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A Multi-Type Branching Model with Varying Environment for Bacterial Dynamics with Postantibiotic Effect

Patricia Geli*

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Abstract

In this paper, a multi-type branching process with varying environment is constructed for describing the growth of bacterial populations under treatment of antibiotics. The model captures the phenomenon of delayed growth, postantibiotic effect (PAE).

PAE is the phenomenon of continued suppression of bacterial growth after a short exposure of bacteria to antimicrobial agents.

The clinical implication of long PAEs lies in the possibility of increasing the intervals between drug administrations, thus allowing for fewer daily doses and thereby potentially reducing treatment costs, increasing patient compliance and decreasing drug exposure.

In spite of the increasing interest in the PAE as an important parameter for the dosage and frequency of administration of a drug, knowledge on this phenomenon is still incomplete.

The model is applied to data from an in vitro study with *E. coli* exposed to different dosing regimens of antibiotics.

The model and results provide a common framework to better understand bacterial populations evolving under different selection pressures.

KEY WORDS: Antibiotic resistance, multi-type branching processes, varying environment, penicillin binding proteins, postantibiotic effect.

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1 Introduction

Postantibiotic effect[1] (PAE) is the phenomenon of continued suppression of bacterial growth after a short exposure of bacteria to antimicrobial agents ([3], [20]).

The clinical implication of long PAEs lies in the possibility of increasing the intervals between drug administrations, thus allowing for fewer daily doses without the loss of therapeutic efficacy [5]. Extended dosing intervals of an antimicrobial has several potential advantages, among them reduced cost, less toxicity, and better compliance among outpatients receiving antimicrobial therapy, which in turn reduces the risk for selection of resistance due to suboptimal doses of antibiotics.

The therapy of patients with tuberculosis, is one example where non-compliance with anti-tuberculosis drug therapy has been recognised as a major cause of treatment failure, drug resistance and relapse [2]. Hence, in managing patients with tuberculosis, administration of drugs at intermittent intervals would reduce cost and possibly toxicity of drugs, as well as enhance adherence through greater feasibility of directly observed therapy [10].

Although there is an increasing interest in the PAE as an important parameter for the dosage and frequency of administration of a drug, knowledge on this phenomenon is still incomplete. The aim in this paper is therefore to construct a stochastic model that describes the dynamics of a bacterial population under the influence of different dosing regimens, which correctly takes into account the PAE. The PAE is probably the result of several mechanisms. One explanatory theory for PAE is that it represents the time required for synthesis of new penicillin binding proteins (PBP), before growth of bacteria ([13],[19],[21]). To describe the PBP dynamics, a model previously described in [1] was used. In reality however, PBP dynamics is difficult to observe directly, and hence we have to rely on the data observed in terms of PAE of the bacterial populations. Therefore to capture the PBP dynamics in the dynamics of the bacterial population, a multi-type branching process (MBP) model with varying environments is used.

The PAE is influenced by several factors, including the microorganism, the inoculum (initial population size), the type of antibiotic, the concentration

[1] In vitro, the PAE is typically measured as the delayed bacterial growth after a short on-off exposure to an antibiotic for 1 or 2 h [6]. Such exposure does not reflect the situation in humans under clinical conditions, where bacteria are exposed to antibiotic concentrations that decline only slowly over time, with half-lives of up to several hours [8]. To capture the additional effects from a varying concentration which might at some time fall below the minimum inhibitory concentration (MIC), the term post-MIC effect (PME) is used [14]. For convenience, we will in this paper use the common term PAE to refer to the continued suppression of bacterial growth after any kind of exposure to antimicrobial agents.

of antibiotic, and the duration of exposure [4]. In this paper data from in vitro experiments with *E. coli* subject to different antibiotic dosing regimens of cefotaxime was used to compare and validate the model. The background of the experiments has been described in detail in [12].

Apart from serving as a theoretical framework for understanding the dynamics influence between different dosing regimens (PAE), the model may also be useful to explore optimal dosing regimens. Furthermore, it highlights the importance of taking the stochasticity into account in pharmacokinetic/pharmacodynamic models.

2 Model description

The objective is to construct a model for the bacterial population dynamics under treatment of antibiotics that also explains the phenomenon of delayed regrowth after antibiotics has declined to subinhibitory levels, the PAE.

Branching processes is a convenient class of models for the dynamics of bacterial populations, which consist of only one type of bacteria, each having the same probability for cell division [15]. In order to capture the delayed regrowth of bacteria in the model, we will rely on the theory that the PAE corresponds to the time required for synthesis of new unsaturated PBPs, sufficient for cell division ([13],[19],[21]).

In this paper, we will assume a special case where the probability for cell division of a bacterium depends on the level of saturation of antibiotics. So, assuming that a bacterium has a fixed total number of PBPs, n, there will be $n+1$ different possible levels of saturation in the bacterial population. In other words, the bacterial population will consist of $n+1$ types of bacteria determined by the number of saturated PBPs. The type of bacteria will affect the distribution of the number of offspring and therefore we will describe the reproduction using the theory of multi-type branching processes (MBP).

Let us start by introducing some notation. The population in generation m is characterized by a vector $\mathbf{Z}_m = (Z_{m0}, ..., Z_{mn})$, where Z_{mj} denotes the number of bacteria of type j, i.e. bacteria having j saturated PBPs in the mth generation. Realistically the total number of PBPs (n) is between 100-1000.

Now, the total population size in generation m, $|\mathbf{Z}_m|$, is the sum of the vector components

$$|\mathbf{Z}_m| = Z_{m0} + \ldots + Z_{mn}.$$

Now, let $\xi_{mjk}^{(i)}$ denote the number of offspring of type j in generation m, given by bacterium k of type i. Then, by summing the number of children given by parent k with type i in generation m, the population size of type j in the $(m+1)$th generation is

$$Z_{m+1,j} = \sum_{i=0}^{n} \sum_{k=1}^{Z_{mi}} \xi_{mjk}^{(i)}$$

and the total population size in generation $m+1$ can now be expressed by

$$|\mathbf{Z}_{m+1}| = \sum_{j=0}^{n} Z_{m+1,j}.$$

For a complete model we need a specification of the distribution of the offspring vector for each type distinguished.

Assume that each bacteria lives for a fixed time τ and that there is no overlapping generations. Hence, we consider a process in discrete time at the time points $t = 0, \tau, 2\tau, ...$ One generation of a bacterium will be defined by three phases, saturation, reproduction and distribution of saturated PBPs among its offspring. These three events are described below in more detail.

Before we continue, we will need some notation for the number of saturated PBPs that a bacterium has in each of the three phases. Let us therefore introduce the following stochastic variables:

$Y_1^{(m)}$ = Number of saturated PBPs of one bacterium at the beginning of its generation. Also equal to the type.

$Y_2^{(m)}$ = Number of saturated PBPs at the end of its generation before reproduction.

1. **Saturation process** As long as the antibiotic concentration is positive, we will assume that each generation starts with a saturation process (unsaturated PBP may become saturated). So, given that a bacterium starts with i saturated PBPs, we will have to formulate a probability of having u saturated PBPs at the end of its lifetime (after the saturation process).

   In [1], a stochastic model for the background mechanism of PAE was constructed. This model included the process of synthesis, saturation and death of PBPs as a function of the concentration dynamics. According to this model, newly synthesized PBPs are assumed to be created with an intensity β. PBPs which are unsaturated will eventually

become saturated with an intensity $\gamma c(t)$, where $c(t)$ denotes the antibiotic concentration at time t, and finally saturated PBPs will eventually become removed from the bacteria with an intensity μ. See Figure 1 for a schematic picture of the model.

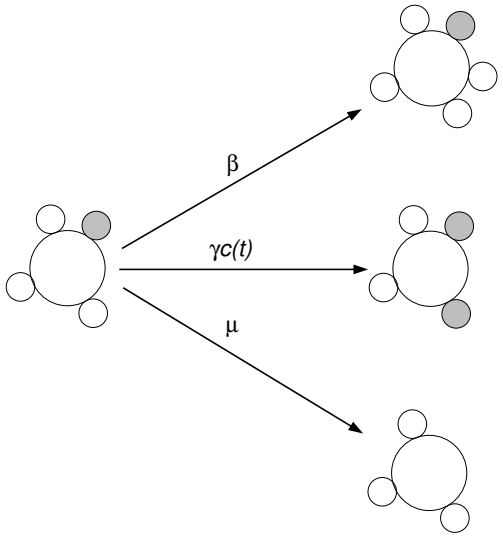

Figure 1: A schematic picture of the general model. PBPs are created (upper arrow) with an intensity β, they become saturated (middle arrow) with an intensity $\gamma c(t)$ and they are removed (lower arrow) from the bacterium with an intensity μ. In this paper it is assumed that only saturation (middle arrow) is possible.

In this paper we will for simplicity consider a special case of the model when there is no new synthesized PBPs and no death of PBPs, only the saturation of already existing PBPs is described. For the further development of the model, the probability of cell division will depend on the level of saturation for in each bacterium. Hence, the constants β and μ are 0. Under these assumptions, we are interested in the probability distribution $p_{iu}(m)$, which denotes the probability that a bacterium with $Y_1^{(m)} = i$ PBPs in generation m, will have $Y_2^{(m)} = u$ after the saturation.

How likely the PBPs are to become saturated after adding antibiotics to the system depends on the dosing regimen of antibiotics, i.e. the concentration at time t expressed by $c(t)$. Each bacterium have a fixed total number of PBPs (n), each assumed to become saturated independently with identical saturation probabilities given the generation. This implies that $p_{iu}(t)$ can be regarded as a result of $n - Y_1^{(m)}$ independent trials, where the probability of having success (i.e. saturation) at each trial is denoted $\pi(t_1, t_2)$. In other words, the number of unsaturated PBPs that have become saturated during one generation is described by the following binomial distribution,

$$(Y_2^{(m)} - Y_1^{(m)})|Y_1^{(m)} = i \sim \text{Bin}(n-i, \pi(m\tau, (m+1)\tau)). \quad (1)$$

Thus,

$$\begin{aligned} p_{i,u}(m) &= P(Y_2^{(m)} - Y_1^{(m)} = u - i | Y_1^{(m)} = i) \quad (2) \\ &= \frac{(n-i)!}{(u-i)!(n-u)!} \pi(m\tau, (m+1)\tau)^{u-i} (1 - \pi(m\tau, (m+1)\tau))^{n-u}, \end{aligned}$$

where $0 \leq i \leq u \leq n$.

Since the concentration of antibiotics is allowed to vary with time, the probability of saturation will also depend on the time t. The dependency on time is described by the following differential equation

$$\frac{d\pi(t_1, t_2)}{dt_2} = -\gamma c(t_2) \pi(t_1, t_2). \quad (3)$$

Let us denote the initial antibiotic concentration added to a system with C_0. The most common dosing regimen of in vitro studies is letting the concentration remain constant throughout the experiment, $c(t) = C_0$. In this case, solving Equation (3), yields

$$\pi(t_1, t_2) = 1 - e^{-\gamma C_0 (t_2 - t_1)}.$$

A more realistic situation, considering the dynamics of human pharmacokinetics is that of exponentially declining concentration.

If instead the initial dose C_0 is declining exponentially so that $c(t) = C_0 e^{-kt}$, the probability of saturation is given by

$$\pi(t_1, t_2) = 1 - \frac{e^{\frac{\gamma C_0 e^{-kt_2}}{k}}}{e^{\frac{\gamma C_0 e^{-kt_1}}{k}}}.$$

This is the case we will consider throughout this paper.

2. **Reproduction**

At the end of the lifetime, a bacterium either reproduces (creating two offspring) or dies (yielding no offspring).

The conditional probability that a bacterium does not reproduce, given that it has $Y_2^{(m)} = u$ saturated PBPs only depends on the number u. It will be given by the following probability

$$q_u = 1 - P(\text{cell division} | Y_2^{(m)} = u)$$

and the probability for reproduction is hence $(1 - q_u)$.

The probability for death is assumed to follow a generalized logistic (also called Richard's) model [16].

$$q_u = k_1 + \frac{k_2}{(1 + se^{-b(u-a)})^{1/s}} \tag{4}$$

The more saturated a bacterium is, the higher the probability will be for cell death.

The motivation for choice of model is the flexibility, which allows us to control the asymptotes, its position and slope. One example of how the generalized logistic curve might look like (with parameters as presented in Section 4) is shown in Figure 2.

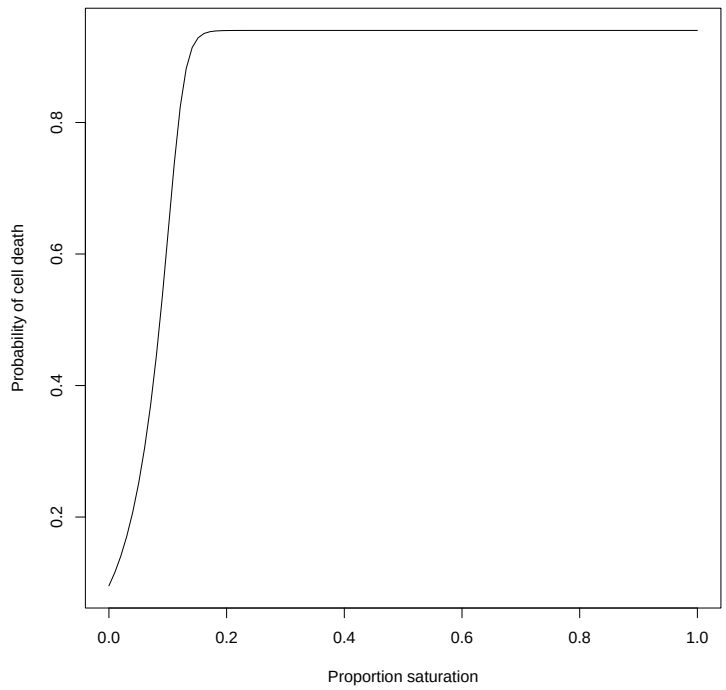

Figure 2: One example of how the generalized logistic curve might look like for an example with parameters as presented in Section 4. The plot shows how the cell death probability varies with the level of saturation.

3. Distribution of saturated PBPs of the parent among its offspring

In case of cell division after the second phase of the cell cycle, the parent dies and produces two offspring having $Y_1^{(m+1)}$ and $Y_2^{(m)} - Y_1^{(m+1)}$ saturated PBPs, respectively and otherwise dies with no offspring produced.

Each offspring will still have a total number n of PBPs, but the number of saturated PBPs of the parent will be randomly distributed among the two offspring. We will denote the conditional probability $r_{u,j}$ of having an offspring with $Y_1^{(m+1)} = j$ saturated PBPs starting its life in generation $m+1$, given that it had a parent with $Y_2^{(m)} = u$ saturated PBPs. We will assume that this follows a hypergeometric distribution. Hence,

$$Y_1^{(m+1)}|Y_2^{(m)} \sim \text{HypGeo}(2n, n, u)$$

which implies that

$$r_{u,j} = P(Y_1^{(m+1)} = j|Y_2^{(m)} = u) = \frac{\binom{2n-u}{n-j}\binom{u}{j}}{\binom{2n}{n}}. \tag{5}$$

with $0 \leq j \leq u \leq n$.

An illustration of how different types of bacteria in the case of three possible types may evolve is shown in Figure 3.

3 Reproduction numbers

We now wish to say something about how the bacterial population will evolve. Many interesting properties describing the population development is determined by the mean reproduction. In Equation (2) we stated that given the size of the preceding generation, the present population size is the sum of the number of offspring of each potential parent and type. Because antibiotic concentration is varying with time, the expectation of these numbers will vary from generation to generation. For this reason, we will introduce a matrix with entry, $\mu_{ij}(m)$, for an i-bacteria's expected number of offspring of type j in generation m. We will refer to this as the mean matrix and denote it by

$$\mathbf{M}(m) = \{\mu_{ij}(m)\}_{i,j=1}^{d}.$$

By the probabilities defined in Section 2 for saturation, reproduction and distribution of saturated PBPs among offspring in Equations (2), (4) and (5), respectively, together with the law of total probability, it follows that

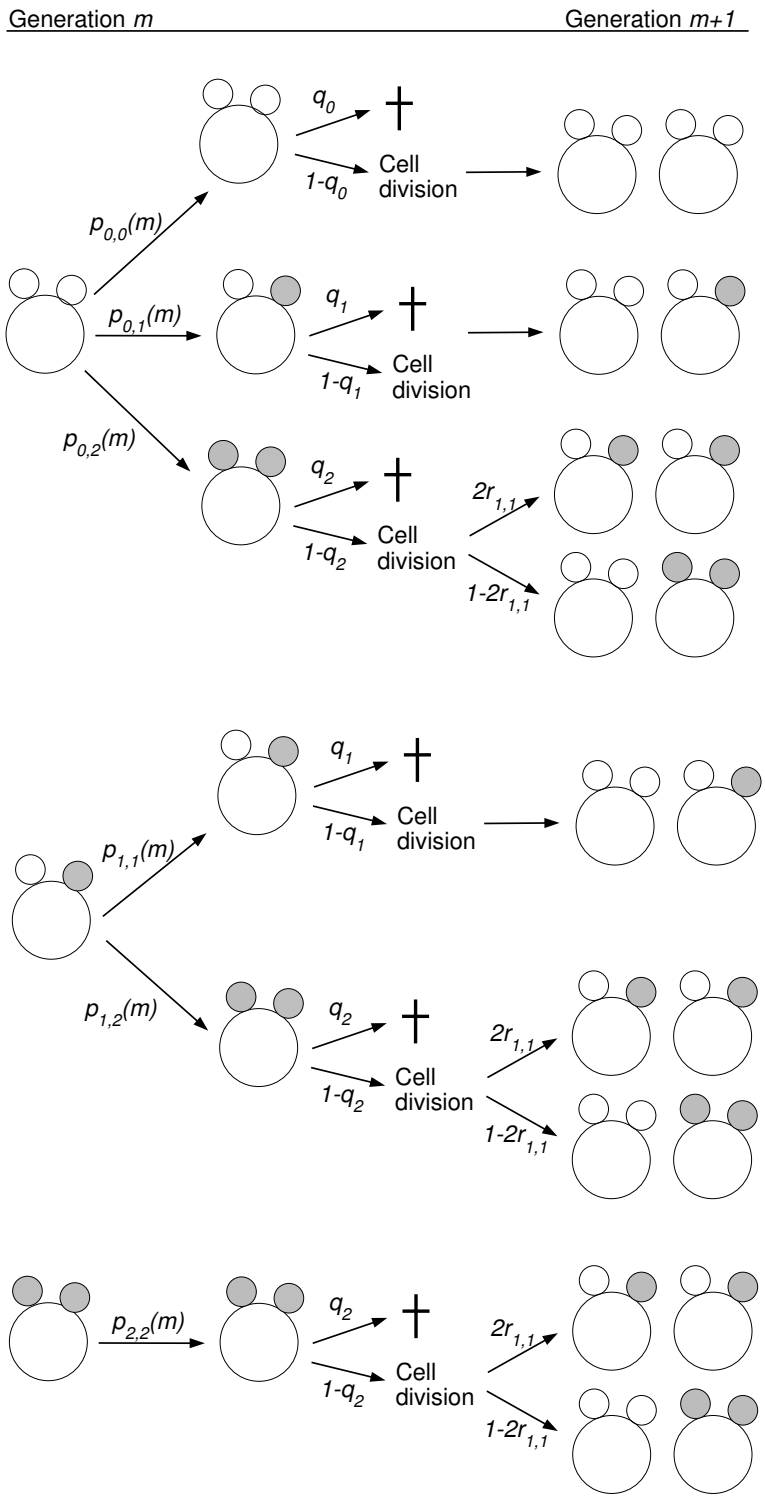

Figure 3: Illustration of possible outcomes from one generation to another for the three different types of bacteria in the case when $n = 2$. Each bacterium lives for a fixed generation life-time and may undergo a change to another type during this time. At the end of the generation the cell either dies with no outcome or generates two offspring.

$$\mu_{i,j}(m) = \sum_{v=i}^{n} p_{i,v}(m)(1-q_v)(r_{v,j} + r_{v,v-j}).$$

The intuitive understanding for the last term, $r_{v,j} + r_{v,v-j}$, in $\mu_{i,j}$ is given by the following: If a parent, which has v saturated PBPs when it splits, gets one child with j saturated PBPs, then the other child must have $v - j$. Also the other way around holds, if a the parent gets one child with $v - j$ saturated PBPs, then the second child must have j saturated PBPs. Hence, we get a contribution to the expected number of j-children both from the probability of having one j-child, $r_{v,j}$, and from the probability of having one $v - j$-child, $r_{v,v-j}$.

Let us introduce the following notation

$$E[\mathbf{Z}_m] = (E[Z_{m0}], \ldots, E[Z_{mn}])^T$$

where T denotes the transpose. Then the relations in vector-matrix form is,

$$E[\mathbf{Z}_m]^T = \mathbf{M}(m)E[\mathbf{Z}_{m-1}]^T$$

and

$$E[\mathbf{Z}_m]^T = \mathbf{M}(m)\mathbf{M}(m-1)\cdots\mathbf{M}(1)E[\mathbf{Z}_0]^T, \tag{6}$$

and since the initial population size, the inoculum, is known, $E[\mathbf{Z}_0]$ can be replaced by $\mathbf{Z}_0$.

4 Data and parameter values

As mentioned in Section 1, there are several factors affecting the PAE. The presence or duration of PAE can differ significantly for specific antimicrobial/organism combinations [6].

The model described in Section 2 can be used for any antimicrobial-organism combination, where the PAE can be defined as above (the time required for synthesis of new PBPs before growth). The parameters in the model will however differ depending on the antimicrobial-organism combination and in order to get any practical use of the model, we have to determine the values of the parameters in the model. For this purpose we use data from a set of in

vitro kinetic experiments with *E. coli* strains which were exposed to different dosing regimens of *cefotaxime*. The data is described in more detail in [12].

In some of the experiments described in [12], the appearance of mutants was shown. Therefore, to avoid the unnecessary impact of other factors not considered in this model, we chose data from the experiments were the mutants were less likely to appear. These experiments will be referred to as, A-F. The initial concentration and half-life associated with each of these trials are presented in Table **??** and in Figure 4 the resulting concentration lapse of the experiments is shown.

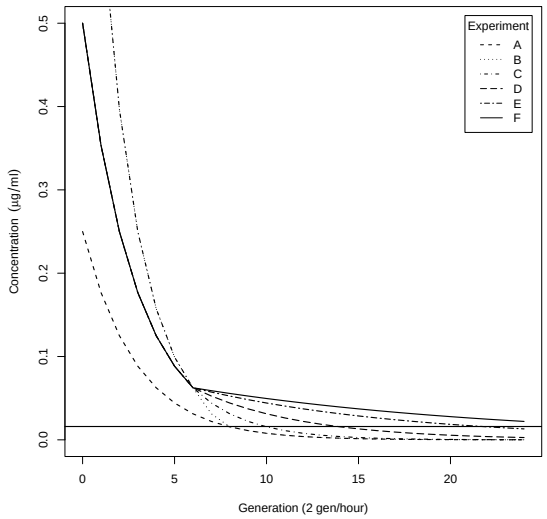

Figure 4: Concentration profiles of cefotaxime for experiments A-E. The horizontal line represents the MIC.

Furthermore, for the purpose of this paper, we only use data for one of these strains (TEM-1). This strain has a minimum inhibitory concentration (MIC) of 0.012 mg/L, which means that the bacterial population, if no PAE is present, would be expected to grow after the antibiotic concentration has declined below this level.

The parameters were estimated using data from experiment C and D, and the experiments E and F were used to validate the model. Additionally, data from experiments without any pressure of antibiotics, see Figure 5 [2] were used for validation. These data gives a check of the maximal growth rate for bacteria without any saturated PBPs.

[2] The time-delay seen in the figure for the growth of bacteria is perhaps a result of the time it takes for bacteria to adapt to the new milieu. The maximal cell division probability is therefore compared to data between 2 and 8 hours.

Table 1: The initial concentration, C_0, the half-life of he concentration, $T_{1/2}$, and the inoculum, Z_{00} (all bacteria are assumed to be unsaturated, and hence of type 0 at the beginning of the experiment), that were used in each of the experiments A-F. The half-life of antibiotics, $T_{1/2}$, is related to the elimination rate, k, by $k < -ln(2)/T_{1/2}$.

Experiment	C_0 (μ g/ml)	$T_{1/2}$ (h)	Z_{00} (log_{10} cfu/ml)
A	0.25	1 (0-12)	5.31
B	1	0.7 (0-3), 0.5 (3-4), 1 (4-12)	5.05
C	1	0.75 (0-3), 1 (3-12)	5.1
D	0.5	1 (0-3), 2 (3-12)	5.17
E	0.5	1 (0-3), 4 (3-12)	5.24
F	0.5	1 (0-3), 6 (3-12)	5.23

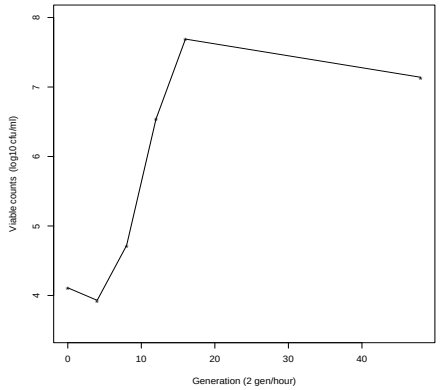

Figure 5: Control curve for growth rate without antibiotics. The time-delay for the growth of bacteria seen between $0-4$ in the graph is perhaps a result of the time it takes for bacteria to adapt to the new milieu. The decrease in the bacterial growth after 24 generations is likely due to limited space in the system.

Due to the extensive amount of time required for numerical calculations with the number of PBPs $n > 10$, the main results will be based on the assumption that $n = 10$, as compared to the realistic number which is between 100-1000.

The parameters γ, k_1, k_2, a, b and s were estimated by minimizing the mean-square errors of the log_{10}-counts of data compared to the corresponding expected value of the bacterial population which was defined in Equation (6). The optimization was made using the optim-routine in R (version 2.5.0) [7].

The following estimates were obtained: $\hat{\gamma} = 1.57$, $\hat{k}_1 = 1.41 \cdot 10^{-5}$, $\hat{k}_2 = 0.94$, $\hat{a} = 1.02$, $\hat{b} = 8.88$ and $\hat{s} = 4.63$. With these estimates the maximal growth

probability $1 - q_0$ achieved from Equation 2 is 0.90, which fits the growth curve of bacteria in absence of any drug effect Figure 5 rather well (fit not shown).

5 Numerical results and simulations

Using the parameters estimated from data from the experimental settings B and C, the expected bacterial growth (based on Equation 6) has been calculated. The results of these calculations are shown in Figure 6.

The data in experiments B, C and D are rather well predicted by the expected outcome of the model. However, in experiments E and F the deviation is larger. In order to get an idea of how much of the deviation that could be explained by the model variance we simulated the outcome ten times.

Experiment A was repeated ten times and in order to investigate how much of the variation seen in the experiments (see Figure 6), we simulated the outcome of the MBP model for the experimental setting A using the parameter estimates from Section 4.

In Figure 7, both data from the ten replicates of experiment A are shown and the result of ten simulations for this experiment are shown.

As seen from the simulated data in Figure 7, the behavior is more or less deterministic before the turning-point to growth. At the turning-point, the counts of the bacterial population are close to the detection limit of 10 bacteria and all variation explained by the model occurs after this turning-point from negative to positive growth. The variation seen in data before the turning-point and partly after the turning-point is perhaps a results of several factors. It is not possible to start with exactly the same inoculum in different repetitions of an experiment. In these repetitions the inoculum varied in the range of $10^{4.94} - 10^{5.35}$ and hence some of the variation before the turning point seen in the data, but not explained by the model is perhaps a result of the varying inoculum. Other variation that might be associated with the experimental methods, such as variation in the initial concentration and the half-life of the concentration, is also not explained by the model.

In another simulation of experiment D (see Figure 8), we see a larger variation after the turning-point as compared to the variation for the simulations of experiment A. In experiment D, the concentration is still relatively high around the turning-point as compared to experiment A and hence the chance that the bacterial population therefore dies out is higher. The bacterial population died out in 7 simulations out of 10 for experiment D, as compared to experiment A in which the bacterial population survived in all simulations.

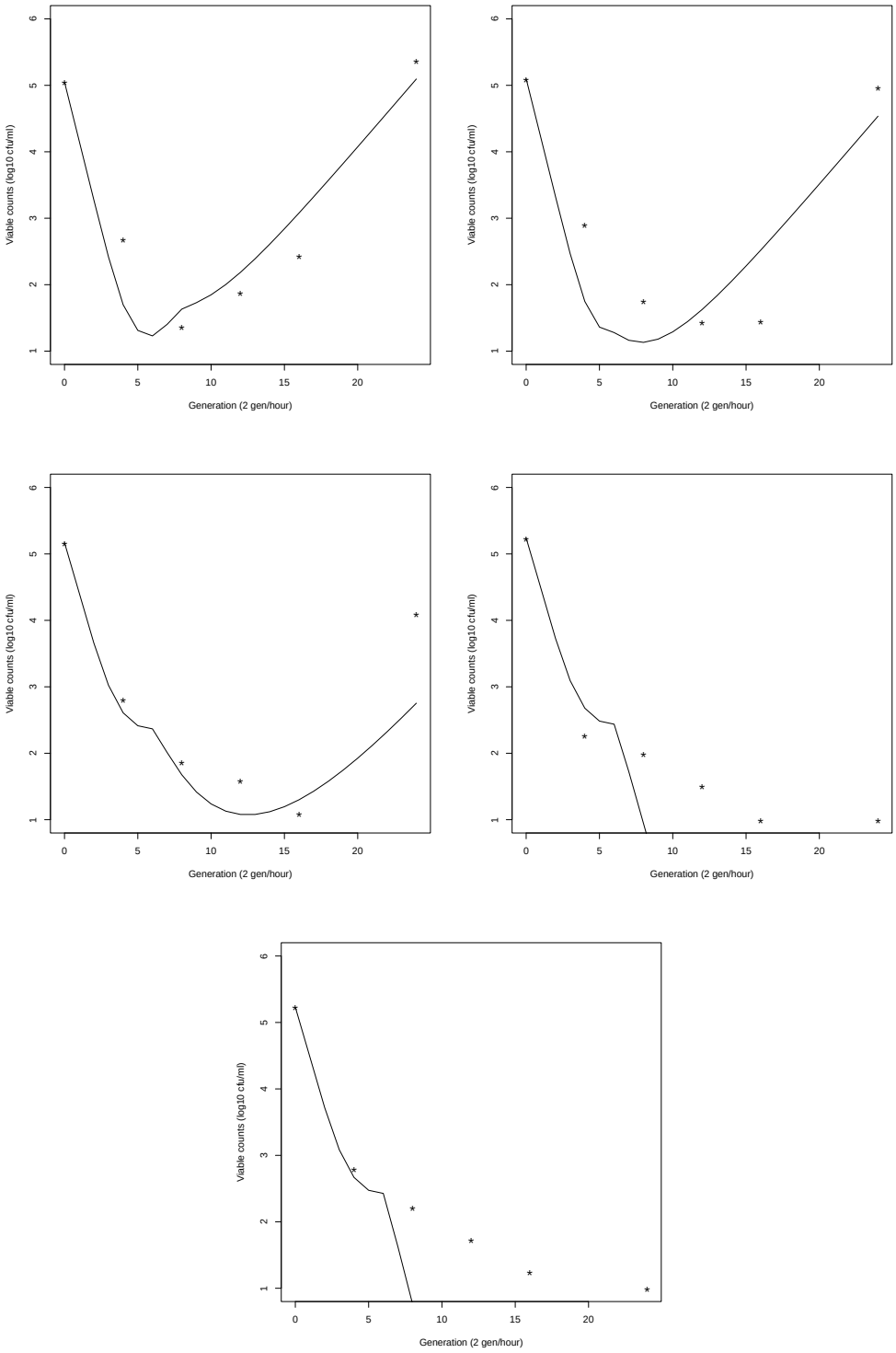

Figure 6: Predicted and experimental data from experimental settings B-F seen from upper left to lower right figure.

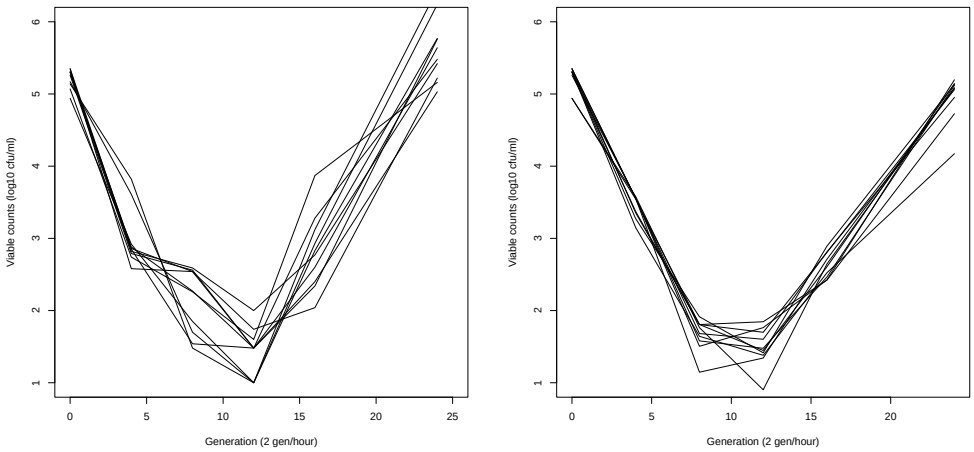

Figure 7: Left: Data from ten repetitions of experiment A. Right: Data from 10 simulated outcomes of experiment A.

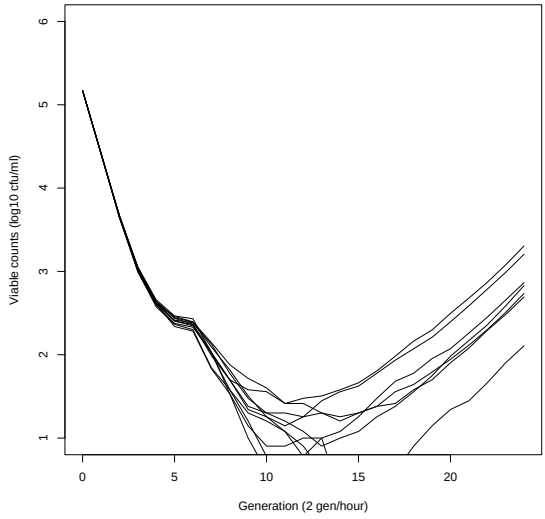

Figure 8: Data from 10 simulated outcomes of experiment D.

6 Discussion

In drug therapy with antibacterial drugs, effective dosage strategies are needed to maintain target drug effects. Mathematical models for the relationship between drug dose and drug effect, so called pharmacokinetic/pharmacodynamic (PK/PD) models, has been studied extensively over the years ([9], [11], [17]).

In this study, we have presented a stochastic model for describing the growth of bacterial populations under treatment of antibiotics, which also captures the phenomenon of delayed growth, the PAE. The model is a multi-type branching process with varying environments and the bacterial growth in this model depends on the saturation of PBPs, which in turn depends on the antibiotic concentration in the system. The main difference from previously published papers on PK/PD models is 1) the stochasticity and 2) the possibility of a delayed effect, PAE. To our knowledge, this is the first stochastic model presented for describing the phenomena PAE.

The model was constructed to be used for any antimicrobial-organism combination, where the PAE can be explained by the theory that the PAE represents the time required for synthesis of new PBPs before growth. However, in this paper, data from a set of in vitro kinetic experiments with *E. coli* strains which was exposed to different dosing regimens of *cefotaxime* was used to test and validate the model.

These experiments were not originally designed for discovering how the PAE varies with different dosing regimens and therefore a range of other factors than the PAE might influence the outcome of the data seen from the bacterial experiments.

In the case of no PAE, the bacterial population would grow immediately after concentrations have declined below the so called MIC. By subtracting the times for which MIC was reached in the experiments A-F with the times of the tuning point as predicted by the model for the corresponding experiments, showed PAEs ranging from -45 minutes to +1 hour in experiments A-D. In experiments E and F, the bacterial populations were predicted to die out before the concentration had declined below the MIC.

There are several explanations for this outcome. In some experiments described in newborn mutations that affected the outcome was seen. The reason for choosing the experiments B-F was that the initial dose of antibiotics was increased to a level above the MICs for the newborn mutants, which would make these variants less likely to be represented in the bacterial population. Despite the increased dose of antibiotics, still newborn mutants were observed for experiment D. Newborn mutants would yield an earlier re-growth of a subpopulation as compared to what would be predicted by the model and could explain the negative PAE predicted for this experiment.

Also, variation arising from the performance of the experiments might influ-

ence the outcome. One source of variation is the initial concentration. The mean initial concentration had a coefficient of variation of 11%. The affect of this variation on the expected length of the PAE has not been investigated further in this paper.

Irrespective of these factors, the simulations showed that the PAEs seen for different experiments are in the range of could be expected just as a result of random variation. As deterministic differential equations has been the mainstay of PK/PD modeling [18], we want to highlight the importance of taking the random fluctuations of the microscopic processes underlying PBP dynamics, mutations and replications into account when modeling. In the limit where the number of bacteria in a PK/PD system is large, these random fluctuations are of negligible magnitude compared with the average bacteria and the deterministic description provides an approximation with negligible error. On the other hand, the deterministic description can lose its validity when the bacterial population becomes small: which is the case after the turning-point where bacteria typically are below the limit of detection for viable counts of 10 cfu/ml.

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