arms of chromosomes. Some useful references on mutation rates and experimental methods to determine them are [18, 32, 33].

On the other hand, another way to become resistant is that in which the bacterium copies a segment of its genetic material and transfers it to other bacteria (extrachromosomal acquisition). In this case, transmission requires contact between cells carrying that genetic material and cells free of it through the processes known as conjugation and horizontal transfer [37], as illustrated in Fig. 1. Actually, several different proteins can be acquired with action against different antibiotics (some attack beta-lactamases, others avoid the income of antibiotic to the nucleus, others avoid ribosomes being attacked by the antibiotic, etc.). That is the reason that explains the fact that there are bacteria which can collect multiple resistance mechanisms over time, becoming resistant to many different families of antibiotics [9].

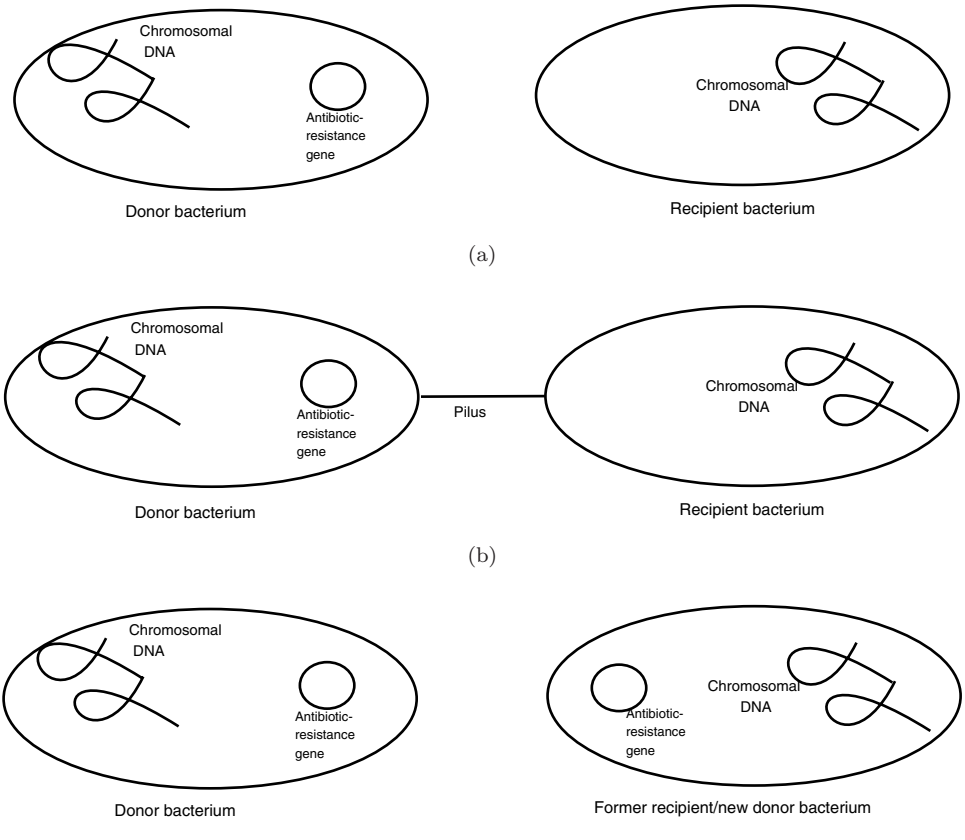


Fig. 1. Schematic representation of horizontal gene transfer. (a) A donor bacterium *meets* a recipient one; (b) Bacterial conjugation consists in the formation of a pilus, through which genetic material can be transferred; (c) Both bacteria contain an antibiotic-resistant gene.

To make matters even worse, it is worth stressing that genetic material is not only transmitted between bacteria from the same species but also between different families of bacteria that share certain compatibility conjugation features with each other. Thus, different bacteria that are responsible for different diseases are getting more and more difficult to attack. To give an idea of the problem we are dealing with, according to the most recent annual report on global risks, the World Economic Forum (WEF) [36] estimates "that in the United States at least two million people acquire serious infections with bacteria that are resistant to one or more of the antibiotics designed to treat them, and at least 23,000 people die each year as a direct result of these antibiotic-resistant infections", while the burden in low- and middle-income countries is much higher. An interesting reference that tackles the environmental framework of antibiotic resistance is [6], where the main harmful human habits that worsen the problem are explored and some remedial solutions

are proposed. Also, some recent developments on experimental efforts in this aspect are addressed in [10, 16].

If we had to compare the relative frequencies of both mechanisms, horizontal transfers are much more frequent than mutations. Mutations, rare spontaneous changes of the bacteria's genetic material, are thought to occur in about one in one million to one in 10 million cells. Different genetic mutations yield different types of resistance. Some mutations enable the bacteria to produce potent chemicals (enzymes) that inactivate antibiotics, while other mutations eliminate the cell target that the antibiotic attacks. Still others close up the entry ports that allow antibiotics into the cell, and others manufacture pumping mechanisms that export the antibiotic back outside so it never reaches its target. On the other hand, there is about one conjugation and transfer event at every 10,000 receptor cells [35].

Finally, let us mention that resistant cells can also lose this capability: this could happen as a consequence of another mutation or, more frequently, because of the segregation of the genetic material that confers resistance [30].

There exist a few mathematical models dealing with the matter of horizontal transfer of plasmids and treatment protocols to prevent antibiotic resistance, like [7, 23–25, 29, 34, 35], though there are not many references in the literature about mathematical models for antibiotic resistance. Some valuable contributions on horizontal gene transfer are [27], where authors introduce a model based on population dynamics for natural transformation and study its dynamic characteristics with nonlinear tools and simulations; while paper [19] proposes a model of horizontal gene transfer using genetic trees tools. In this paper, we propose a model that takes into account — in addition to horizontal transfer and antibiotic resistance issues — the presence of the immune system that also tries to contrast the development of a disease. Our mathematical approach is based on the kinetic theory for active particles (KTAP) that considers the complexity features of the system under consideration. This theory has so far been successfully applied to model complex living systems constituted by a large number of particles interacting according to rules modeled by theoretical game theory [3], among which we can mention the modeling of social systems [1, 21], opinion formation with dynamics over networks [15, 22], molecular genetics [14], selective mutations in epidemiology [12, 13] and Darwinian mutation and selection on cancer phenomena contrasted by immune cells [4]. We refer to [2] and references therein for an interesting critical analysis on the derivation of mathematical structures developed to capture the complexity of biological systems, in particular with Darwinian-type dynamics. An important recent contribution of the KTAP that is applied in our framework refers to collective learning [8], since it is a major complexity feature of the biological system under consideration. Mayr introduced the concept of population thinking [28] that, linked to the concept of mutations and selection, can explain many aspects of the theory of evolution and motivated the development of evolutionary game theory [31].

In our approach we consider a system constituted by a large number of interacting cells that are divided into three subpopulations: resistant bacteria, non-resistant

bacteria and immune cells. Interactions are modeled at the microscopic state that is described by a scalar variable and the system is thus described by a generalized distribution function depending on time and on the microscopic state. The overall dynamics is obtained as a result of these interactions: immune cells try to fight against bacteria, while bacteria can conjugate among them and horizontally transfer the ability to resist against an antibiotic, that is supplied and interpreted as an external action.

This paper is organized as follows: Sec. 2 describes the system under consideration and introduces the general mathematical framework for the derivation of the model. Section 3 introduces the mathematical model and the modeling of interactions among cells. In Sec. 4, we perform a computational analysis of the proposed model and report some simulations addressed to show emerging behaviors. Finally, Sec. 5 contains conclusions and highlights some research perspectives.

2. Representation of the Cellular System and Formulation of the Mathematical Model

2.1. *Description of the system and mathematical representation*

Let us consider a system composed of a large number of cells. On the one hand, we have pathogenic bacterial cells, all of them belonging to the same species, which are spatially homogeneously distributed in a given tissue or culture. These cells can be essentially divided into two groups of strains, namely those that are susceptible to be killed by a certain antibiotic A and those that have acquired resistance to it. This can be explained by the presence or absence of a given genetic material (for instance a plasmid) inside its cytoplasm. This material is responsible for conferring to the bacterium resistance to A. Consequently, bacteria containing P share a common strategy that let them resist to antibiotic supply. On the other hand, we have immune cells, whose major action is that of recognizing and destroying foreign substances in the body, in this case bacteria.

Thus, our system is made up of a large number of interacting cells, called active particles in the context of the KTAP, whose physical microscopic state is described by a variable called activity, that represents the individual ability to express a specific biological function. The system can be divided into functional subsystems according to the following criteria:

- $i = 1$ labels bacteria that are susceptible to be killed by a certain antibiotic A;
- $i = 2$ refers to those bacteria that have acquired resistance to A;
- $i = 3$ denotes cells of the immune system that can acquire, by a learning process, the ability to contrast the spread of bacterial cells.

Remark 2.1. The mathematical and numerical analysis of the model can be also performed by considering no immune reaction, for instance in the case in which only resistance to antibiotics is studied in *in vitro* experiments.

Within each group of bacteria, it is assumed that — if not contrasted by the immune system or by antibiotic supply — cells can evolve toward more aggressive states, leading to an increase of its activity. Analogously, immune cells can also evolve toward stronger states as they acquire the ability to recognize more aggressive bacteria.

The activity variable describes these progression states, taking values in the discrete set

$$I_u = \{0 = u_1, \ldots, u_m = 1 : u_1 < \cdots < u_m\}.$$

The activity is heterogeneously distributed and is such that increasing values of the variable represent an increasing ability of cells to express their biological function: more aggressive states (for functional subsystems 1 and 2) or stronger immune reaction (for functional subsystem 3).

In order to describe the overall state of the system, let us now introduce the discrete generalized distribution functions

$$f_{ij} = f_{ij}(t), \quad i = 1, 2, 3, \;\; j = 1, \ldots, m,$$

which denote the number of active particles from functional subsystem i that have, at time t, the state u_j. Consequently, the zeroth-order moment

$$n_i[f](t) = \sum_{j=1}^{m} f_{ij}(t), \quad i = 1, 2, 3, \tag{2.1}$$

gives the number of particles that, at time t, belong to the ith-functional subsystem. Therefore, $N(t) = n_1(t)+n_2(t)$ represents the total number of living bacteria, where $n_1(t)$ is the number of those non-resistant to A, while $n_2(t)$ is the number of resistant ones. It is worth remarking at this point that increasing values of n_2 constitute one of the main concerns of medicine nowadays [36].

Finally, as it was previously stated, we want to study the action of antibiotic supply. Within the present framework, this is introduced as an external action given by a function $c(t)$ that refers to the concentration of A. The drug attacks bacteria from the first functional subsystem, but has no effect against those from the second one. The form of $c(t)$ depends on a drug administration protocol previously established and supposed to be known.

Now, we are able to obtain the general mathematical structure that describes the temporal evolution of distribution functions f_{ij}. Following the ideas exposed in [4, 13], we can identify two main sources of transitions in the microscopic state of cells:

- A self-evolution not mediated by binary interactions that depends only on the current state of particles. This approach constitutes realistic scenario to model cell proliferation and mutations.
- Binary interactions between particles from the same or different functional subsystems.

Accordingly, the evolution in time of the distribution function f_{ij} is obtained by performing a balance of particles in the state u_j of functional subsystem i, and the balance equation can be summarized as

$$\frac{df_{ij}}{dt} = C_{ij}[\mathbf{f},\mathbf{f}](t) + P_{ij}[\mathbf{f}](t) - D_{ij}[\mathbf{f},\mathbf{f}](t) - L_{ij}[\mathbf{f}](t) + Q_{ij}[\mathbf{f}](t), \qquad (2.2)$$

for $i = 1, 2, 3$ and $j = 1, \ldots, m$, where $\mathbf{f}$ denotes the set of all distribution functions and square brackets represent the dependence on $\mathbf{f}$.

Specifically:

- $C_{ij}[\mathbf{f},\mathbf{f}](t)$ is the net flux, at time t, into the state u_j of the functional subsystem i, due to conservative interactions.
- $P_{ij}[\mathbf{f}](t)$ is the gain, at time t, into the state u_j of the functional subsystem i, due to proliferative events, through a self-mediated process.
- $D_{ij}[\mathbf{f},\mathbf{f}](t)$ is the loss, at time t, in the state u_j of the functional subsystem i, due to destructive events.
- $L_{ij}[\mathbf{f}](t)$ is the natural self-relaxation of the immune system, at time t and in the state u_j of the functional subsystem i, to a given healthy state.
- $Q_{ij}[\mathbf{f}](t)$ refers to the external action of the antibiotic A that is administered in such a dose that its concentration is $c(t)$.

Remark 2.2. Since a cellular system is characterized by a rapid replication (eventually exhibiting mutation phenomena) and immune action and drug treatment are able to deplete bacterial cells, the above-described system is not conservative, as [4]. This constitutes a major difference with the model proposed in [13]. In that model, authors took as active particles those individuals (people) carriers of the infectious agent. In this present model, however, active particles are cells.

2.2. *Description of self- and binary-mediated terms*

Let us now specify each of the terms in Eq. (2.2) in order to model the above-described cellular system.

• The term $C_{ij}[\mathbf{f},\mathbf{f}](t)$ describing conservative interactions between cells considers interactions between particles. A *candidate* particle belonging to functional subsystem h with microstate u_p (hp-particle from now on) can undergo a transition into the state of the *test* particle ij after an interaction with a *field* kq-particle. In addition, the test particle can lose its state as a consequence of an interaction with a field particle. Binary interactions are described by the following interaction terms:

— $\eta_{hp}^{kq} \geq 0$ denotes the encounter rate between the candidate hp and the field kq-particles.
— The discrete probability transition functions $\mathcal{B}_{hk}^{pq}(ij)$ denote the probability that a candidate hp-particle falls into the state ij of the test particle after

an interaction with a field kq-particle. These transition functions satisfy:

$$\mathcal{B}_{hk}^{pq}(ij) \geq 0, \quad \forall\, i,h,k = 1,2,3, \ \ \forall\, j,p,q = 1,\ldots,m,$$

$$\sum_{i=1}^{3}\sum_{j=1}^{m} \mathcal{B}_{hp}^{kq}(ij) = 1, \quad \forall\, h,k = 1,2,3, \ \ \forall\, p,q = 1,\ldots,m.$$

Then, the balance of particles due to conservative interactions reads

$$C_{ij}[\mathbf{f},\mathbf{f}](t) = \sum_{h,k=1}^{3}\sum_{p,q=1}^{m} \eta_{hp}^{kq}\mathcal{B}_{hp}^{kq}(ij) f_{hp}(t) f_{kq}(t) - f_{ij}(t)\sum_{k=1}^{3}\sum_{q=1}^{m}\eta_{ij}^{kq} f_{kq}(t). \quad (2.3)$$

• Regarding to the modeling of the term $P_{ij}[\mathbf{f}](t)$ for bacterial strains $i = 1,2$, we introduce the proliferation rate $\mu_{hp}(ij)$ that denotes the rate at which hp-cells divide, giving a daughter ij-cell. Let us stress that if $i = h$ and $j = p$, then daughter cells are identical to the mother cell. However, if one of these variables changes during replication, then a mutation has occurred. Consequently, we have

$$P_{ij}[\mathbf{f}](t) = \sum_{h=1}^{3}\sum_{p=1}^{m}\mu_{hp}(ij) f_{hp}(t), \quad i = 1,2. \quad (2.4)$$

Notice that, in this case, we indeed have a self-mediated process. This is inspired in the fact that it is well known that the rate of change in cell population due to replication is proportional to its instantaneous size [31]. The proliferation rate may depend, for instance, on the cell type and nutrient availability.

• The term $P_{3j}[\mathbf{f}](t)$ describing the proliferation of immune cells is quite different, since immune system is indeed able to acquire immunity in the presence of foreign agents (in this case bacteria). Consequently, this term must consider binary interactions with bacteria in the sense that their presence stimulates immune cells proliferation. Thus, we have

$$P_{3j}[\mathbf{f}](t) = \sum_{k=1}^{2}\sum_{q=1}^{m}\eta_{3p}^{kq}\kappa_{3p}^{kq}(3j) f_{3p} f_{kq}(t), \quad (2.5)$$

where $\kappa_{3p}^{kq}(3j)$ is the proliferation rate of immune cells with state p into state j induced by interactions with bacterial kq-cells.

• The term $D_{ij}[\mathbf{f},\mathbf{f}](t)$ models cell death due to binary interactions. Basically, it refers to the action of the immune system over bacterial cells. Introducing the net destruction rate of ij-cells ν_{ij}^{kq} due to binary interactions, occurring with a frequency η_{ij}^{kq} with field kq-particles, we have

$$D_{ij}[\mathbf{f},\mathbf{f}](t) = f_{ij}(t)\sum_{k=1}^{3}\sum_{q=1}^{m}\eta_{ij}^{kq}\nu_{ij}^{kq} f_{kq}(t). \quad (2.6)$$

• For the term $L_{ij}[\mathbf{f}](t)$ that represents the relaxation of the immune system, we introduce the parameter λ, that refers to the natural tendency of the immune system

to relax to a given healthy state [4, 11], that is defined as a given distribution of immune cells in a healthy individual. In general, it is a good choice for the healthy state to take the initial value of the distribution functions f_{ij}^0, so that

$$L_{ij}[\mathbf{f}](t) = \lambda\big(f_{ij}(t) - f_{ij}^0(t)\big). \tag{2.7}$$

• Finally, the antibiotic efficiency is described by the parameter φ_A that may depend on the provided drug A. Thus, its action over bacteria is given by

$$Q_{ij}[\mathbf{f}](t) = -\varphi_A g(c(t)) f_{ij}(t), \tag{2.8}$$

where g is an increasing non-negative function, meaning that A is a concentration-dependent antibiotic that achieves an increasing bacterial killing ability with increasing levels of drug (e.g. azithromycin, quinolones).

In the next section, all the above-introduced terms will be specified in order to obtain the general expression that models the interaction among bacteria, immune cells and antibiotic system.

3. Derivation of the General Model

Let us recall that our system is constituted by three functional subsystems, labeled by $i = 1, 2, 3$, where the first two are composed of bacterial cells (non-resistant and resistant to antibiotic A, respectively), while the last one is formed by immune cells. Each population is characterized by the discrete activity variable u_j, $j = 1, \ldots, m$, that represents the aggressivity for the populations of bacteria, and the acquired capability to contrast them for immune cells.

The main features to be considered in the model are:

- *Immune evasion*: If not contrasted by the immune system or by the action of the antibiotic, bacterial cells evolve toward more aggressive states, leading to an increase of their state within the same population. Indeed, bacteria have developed complex and efficient methods to overcome innate and adaptive immune mechanisms [17].
- *Immune recognition and action*: When immune cells are able to recognize bacteria, they act destroying them. In general, new strains of immune cells are generated — via a learning process — in order to contrast the action of more evolved bacteria.
- *Extrachromosomal resistance acquisition*: Interactions between resistant (containing a specific genetic material) and non-resistant (not containing it) bacteria can induce, with a certain probability, the formation of a conjugation channel joining them. Then, the antibiotic-resistance gene (usually a plasmid) is horizontally transferred from one cell to the other, giving as a result two resistant bacteria.
- *Proliferation and chromosomal resistance acquisition*: Bacteria divide at a given rate in a self-regulated process. In general, daughters are identical to mother cells but in some cases, even with a small probability, replication can produce a mutation from a non-resistant to a resistant bacterium.

- *Antibiotic action*: If antibiotic is supplied, it is only able to kill non-resistant bacteria. This action depends on its efficiency and on the dose.

In the following subsections, the above-mentioned features are taken into account in order to model those terms introduced in Sec. 2.

3.1. *Modeling conservative interactions*

Encounter rate: Only the modeling of non-trivial encounters is taken into account. In other words, those encounters between cells that do not produce state transitions are not necessary to be introduced. In this sense, we need to model encounter rates between resistant and non-resistant bacteria that can conjugate for horizontal transfer, and between bacterial and immune cells. In particular, we assume constant interaction rates $\eta_0, \tilde{\eta}_0 \geq 0$ such that:

$$\eta_{1p}^{2q} = \eta_{2q}^{1p} = \eta_0, \quad \forall\, p, q = 1, \ldots, m, \tag{3.1}$$

$$\eta_{hp}^{3q} = \eta_{3q}^{hp} = \tilde{\eta}_0, \quad h = 1, 2, \ \forall\, p, q = 1, \ldots, m. \tag{3.2}$$

An alternative modeling approach for these terms could be referred to a certain notion of distance between functional subsystems and states, see [3].

Transition probability density: Let us now consider those interactions that undergo a conservative transition. More in details, we have:

- *Interactions between functional subsystems* 1 *and* 2. These interactions are responsible of the horizontal transfer of genetic material from a field bacterium belonging to functional subsystem $k = 2$ to a candidate one belonging to functional subsystem $h = 1$. If this transfer effectively occurs — with probability α — then candidate bacterium acquires antibiotic resistance, undergoing a transition into subsystem 2:

$$\mathcal{B}_{1p}^{2q}(ij) = \begin{cases} \alpha, & i = 2, \ j = p, \ 0 \leq \alpha \leq 1, \\ 1 - \alpha, & i = 1, \ j = p, \\ 0, & \text{otherwise.} \end{cases} \tag{3.3}$$

  Recall that Fig. 1 illustrates this phenomenon biologically, while Fig. 2(a) exemplifies the dynamics modeled in Eq. (3.3).
- *Interactions between functional subsystems* $h = 3$ *and* $k = 1, 2$. Immune cells progressively acquire the ability to identify bacterial cells. Consequently, they may increase their activity state with probability β in order to contrast more aggressive bacteria, as modeled in Eqs. (3.4)–(3.5):

$$\mathcal{B}_{3p}^{kq}(ij) = \begin{cases} \beta, & i = 3, \ j = p + 1, \ p < q, \ 0 \leq \beta \leq 1, \\ 1 - \beta, & i = 3, \ j = p, \\ 0, & \text{otherwise,} \end{cases} \tag{3.4}$$

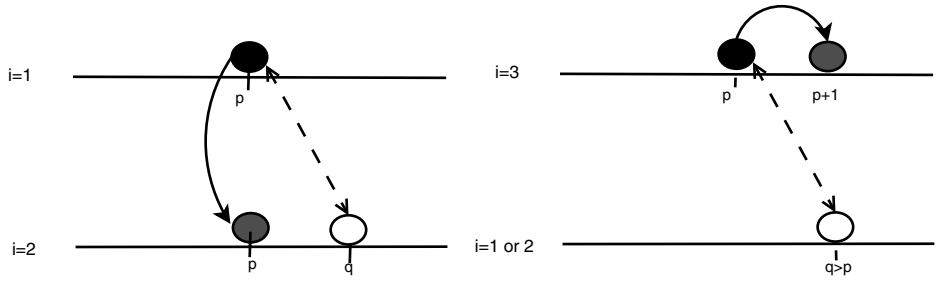


Fig. 2. Dynamics of conservative interactions. (a) A candidate p-bacterium from subsystem 1 (black bullet) interacts with a field q-bacterium from subsystem 2 (white bullet). As a result, genetic material that confers antibiotic resistance can be transferred (with probability α) through conjugation and horizontal transfer. In this way, the candidate bacterium undergoes a transition into functional subsystem 2, with the same activity state (grey bullet). (b) A candidate p-immune cell (black bullet) interacts and recognizes a field q-bacterium (with $q > p$). Then, the immune response consists in a transition (with probability β) to the next activity state $p+1$ (grey bullet).

for $k = 1, 2$, and

$$\mathcal{B}_{3p}^{kq}(3p) = 1 \quad \text{if } p \geq q, \tag{3.5}$$

for $k = 1, 2$.

This dynamics is illustrated in Fig. 2(b).

3.2. *Modeling proliferative and destructive events*

Proliferative events and mutations: As already stated, these events are regarded as self-mediated, since cells can divide by themselves under suitable conditions (e.g. nutrient, oxygen and space availability).

Proliferation occurs within the same functional subsystem giving identical daughter cells. It is assumed that this occurs at a rate μ_0 for bacteria and κ for immune cells. In the case of the latter, proliferation of immune cells with activity u_j is stimulated by the presence of bacterial cells with the same activity, see Eqs. (3.6)–(3.8).

On the other hand, bacterial replication can give mutant daughters; these are rare but extremely important events that can drastically change the overall dynamics. For the sake of simplicity, we divide these events in two main categories:

- Type I mutations that refer to the ability of bacteria to become more aggressive and to acquire stronger strategies to avoid immune response. These mutations occur at a rate μ_1.
- Type II mutations that refer to the ability of bacteria to acquire resistance to antibiotic A. These occur at a rate μ_2.

Finally, resistant bacteria can eventually lose the genetic material responsible of conferring antibiotic resistance through segregation. This is a complex process

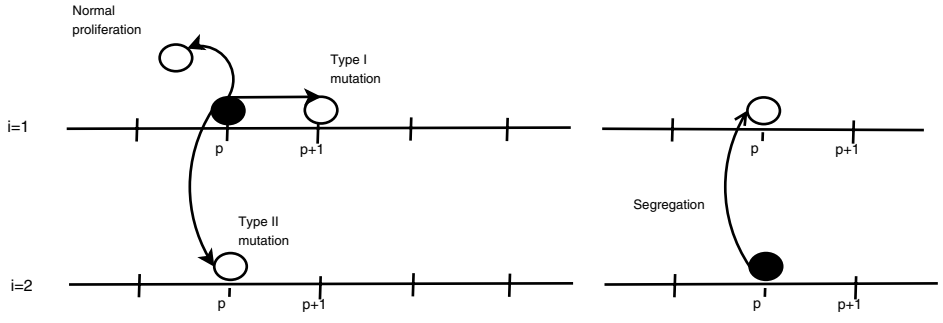


Fig. 3. Dynamics of proliferative events. (a) A candidate p-bacterium from subsystem 1 can divide into three ways: giving identical daughters with rate μ_0, becoming more aggressive (Type I mutation) with rate μ_1 or becoming resistant, i.e. daughters belong to functional subsystem 2 (Type II mutation) with rate μ_2. (b) Offspring of a resistant bacterium can lose the conferring genetic material through segregation with rate μ_{seg}.

that can occur involving several mechanisms [30] and it is assumed a constant low rate μ_{seg}.

Equations (3.6)–(3.8) model the above-described phenomena and Fig. 3 shows a schematic representation:

$$\mu_{1p}(ij) = \begin{cases} \mu_0, & i = 1,\ j = p, \\ \mu_1, & i = 1,\ j = p+1,\ p = 1, \ldots, m-1, \\ \mu_2, & i = 2,\ j = p, \end{cases} \tag{3.6}$$

$$\mu_{2p}(ij) = \begin{cases} \mu_0, & i = 2,\ j = p, \\ \mu_1, & i = 2,\ j = p+1,\ p = 1, \ldots, m-1, \\ \mu_{\text{seg}}, & i = 1,\ j = p, \end{cases} \tag{3.7}$$

$$\kappa_{3p}^{kq}(ij) = \kappa, \quad i = 3,\ j = p = q,\ k = 1, 2. \tag{3.8}$$

Destructive events: Regarding to destructive interactions, immune system is able to kill those bacteria that it is able to recognize. This is, bacterial cells with activity p can be contrasted and killed by immune cells with activity $q \geq p$:

$$\nu_{hp}^{3q} = \gamma, \quad q \geq p,\ \gamma > 0. \tag{3.9}$$

3.3. *External action dynamics*

This subsection is devoted to the action of antibiotic A over non-resistant bacteria. This constitutes not an easy task that is deeply studied by pharmacokinetics and pharmacodynamics [5, 26].

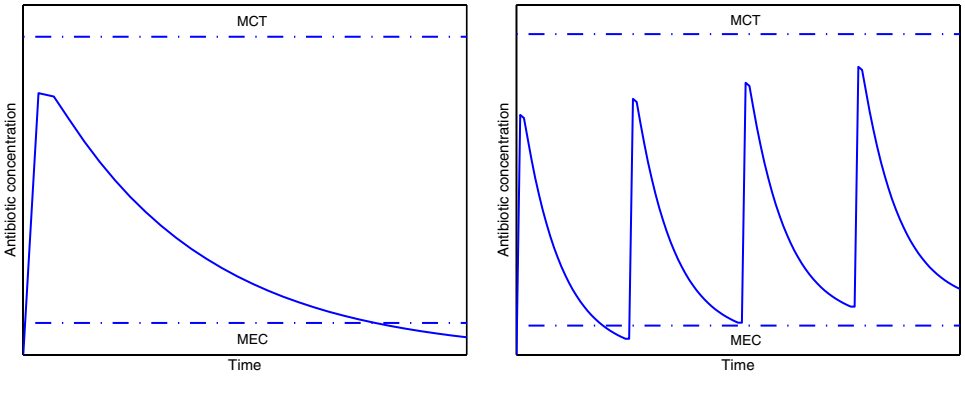
In a rather simple but also representative approach, as stated in the previous section, we assume that A is a concentration-dependent antibiotic, which means

that it achieves an increasing bacterial killing ability with increasing levels of drug. So, this killing ability of the drug described in Eq. (2.8) is increasing with respect to its instantaneous concentration $c(t)$ through the increasing function $g(c)$ and to its efficiency φ_A. In general, we can simply assume that g is the identity function, meaning that killing rate is proportional to c.

Moreover, for an extravascular administration of antibiotic in an *in vivo* patient, Bateman's equation provides an accurate description of the concentration of drug in the body:

$$c(t) = c_0\big(e^{-k_e t} - e^{-k_a t}\big), \tag{3.10}$$

where c_0 is the concentration of the supplied drug, k_a is the absorption rate and k_e is the elimination rate via renal excretion and hepatic metabolism. Here, we are considering a monocompartimental model, which assumes that blood plasma concentrations of the antibiotic are equal to the concentration in other fluids or tissues. In Fig. 4(a), we show Bateman's curve for a given choice of parameters. It can be observed that if drug is supplied at $t = 0$ there is an absorption period in which c grows until reaching the maximal concentration, and then c starts to decay due to excretion. In a clinical situation, however, drug is supplied many times at every given time interval. The dosage is the amount of drug needed to be administered to maintain the plasma concentration above the minimum effective concentration (MEC) and below the minimum toxic concentration (MTC), see Fig. 4(b) for a repeated dosage of antibiotic satisfying Bateman's kinetics. The area between both concentrations MTC and MEC is known as *mutant selection window*, and in practice it is desirable that both of them were close in order to prevent mutations.



(a) (b)

Fig. 4. (a) Antibiotic concentration for a dosage following Bateman's scheme. Drug is supplied at $t = 0$ and after a period of absorption in which maximum concentration is reached its concentration starts to decay due to elimination. Dashed lines correspond to the MEC and the MTC. (b) Bateman's scheme for multiple dose of antibiotic.

It is worth stressing, however, that physicians usually consider a simplified version of this curve that consists in a linear interpolation for each interval of increasing and decreasing concentration.

3.4. *Obtention of the explicit model*

We are now able to derive explicitly the system of equations describing the phenomena under consideration. Substitution of Eqs. (3.1)–(3.9) into Eqs. (2.3)–(2.8) and the general balance equation (2.2) leads to:

$$\frac{df_{1j}}{dt}(t) = \left(-\alpha\eta_0 n_2 + \mu_0 - \gamma\tilde{\eta}_0 \sum_{q=j}^{m} f_{3q}(t) - \varphi_A g(c(t))\right) f_{1j}(t) + \mu_1 \chi_{\{j\geq 2\}}(j) f_{1,j-1}(t) + \mu_{\text{seg}} f_{2j}(t), \tag{3.11}$$

$$\frac{df_{2j}}{dt}(t) = (\alpha\eta_0 n_2(t) + \mu_2) f_{1j}(t) + \left(\mu_0 - \gamma\tilde{\eta}_0 \sum_{q=j}^{m} f_{3q}(t) - \mu_{\text{seg}}\right) f_{2j}(t) + \mu_1 \chi_{\{j\geq 2\}}(j) f_{2,j-1}(t), \tag{3.12}$$

$$\frac{df_{3j}}{dt} = \beta\tilde{\eta}_0 \left(\chi_{\{j\geq 2\}}(j) f_{3,j-1}(t) \sum_{q=j}^{m} f_{kq}(t) - f_{3j}(t) \sum_{k=1}^{2} \sum_{q=j+1}^{m} f_{kq}(t)\right) + \kappa\tilde{\eta}_0 f_{3j}(t)(f_{1j}(t) + f_{2j}(t)) - \lambda(f_{3j}(t) - f_{3j}^0), \tag{3.13}$$

where χ denotes the indicator function. This system of ODEs must be coupled with initial conditions $f_{ij}(0) = f_{ij}^0$, $i = 1, 2, 3$, $j = 1, \ldots, m$. The biological meaning of the parameters introduced in the model are summarized in Table 1.

Table 1. Model parameters.

Parameter	Biological meaning
$\eta_0, \tilde{\eta}_0$	Interaction rates
α	Probability of occurrence of horizontal transfer from resistant to non-resistant bacteria
β	Probability of conservative progressions within immune cells
γ	Suppression rate
μ_0, κ	Normal proliferation rate of bacteria and immune cells
μ_1	Type I mutation rate: mutation leads to more aggressive bacteria
μ_2	Type II mutation rate: mutation leads to antibiotic resistant bacteria
μ_{seg}	Segregation rate of genetic material responsible for antibiotic resistance
λ	Relaxation of the immune system
φ_A	Antibiotic efficiency
c_0	Maximum attainable antibiotic concentration in Bateman's scheme
k_a, k_e	Absorption and elimination rates of antibiotic in Bateman's scheme

4. Numerical Simulations

In this section, we perform some numerical simulations and computational analysis of the model proposed in Sec. 3. The analysis is possible since the problems (3.11)–(3.13) is well-posed and has a unique large-time solution. We refer to [4] for a detailed proof of this fact that follows from the use of classical fixed point theorems for ODEs.

The aim of the simulations is to test the predictive ability of the model, to contrast the numerical results with empirical evidence and to explore some interesting emerging behaviors that particularly concern medical applications.

In particular, we consider the following two scenarios, depending on the ability of the body to produce a normal immune response following an exposure to an antigen, in this case pathogenic bacteria:

(a) *Immunocompetent patient*: This case corresponds to a young, healthy individual, without associated comorbidities (i.e. nondiabetic, non-hypertensive, etc.), whose immune system has the ability to detect and attack antigens normally. It is supposed that the individual is initially exposed to the bacterial pathogen and we study the temporal evolution of bacterial populations, according to the interaction between immune response and antibiotic effectiveness. In this case, the joint action of the antibiotic and immune system should keep under control the bacterial population.

(b) *Immunodeficient patient*: This scenario corresponds to an immunosuppressed patient, i.e. a patient such that his/her immune system is much weaker than in the previous case. Here, it is expected that the antibiotic effectiveness is the crucial factor to reduce the total bacteria count, without having much support from the immune system. In this case one would expect that if a resistant strain appears, then its population will grow without being completely depleted.

Simulations have been performed with the parameters in Table 1 taking different sets of values according to the specific case under consideration. Regarding to the initial conditions, in all the cases we consider an infection by non-resistant bacteria with the lowest activity value, while the population of resistant bacteria is set to zero. Immune system has only an initial sentinel state with cells belonging to the lowest activity value and it can be activated by more aggressive bacteria. Parameters have been chosen in such a way that time is measured in minutes, while cellular populations and antibiotic concentration are normalized with respect to the initial population and a maximum concentration $c_{\max}$, respectively.

Case I: *Immunocompetence*

As already stated, let us consider the case of a strong immune response. Basically, this feature is described — in the present model — by the probability of progression β and the relaxation factor λ. So, these are the two parameters that differ in both case studies. Simulations are developed for the following values of parameters: $\alpha = 10^{-4}$, $\beta_1 = 10^{-1}$, $\gamma = 5 \times 10^{-1}$, $\mu_0 = 10^{-2}$, $\mu_1 = 10^{-4}$, $\mu_2 = 10^{-6}$, $\kappa = 10^{-2}$,

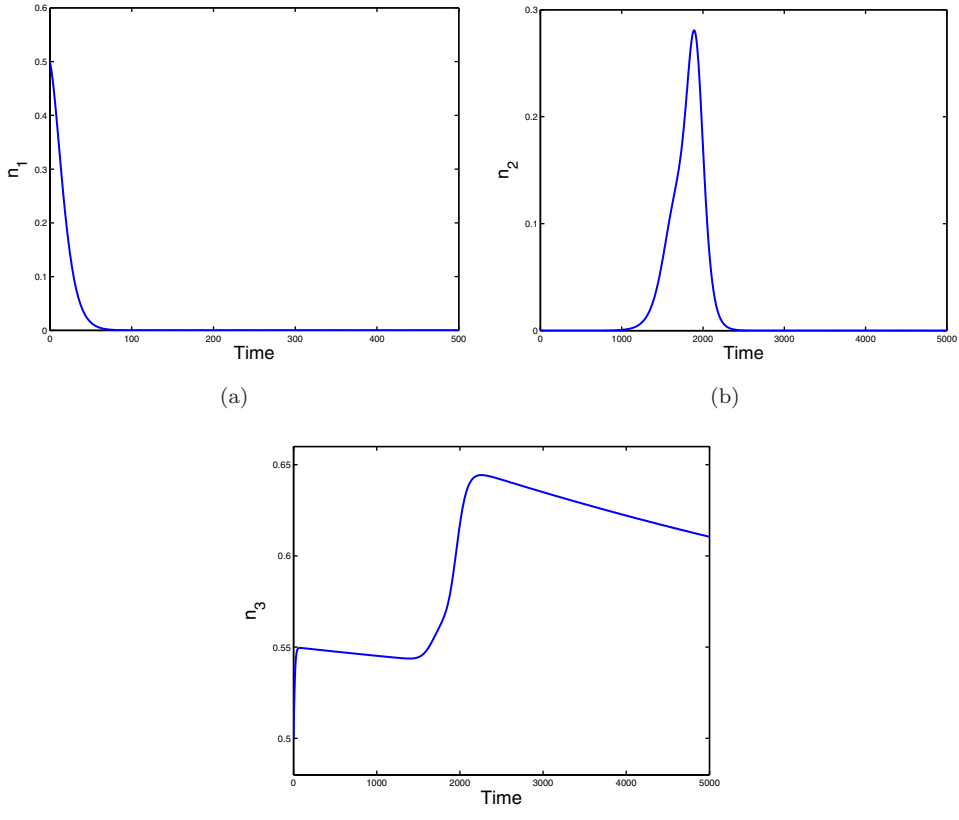


Fig. 5. Temporal evolution of (a) $n_1(t)$, (b) $n_2(t)$, and (c) $n_3(t)$ for an immunocompetent patient.

$\mu_{\text{seg}} = 10^{-4}$, $\lambda = 10^{-4}$, $\eta_0 = \tilde{\eta}_0 = 10^{-1}$ and antibiotic is supplied with a multiple Bateman's scheme with $c_0 = 1$ and efficiency $\varphi_A = 10^{-1}$ at every 8 h since the beginning of the infection, with absorption and elimination rates $k_a = 2.5\,\text{h}^{-1}$ and $k_e = 10^{-1}\,\text{h}^{-1}$, respectively, and g is the identity function.

Results are shown in Fig. 5. We can observe that the joint action of the antibiotic and the immune system quickly and completely depletes the non-resistant population of bacteria n_1. However, mutations and horizontal transfer let resistant bacteria grow. In this case, it is the immune strength that acts contrasting them, accomplishing its task in about two days from the beginning of the infection and of the treatment. From that moment, immune system begins to slowly relax to its sentinel state.

Case II: *Immunodepression*

This case considers a scenario in which the immune system has a rather weak capability to contrast the pathology. As already mentioned, this is reflected in the

fact that it has a reduced ability to learn, cells are prevented to increase their state toward the acquired immunity and relaxation is faster.

Simulations were performed with the same parameter values as in the previous case, except from those that weaken the immune response, namely $\beta = 5 \times 10^{-2}$ and $\lambda = 3 \times 10^{-2}$. In addition, different antibiotic efficiencies were considered and antibiotic was supplied with constant concentration $c = 0.1$.

Figure 6 shows how populations evolve for $\varphi_A = 0.2$ and $\varphi_A = 0.02$. It can be observed that in both cases bacteria are able to prosper under this weakened immune system. If the antibiotic is efficient enough, then it is able to deplete the non-resistant population, but resistant bacteria are generated and cannot be contrasted by the immune action. On the other hand, for a smaller antibiotic efficiency it is the first population that is the one not completely depleted. This is a really interesting result that is confirmed in Fig. 7, which shows the asymptotic values of both bacterial populations for different values of φ_A. An inspection of this figure

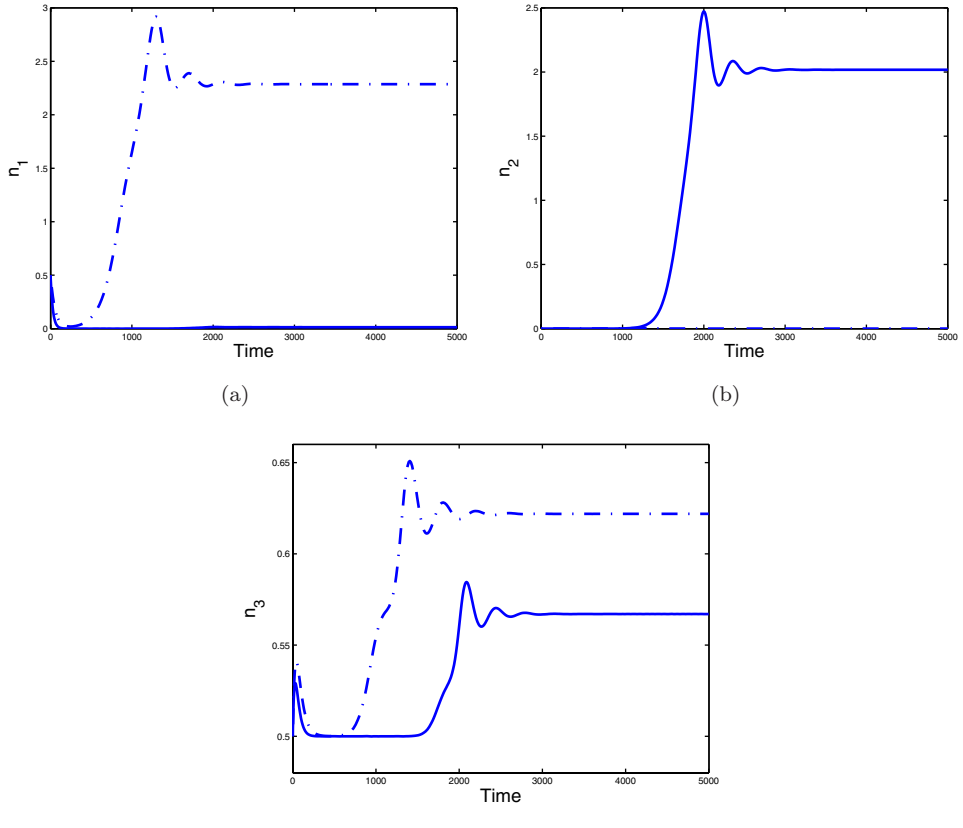
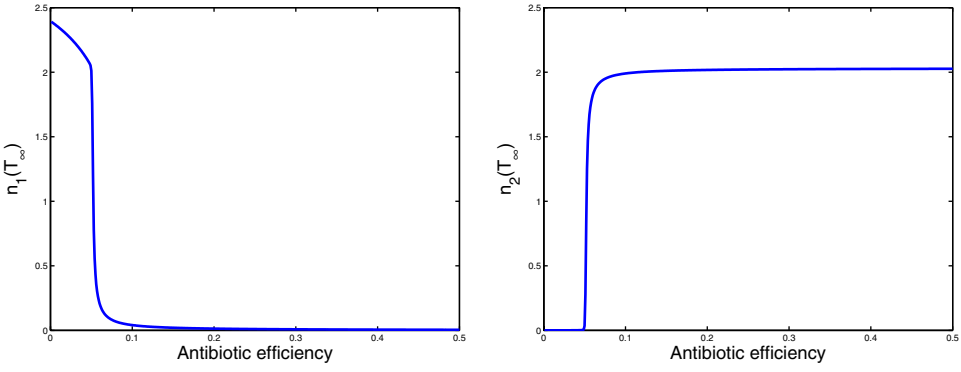


Fig. 6. Temporal evolution of (a) $n_1(t)$, (b) $n_2(t)$, and (c) $n_3(t)$ for an immunosuppressed patient for two different values of antibiotic efficiency. Continuous lines correspond to $\varphi_A = 0.2$ and dashed lines to $\varphi_A = 0.02$.



(a) (b)

Fig. 7. Asymptotic values of (a) non-resistant and (b) resistant bacterial populations for different values of antibiotic efficiency φ_A, for an immunosuppressed patient.

allows us to state that growth of resistant strains is a really serious matter, since immunodepressed patients will actually not be able to fight against the infection.

5. Conclusions and Looking Forward

A mathematical model for bacterial immune competition and antibiotic resistance has been proposed in this paper. The approach is based on tools of the KTAP that turns out to be useful in the modeling of complex living system composed of a large number of interacting particles. In particular, a main contribution of the present model is the mutations that bacteria undergo, modeled as Darwinian-type processes. In addition, horizontal transfer of genetic material between bacteria is considered, and it is claimed to be one of the most troubling issues in modern medicine. The numerical simulations performed for two particular case studies confirm the empirical evidence of this problem. Indeed, they clearly show that a bacterial infection may not be contrasted by the action of antibiotics only, but it also requires the immune response. However, if for any reason a patient has her immune system weakened, then antibiotics will be inefficient if bacteria had developed resistance.

Since the kinetic approach has shown to be accurate enough to take into account the complexity features of the system, it could eventually be used to model general horizontal genetic transfer between cells that is responsible not only for drug resistance acquisition processes, but is also important in other phenomena like adaptation, evolution and cooperation in ecology. Also, in genetic engineering, artificial horizontal transfer has so far been used to introduce genetic sequences into a wide variety of animal genomes. Moreover, some interesting problems for further research activity arise focusing not only on the mathematical but also on the medical fields. In the first case, much has to be done regarding to model validation, simulations and analytical aspects; while in the latter, it will be crucial to work on clinical data and the design of treatment protocols that can be numerically and inexpensively tested

in silico. Also, moving forward in further developments of this line of research, our model can be used to design treatment protocols by solving an optimal control problem: in other words, we ask whether it is possible to find an antibiotic dosing strategy such that: (i) antibiotic concentration levels remain always within the permitted limits (MCT, MECT) and (ii) maximizes its performance (killing rate) even when considering the acquired resistance.

In conclusion, the proposed model and simulations essentially support the idea that even with an efficient treatment, if bacteria acquire resistance, then they are able to grow in a suitable environment. It is true that some resistance occurs without human action, as bacteria can produce and use antibiotics against other bacteria, leading to a low level of natural selection for resistance to antibiotics. However, the current higher levels of antibiotic resistant bacteria are attributed to the overuse and abuse of antibiotics. In some countries and over the Internet, antibiotics can be purchased without a doctor's prescription. Patients sometimes take antibiotics unnecessarily, to treat viral illnesses like the common cold. Considering then that bacteria can spread from one individual to another, we uphold the major prevailing concern in the field of health, namely antibiotic resistance. From now on, it is of utmost importance to make renewed efforts to seek for new effective antibiotics against resistant pathogenic bacteria.

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References

[1] G. Ajmone Marsan, N. Bellomo and L. Gibelli, Stochastic evolutionary differential games toward a systems theory of behavioral social dynamics, *Math. Models Methods Appl. Sci.* **26**(6) (2016) 1051–1093.

[2] N. Bellomo, A. Ełaiw, A. M. Althiabi and M. A. Alghamdi, On the interplay between mathematics and biology: Hallmarks toward a new systems biology, *Phys. Life Rev.* **12** (2015) 44–64.

[3] N. Bellomo, D. Knopoff and J. Soler, On the difficult interplay between life, "complexity", and mathematical sciences, *Math. Models Methods Appl. Sci.* **13** (2013) 1861–1913.

[4] A. Bellouquid, E. De Angelis and D. Knopoff, From the modeling of the immune hallmarks of cancer to a black swan in biology, *Math. Models Methods Appl. Sci.* **23** (2013) 949–978.

[5] C. Beltrán, Pharmacokinetics and pharmacodynamics of antibiotics: Clinical usage, *Rev. Chil. Infect.* **21**(1) (2004) S39–S44.

[6] T. U. Berendonk *et al.*, Tackling antibiotic resistance: The environmental framework, *Nat. Rev. Microbiol.* **13**(5) (2015) 310–317.

[7] S. Bonhoeffer, M. Lipsitch and B. R. Levin, Evaluating treatment protocols to prevent antibiotic resistance, *Proc. Natl. Acad. Sci. USA* **94**(22) (1997) 12,106–12,111.

[8] D. Burini, S. De Lillo and L. Gibelli, Collective learning modeling based on the kinetic theory of active particles, *Phys. Life Rev.* **16** (2016) 123–139.

[9] T. Cohen and M. Murrat, Modeling epidemics of multidrug-resistant M. tuberculosis of heterogeneous fitness, *Nat. Med.* **10**(10) (2004) 1117–1121.
[10] M. J. Culyba, C. Y. Mo and R. M. Kohli, Targets for combating the evolution of acquired antibiotic resistance, *Biochemistry* **54**(23) (2015) 3573–3582.
[11] E. De Angelis and B. Lods, On the kinetic theory for active particles: A model for tumor-immune system competition, *Math. Comput. Model.* **47** (2008) 196–209.
[12] S. De Lillo, M. Delitala and M. C. Salvatori, Modelling epidemics and virus mutations by methods of the mathematical kinetic theory for active particles, *Math. Models Methods Appl. Sci.* **19**(1) (2009) 1405–1425.
[13] M. Delitala, P. Pucci and C. Salvatori, From methods of the mathematical kinetic theory for active particles to modelling virus mutations, *Math. Models Methods Appl. Sci.* **21** (2011), 843–870.
[14] M. Dolfin and M. Lachowicz, Modeling DNA thermal denaturation at the mesoscopic level, *Discrete Contin. Dynam. Syst. Ser. B* **19**(8) (2014) 2469–2482.
[15] M. Dolfin and M. Lachowicz, Modeling opinion dynamics: How the network enhances consensus, *Netw. Heterog. Media* **10** (2015) 877–896.
[16] J. Dou, Y. Jiang, J. Xie and X. Zhang, New is old, and old is new: Recent advances in antibiotic-based, antibiotic-free and ethnomedical treatments against methicillin-resistant *staphylococcus aureus* wound infections, *Int. J. Molec. Sci.* **17**(5) (2016) E617.
[17] B. B. Finlay and G. McFadden, Anti-immunology: Evasion of the host immune system by bacterial and viral pathogens, *Cell* **124**(4) (2006) 767–782.
[18] J. C. Galán, M. R. Baquero, M. I. Morosini and F. Baquero, High mutation rate bacteria: Risks of a high-speed life, *Infectio* **10**(1) (2006) 22–29.
[19] N. Galtier, A model of horizontal gene transfer and the bacterial phylogeny problem, *Syst. Biol.* **56**(4) (2007) 633–642.
[20] P. F. Harrison and J. Lederberg (eds.), *Antimicrobial Resistance*: *Issues and Options* (National Academic Press, 1998).
[21] D. Knopoff, On the modeling of migration phenomena on small networks, *Math. Models Methods Appl. Sci.* **23**(3) (2013) 541–563.
[22] D. Knopoff, On a mathematical theory of complex systems on networks with application to opinion formation, *Math. Models Methods Appl. Sci.* **24** (2014) 405–426.
[23] S. M. Krone, R. Lu, R. Fox, H. Suzuki and E. Top, Modelling the spatial dynamics of plasmid transfer and persistence, *Microbiology* **153**(8) (2007) 2803–2816.
[24] C. Lagido, I. J. Wilson, L. A. Glover and J. I. Prosser, A model for bacterial conjugal gene transfer on solid surfaces, *FEMS Microbiol. Ecol.* **44**(1) (2003) 67–78.
[25] B. R. Levin and F. M. Stewart, The population biology of bacterial plasmids: *A priori* conditions for the existence of mobilizable nonconjugative factors, *Genetics* **94**(2) (1980) 425–443.
[26] M. E. Levison and J. H. Levison, Pharmacokinetics and pharmacodynamics of antibacterial agents, *Infect. Diseases Clin. N. Amer.* **23**(4) (2009) 791–815.
[27] J. Mao and T. Lu, Population-dynamic modeling of bacterial horizontal gene transfer by natural transformation, *Biophys. J.* **110**(1) (2016) 258–268.
[28] E. Mayr, *Populations*, *Species*, *and Evolution*: *An Abridgment of Animal Species and Evolution* (Harvard University Press, 1970).
[29] S. E. McGinty, D. J. Rankin and S. P. Brown, Horizontal gene transfer and the evolution of bacterial cooperation, *Evolution* **65**(1) (2011) 21–32.
[30] S. Million-Weaver and M. Camps, Mechanisms of plasmid segregation: Have multicopy plasmids been overlooked? *Plasmid* **75** (2014) 27–36.

[31] M. A. Nowak, *Evolutionary Dynamics* (Belknap Harvard University Press, Cambridge, MA, 2006).
[32] L. Pray, Antibiotic resistance, mutation rates and MRSA, *N. Educ.* **1**(1) (2008) 30.
[33] W. A. Rosche and P. L. Foster, Determining mutation rates in bacterial populations, *Methods* **20**(1) (2000) 4–17.
[34] S. J. Sørensen, M. Bailey, L. H. Hansen, N. Kroer and S. Wuertz, Studying plasmid horizontal transfer *in situ*: A critical review, *Nat. Rev. Microbiol.* **3**(9) (2005) 700–710.
[35] F. M. Stewart and B. R. Levin, The population biology of bacterial plasmids: *A priori* conditions for the existence of conjugationally transmitted factors, *Genetics* **87** (1977) 209–228.
[36] The Global Risks Report 2016, 11th edn., World Economic Forum (2016), http://wef.ch/risks2016.
[37] C. von Wintersdorff, J. Penders, J. van Niekerk, N. Mills, S. Majumder, L. van Alphen, P. Savelkoul and P. Wolffs, Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer, *Front. Microbiol.* **7** (2016) 173.