Stochastic Modeling of In Vitro Bactericidal Potency

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Received: 4 May 2021 / Accepted: 29 October 2021 / Published online: 24 November 2021
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Abstract
We provide a Galton–Watson model for the growth of a bacterial population in the presence of antibiotics. We assume that bacterial cells either die or duplicate, and the corresponding probabilities depend on the concentration of the antibiotic. Assuming that the mean offspring number is given by
\[ m(c) = \frac{2}{1 + \alpha c^\beta} \]
for some \( \alpha, \beta \), where \( c \) stands for the antibiotic concentration we obtain weakly consistent, asymptotically normal estimator both for \( (\alpha, \beta) \) and for the minimal inhibitory concentration, a relevant parameter in pharmacology. We apply our method to real data, where *Chlamydia trachomatis* bacterium was treated by azithromycin and ciprofloxacin. For the measurements of *Chlamydia* growth quantitative polymerase chain reaction technique was used. The 2-parameter model fits remarkably well to the biological data.

Keywords Multitype Galton–Watson process · Asymptotically normal estimator · Quantitative PCR · *Chlamydia* · MIC

Mathematics Subject Classification 60J85 · 92C70

Kevei is supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and by the EU-funded Hungarian Grant EFOP-3.6.1-16-2016-00008. Szalai’s research was partially supported by the EU-funded Hungarian Grant EFOP-3.6.2-16-2017-00015 2020, and by the Grant NKFIH-1279-2/2020 of the Ministry for Innovation and Technology, Hungary.

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1 Introduction

Since the discovery of penicillin, antibiotics have been used increasingly worldwide to treat bacterial infections. As the overuse of antibiotics may result in drug-resistant bacteria, determining the bactericidal potency is of the utmost importance.

In the present paper the bacterial population is modeled by a Galton–Watson branching process. We assume that the offspring distribution, in particular the offspring mean $m(c)$ depends on the antibiotic concentration $c > 0$ as

$$m(c) = m_{\alpha, \beta}(c) = \frac{2}{1 + \alpha c^\beta},$$  \hspace{1cm} (1)

where $\alpha > 0$, $\beta > 0$ are unknown parameters. This flexible 2-parameter model captures the basic features of antibiotic dependence: (1) $m(0) = 2$, that is each bacterial cell duplicates in an antibiotic-free environment; (2) $m$ is monotone decreasing and continuous, that is increasing the antibiotic concentration decreases the chance to duplicate; (3) $\lim_{c \to \infty} m(c) = 0$, that is sufficiently large antibiotic concentration kills the bacteria. Under this model the minimal inhibitory concentration (MIC), the smallest antibiotic concentration preventing bacterial growth, is the smallest $c$ for which $m(c) = 1$, that is $\alpha^{-1/\beta}$. Based on measurements at different concentrations we obtain weakly consistent asymptotically normal estimator both for $(\alpha, \beta)$, and for the MIC.

We assume that the bacterial population is homogeneous, all the cells behave similarly. In particular, there is no resistant type. As mutation is rare under normal conditions and in short time, this is a natural assumption for our data set. Long-term evolution of bacterial populations with both resistant and susceptible types was investigated in several papers using deterministic models, see Svara and Rankin (2011) and Paterson et al. (2016), and the references therein. Closest to our model is the deterministic model given by Liu et al. (2004). In Liu et al. (2004) a deterministic expression for the number of colony forming units is obtained in terms of the antibiotic concentration.

Branching processes are classical tools to model cell proliferation, see the monographs by Haccou et al. (2007), Kimmel and Axelrod (2015). However, to the best of our knowledge for estimation of bactericidal potency of antibiotics only deterministic models are used.

In the experiments growth of *Chlamydia trachomatis* bacterial population was analyzed by quantitative PCR (qPCR) method with 12 different antibiotic concentrations and 2 different antibiotics.

*Chlamydiae* are obligate intracellular bacteria that primarily infect epithelial cells of the conjunctiva, respiratory tract and urogenital tract. They have a unique developmental cycle, with two phenotypic bacterial forms, the elementary body (EB) and the reticulate body (RB). The EB is the infectious form that can be found outside of the host cells and it is not capable to multiply. After infection of the host cell, the EB differentiates to RB. The RB multiplies in the host cell by binary fission in a specific area of the infected host cell, the inclusion. After a certain period of time, depending on the chlamydial species, the RB redifferentiates to EB. The EB is then released from the host cell ready to infect new host cells. This unique life-cycle triggered a lot
of mathematical work to model the growth of the population. Wilson (2004) worked out a deterministic model taking into account the infected and uninfected host cells and the extracellular *Chlamydia* concentration. Wan and Enciso (2017) formulated a deterministic model for the quantities of RB’s and EB’s, and solved an optimal control problem to maximize the quantity of EB’s when the host cell dies. The same problem in a stochastic framework was investigated by Enciso et al. (2021) and Lee et al. (2018). In these papers population growth is modeled without the presence of antibiotic.

There is a third form of the bacterium, the aberrant body or persistent body. This form is induced by various adverse environmental stimuli, such as the lack of nutrients and the presence of antibiotics, see Panzetta et al. (2018). The persistent body is not capable to multiply. After elimination of the stress stimuli, the persistent body may reenter the normal developmental cycle, differentiates to RB, multiplies and redifferentiates to EB. If there is an excess of antibiotics reaching the so-called bactericidal concentration, the bacterium is killed, and no multiplication can be observed. A lower antibiotic concentration does not kill all the bacteria, but leads to the formation of non-multiplying aberrant bodies. Further lowering the antibiotic concentration more RB can be observed, while the formation of aberrant body decreases. At very low antibiotic concentration, the antibiotic has no effect on the bacterial growth and all the bacteria enter the normal developmental cycle. Azithromycin and doxycycline are the most commonly used antibiotics in *Chlamydia* infections (Miller 2006), but *Chlamydiae* are also sensitive to quinolone type antibiotics (Vu et al. 2019). In our study *Chlamydia trachomatis* infected cells were treated with azithromycin and the quinolone ciprofloxacin. The dose response curves, the concentration dependent impacts of these antibiotics on chlamydial growth were measured 48 hours post infection. A major challenge is the accurate measurement of chlamydial growth. The golden standard is the immunofluorescent labeling and manual counting of the chlamydial inclusions. This very tedious but precise method was used recently in Lee et al. (2018). Instead of counting the bacterial cells, the quantity of bacterial genomes (which is a constant times the number of bacteria) can also be measured. Chlamydial genome concentration in the infected host cells can be measured by qPCR technique. This method is accurate and theoretically measures the genome of all individual bacteria. Eszik et al. (2016) developed a version of the qPCR, the so-called direct qPCR method for chlamydial growth monitoring. Direct qPCR is capable to perform qPCR measurements without the labor-intensive DNA purification.

If the effectivity of the qPCR is 100%, then in the exponential phase of the PCR, when there are enough reagents the amount of PCR product doubles in each cycle. In a qPCR experiment the amount of the PCR product is monitored continuously after each qPCR cycle. The less is the original amount of qPCR template (here *Chlamydia trachomatis* DNA) the higher number of cycles are needed to reach a certain level of PCR product (in fact fluorescence intensity). Fixing a threshold level the needed cycle number is the Ct (cycle threshold) value, see e.g. Yuan et al. (2006). As an example, if sample A has a Ct value of 22 and sample B has a Ct value of 24, then sample A contains 4 times as much chlamydial DNA than sample B. Therefore, the theoretical Ct value equals $a - \log_2 Z_{i,c,x_0}^{(f)}$, where $a \in \mathbb{R}$ is an unknown constant, which depends on the choice of the threshold level, and can be estimated as described in (14) below.
and $Z_{n,c,x_0}^{(i)}$ stands for the total number of dead and alive bacterial cells at antibiotic concentration $c > 0$, after $n$ generations starting with $x_0$ bacteria, in experiment $i$. Adding a measurement error, the measurements have the form

$$C_i(c, x_0) = a - \log_2 Z_{n,c,x_0}^{(i)} + \varepsilon_{i,c}, \quad i = 1, \ldots, N,$$

where measurement error $\varepsilon_{i,c}$ is assumed to be Gaussian with mean zero, and variance $\sigma^2_{\varepsilon}$. This simple model is suggested by Yuan et al. (2006). Due to the measurement method lower Ct value means higher genome concentration. The dose response curves measured by a direct qPCR method are given in Figs. 3 and 4.

The rest of the paper is organized as follows. The model and some basic properties are given in Sect. 2. The estimator of $m(c)$ for $c$ fixed is provided in Sect. 3, while in Sect. 4 we consider different antibiotic concentrations together. Section 5 contains a small simulation study, and real data is analyzed in Sect. 6. The proofs are gathered together in the Appendix.

2 The Theoretical Model

We consider a simple Galton–Watson branching process where the offspring distribution depends on the antibiotic concentration $c \geq 0$. Each bacterium either dies (leaves no offspring), survives (leaves 1 offspring), or divides (leaves 2 offsprings) with respective probabilities $p_0 = p_0(c)$, $p_1 = p_1(c)$, and $p_2 = p_2(c)$. Let $f(s) = f_c(s)$ denote the offspring generating function and $m = m(c)$ the offspring mean if the antibiotic concentration is $c$, i.e.

$$f(s) = f_c(s) = E s^\xi_c = \sum_{i=0}^{2} p_i(c) s^i, \quad s \in [0, 1],$$

$$m = m(c) = f'_c(1) = E \xi_c,$$

where $\xi_c$ is the number of offsprings. The process starts with $X_0 = x_0$ initial individuals, and

$$X_{n+1,c} = \sum_{i=1}^{X_{n,c}} \xi_{i,c}^{(n)},$$

where $\{\xi_c, \xi_{i,c}^{(n)} : i \geq 1, n \geq 1\}$ are independent and identically distributed (iid) random variables with generating function $f_c$. Note that the offspring distribution does depend on the antibiotic concentration $c$, but here and in the next section we suppress this dependence from the notation.

Using qPCR method the observed quantity is the genom of all individual bacteria, which is a constant times the total number of bacteria, that is live and dead cells together. Therefore, we have to keep track of the dead bacteria too. In order to do this we consider a two-type Galton–Watson branching process $X_n = (X_n, Y_n), n \geq 0,$
where $X_n, Y_n$ stands for the number of alive, dead bacteria, respectively, in generation $n$. Then the total number of bacteria at generation $n$ is $Z_n = X_n + Y_n$. We also write $Z_{n,x_0}$ to emphasize that $X_0 = x_0$. The process evolves as

$$X_{n+1} = \sum_{i=1}^{X_n} \xi_i^{(n)},$$

$$Y_{n+1} = Y_n + \sum_{i=1}^{Y_n} \eta_i^{(n)}, \quad n \geq 0,$$

$(X_0, Y_0) = (x_0, 0)$, where $(\xi, \eta), (\xi_i^{(n)}, \eta_i^{(n)}), n = 1, 2, \ldots, i = 1, 2, \ldots$ are iid random vectors such that $P((\xi, \eta) = (0, 1)) = p_0, P((\xi, \eta) = (1, 0)) = p_1, P((\xi, \eta) = (2, 0)) = p_2$. The offspring mean matrix $M$ has the form

$$M = \begin{pmatrix} E\xi & E\eta \\ 0 & 1 \end{pmatrix} = \begin{pmatrix} m & p_0 \\ 0 & 1 \end{pmatrix}.$$

Next we determine the mean vector of $X_n$.

**Lemma 1** If $x_0 = 1$ then for the mean we have $E X_n = m^n$, and $E Y_n = p_0(1 + m + \cdots + m^{n-1})$, thus

$$\mu_n := EZ_{n,1} = \begin{cases} m^n \left( 1 + \frac{p_0}{m-1} \right) - \frac{p_0}{m-1}, & m \neq 1, \\ 1 + p_0, & m = 1. \end{cases}$$

We note that the covariance matrix of $X_n$ can be determined explicitly. The computation is straightforward but rather lengthy. Since we only need the explicit form of the mean and the finiteness of the second moments, we skip the computation.

The strong law of large numbers and the central limit theorem imply that for each fixed $n$ as $x_0 \to \infty$

$$\frac{Z_{n,x_0}}{x_0} \to \mu_n \quad \text{a.s.}$$

and

$$\frac{Z_{n,x_0} - x_0 \mu_n}{\sqrt{x_0}} \overset{D}{\to} N(0, \sigma_n^2),$$

(3)

where $\overset{D}{\to}$ stands for convergence in distribution, and

$$\sigma_n^2 = \text{Var}(Z_n).$$

It is clear that the geometric growth rate of $EZ_n$ is the offspring mean $m$, while the precise distribution determines only the constant factor. Simple analysis shows that if
\[ m = p_1 + 2p_2 > 1 \text{ then} \]
\[ m^n \leq \mu_n = \frac{p_2m^n - p_0}{m - 1} \leq \frac{m(m^n - 1)}{2(m - 1)} + 1, \tag{4} \]

if \( m = 1 \) then
\[ 1 \leq \mu_n = 1 + p_0n \leq 1 + \frac{n}{2}, \tag{5} \]

while for \( m < 1 \)
\[ 1 \leq \mu_n = \frac{p_0 - p_2m^n}{1 - m} \leq \frac{m(1 - m^n)}{2(1 - m)} + 1. \tag{6} \]

The upper bound is attained at \( (p_0, p_1, p_2) = (1 - m/2, 0, m/2) \), while the lower bound is attained at \( (p_0, p_1, p_2) = (0, 2 - m, m - 1) \) for \( m \geq 1 \), and at \( (p_0, p_1, p_2) = (1 - m, m, 0) \) for \( m \leq 1 \).

The process \( (X_n) \) is a single type Galton–Watson process with offspring mean \( m = p_1 + 2p_2 \). If \( m \leq 1 \) then the process dies out almost surely, that is \( X_n = 0 \) for some \( n \) (if \( m = 1 \) we exclude the degenerate case \( p_1 = 1 \)), while if the process is supercritical, i.e. \( m > 1 \) then the probability of extinction is the smaller root of \( f(q) = q \), which is \( q = p_0/p_2 \); see e.g. Theorem 5.2 in Haccou et al. (2007). By the martingale convergence theorem
\[ \frac{X_n}{m^n} \rightarrow W \text{ a.s.}, \tag{7} \]
where \( W \) is a nonnegative random variable. For \( m \leq 1 \) clearly \( W \equiv 0 \), while if \( m > 1 \) then \( \mathbb{P}(W = 0) = q \), and the distribution of \( W \) is absolutely continuous on \((0, \infty)\).

The process \( X_n = (X_n, Y_n) \) is decomposable, which, in the 2-type case only means that type-2 individual cannot have type-1 offspring. Limit theorems for supercritical decomposable processes were obtained by Kesten and Stigum (1967). The eigenvalues of \( M \) are \( m \) and 1, therefore the process is supercritical if and only if \( m > 1 \). Applying Theorem 2.1 by Kesten and Stigum (1967) we obtain for \( m > 1 \) that
\[ \lim_{n \rightarrow \infty} \frac{1}{m^n}(X_n, Y_n) = W \left(1, \frac{p_0}{m - 1}\right), \]
where \( W \) is the nonnegative random variable from (7).

3 Estimation of the Offspring Mean

Recall that the measurements are given in the form (2), where \( Z_{n; c, x_0}^{(i)} \) stands for the total number of dead and alive bacteria at generation \( n \), starting with \( x_0 \) bacteria under antibiotic concentration \( c \) at experiment \( i \), \( i = 1, 2, \ldots, N \). We assume that the
sequence \{\varepsilon_{i,c} : i \geq 1, c \geq 0\} is iid, independent of the process \(X_n\), and is Gaussian with mean 0 and variance \(\sigma^2\).

By (3), an application of the delta method (see e.g. Agresti (2002) Section 14.1) implies, as \(x_0 \to \infty\), for any \(i = 1, 2, \ldots, N\)

\[
\sqrt{x_0} \log_2 \left( 1 + \frac{Z_{n,c,x_0}^{(i)} - x_0 \mu_n}{x_0 \mu_n} \right) \overset{\mathcal{D}}{\to} N(0, \sigma^2 \mu_n \log 2^{-2}),
\]

in particular, as \(x_0 \to \infty\)

\[
\log_2 Z_{n,c,x_0}^{(i)} - \log_2 (x_0 \mu_n) \overset{\mathbb{P}}{\to} 0,
\]

where \(\mathbb{P}\) stands for convergence in probability. In the following, we frequently use the delta method. Put

\[
\log_2 \mu_n = a - \log_2 x_0 - \frac{\sum_{i=1}^{N} C_i(c, x_0)}{N}.
\]

The next results both \(x_0\) and \(N\) tend to infinity. Taking iterated limits are always understood as first \(x_0 \to \infty\) and then \(N \to \infty\). The next statement is a simple consequence of (9), (8), the law of large numbers, and the central limit theorem.

**Proposition 1** As first \(x_0 \to \infty\) and then \(N \to \infty\)

\[
\log_2 \mu_n \overset{\mathbb{P}}{\to} \log_2 \mu_n,
\]

which implies that \(\mu_n\) is a weakly consistent estimator of \(\mu_n\). Furthermore, as first \(x_0 \to \infty\) and then \(N \to \infty\)

\[
\frac{1}{\sqrt{N}} \mathbb{E} \left[ \log_2 \mu_n - \log_2 \mu_n \right] \overset{\mathcal{D}}{\to} N(0, 1),
\]

which implies that

\[
\frac{1}{\sigma \mu_n \log 2} \sqrt{N} (\mu_n - \mu_n) \overset{\mathcal{D}}{\to} N(0, 1).
\]

Thus we can estimate \(\mu_n\). The problem is that \(\mu_n\) does not determine uniquely \(m\), only gives a possible range for it. This range can be deduced from the sharp bounds in (4), (5), (6). In Fig. 1 we see the corresponding upper and lower bounds for \(\log_2 \mu_n\) for \(n = 10\). If \(\mu_{10} = 8\) we can deduce that \(m\) has to be in the range (1.709, 1.741), while if \(\mu_{10} = 1\), than \(m \in (0.671, 1.072)\). The larger values of \(\mu_n\) imply more precise bounds for \(m\). Furthermore, larger \(n\) also implies more precise bounds. However, for \(m \leq 1\) one cannot determine the value \(m\). This is reasonable, since for both \(p_0 = 1\) and \(p_1 = 1\) we have \(\mu_n = 1\), whereas \(m = 0\) in the former and \(m = 1\) in the latter case.
To overcome this difficulty, we assume that $p_1 \equiv 0$. This is clearly reasonable for bactericide antibiotic, which either kills the bacterium, or lets it duplicate. Bacteriostatic antibiotic may not kill the bacterial cell, only prevents duplication. If a bacteriostatic antibiotic blocks the duplication of a single bacterium then we assume that it keeps blocking in the later generations as well. Therefore, we can equally count a ‘blocked’ bacterium as a dead one.

Assume now that $p_1 \equiv 0$. Then $\mu_n$ is Lemma 1 simplifies to

$$
\mu_n(m) = \frac{m}{2} \left( m^{n-1} + \cdots + 1 \right) + 1 = \begin{cases} 
\frac{m(m^n-1)}{2(m-1)} + 1, & m \neq 1, \\
\frac{n}{2} + 1, & m = 1.
\end{cases}
$$

Thus $\mu_n$ is a strictly increasing convex function, $\mu_n(0) = 1$, $\mu_n(2) = 2^n$. Its inverse function $\psi_n : [1, 2^n] \to [0, 2]$ is continuous and strictly increasing. Define the estimate

$$
\hat{m} = \psi_n(\hat{\mu}_n).
$$

From Proposition 1 it follows that $\hat{m}$ is a weakly consistent estimator of $m$, and by the delta method

$$
\frac{\sqrt{N}}{\psi_n'(\mu_n(m))\sigma_\epsilon \mu_n \log 2} (\psi_n(\hat{\mu}_n) - \psi_n(\mu_n(m)))
\approx \frac{\sqrt{N}}{\psi_n'(\mu_n(m))\sigma_\epsilon \mu_n \log 2} (\hat{m} - m) \xrightarrow{D} N(0, 1).
$$

Noting that $\psi_n'(\mu_n(m)) = 1/\mu_n'(m)$ we obtain the following.
Proposition 2  Assume that \( p_1 = 0 \). As first \( x_0 \to \infty \) and then \( N \to \infty \), \( \hat{m} \) is a weakly consistent estimator of \( m \), and

\[
\frac{\mu_n'(m)}{\sigma_f \mu_n(m) \log 2} \sqrt{N} (\hat{m} - m) \xrightarrow{D} N(0, 1).
\]

4 The Dependence of \( m \) on the Antibiotic Concentration

Assuming \( p_1 \equiv 0 \) we can estimate the mean for \( c > 0 \) fixed as described in Proposition 2. Next we combine our estimator for different concentrations.

We assume that the offspring mean as a function of \( c \) satisfies (1) for some unknown parameters \( \alpha > 0, \beta > 0 \). This is a quite flexible model, and we show that empirical data fits very well to this model. Rewriting (1)

\[
\log \alpha + \beta \log c = \log \left( \frac{2}{m(c)} - 1 \right).
\]

Assume that we have measurements for \( K \geq 2 \) different concentrations \( c_1 < c_2 < \ldots < c_K \), and we obtain the estimator for the offspring mean \( \hat{m}(c_i), i = 1, 2, \ldots, K \).

Standard least square theory implies that the expression

\[
\sum_{i=1}^{K} \left( \log \left( \frac{2}{\hat{m}(c_i)} - 1 \right) - \beta \log c_i - \log \alpha \right)^2
\]

attains its minimum at \( (\alpha, \beta) = (\hat{\alpha}, \hat{\beta}) \), with

\[
\hat{\beta} = \frac{K \sum_{i=1}^{K} h_i \ell_i - \sum_{i=1}^{K} h_i L_1}{KL_2 - L_1^2},
\]

where to ease notation we write

\[
h_i = \log \left( \frac{2}{\hat{m}(c_i)} - 1 \right), \quad \ell_i = \log c_i,
\]

and

\[
L_1 = \sum_{i=1}^{K} \ell_i, \quad L_2 = \sum_{i=1}^{K} \ell_i^2.
\]

Note that by the Cauchy–Schwarz inequality the denominator of \( \hat{\beta} \) is strictly positive for \( K \geq 2 \).
The minimal inhibitory concentration (MIC) is the smallest antibiotic concentration that stops bacterial growth. In mathematical terms

$$\vartheta := \text{MIC} = \min\{c : m(c) \leq 1\},$$

which, under the assumption (1), $$\vartheta = \text{MIC} = \alpha^{-1/\beta}$$. Define the estimator

$$\hat{\vartheta} = \hat{\alpha}^{-1/\hat{\beta}}.$$

In the following statement we summarize the main properties of these estimators. Introduce the notation

$$k_i = \frac{2}{m(c_i)(2 - m(c_i))} \frac{\sigma_n \mu_n(m(c_i)) \log 2}{\mu_n'(m(c_i))}, \quad i = 1, 2, \ldots, K.$$

**Proposition 3** Assume that first $$x_0 \to \infty$$ and then $$N \to \infty$$. Then $$\hat{\alpha}, \hat{\beta},$$ and $$\hat{\vartheta}$$ are weakly consistent estimators of the corresponding quantities. Furthermore, as $$x_0 \to \infty$$ and then $$N \to \infty$$

$$\sqrt{N}(\alpha - \hat{\alpha}, \beta - \hat{\beta}) \xrightarrow{D} (U, V),$$

where $$(U, V)$$ is a two-dimensional normal random vector with mean 0 and covariance matrix

$$\begin{pmatrix} \sigma_\alpha^2 & \sigma_{\alpha\beta} \\ \sigma_{\alpha\beta} & \sigma_\beta^2 \end{pmatrix},$$

where

$$\sigma_\alpha^2 = \frac{\alpha^2}{(KL_2 - L_1^2)^2} \sum_{i=1}^K k_i^2 (L_2 - L_1 \ell_i)^2,$$

$$\sigma_{\alpha\beta} = \frac{\alpha}{(KL_2 - L_1^2)^2} \sum_{i=1}^K k_i^2 (K \ell_i - L_1)(L_2 - L_1 \ell_i),$$

$$\sigma_\beta^2 = \frac{1}{(KL_2 - L_1^2)^2} \sum_{i=1}^K k_i^2 (K \ell_i - L_1)^2,$$

and

$$\sqrt{N}(\vartheta - \hat{\vartheta}) \xrightarrow{D} N(0, \sigma_\vartheta^2),$$

with

$$\sigma_\vartheta^2 = \frac{\vartheta^2 (\log \alpha)^2}{\beta^2 (KL_2 - L_1^2)^2} \sum_{i=1}^K k_i^2 \left( \frac{L_2 - L_1 \ell_i}{\log \alpha} - \frac{K \ell_i - L_1}{\beta} \right)^2.$$
5 Simulation Study

Regardless of the fixed values $c = (c_1, \ldots, c_K)$ the estimator $(\hat{\alpha}, \hat{\beta})$ is weakly consistent and asymptotically normal as $x_0 \to \infty$ and $N \to \infty$. However, the asymptotic variances in Proposition 3 do depend on the specific choice of $K \geq 2$ and the values $c_1 < \ldots < c_K$. If the antibiotic concentration is too low we essentially see a freely growing bacterial population, while for too large concentration the antibiotic already kills all the bacteria, and we only see the initial population. Therefore, intuitively it is clear that we should choose some values for the concentrations $c_i$ such that $m(c_i)$ is not close to 0, nor to 2. Otherwise we cannot tell at which concentration the antibiotic starts to work.

Consider the following example. Assume that

$$\alpha = 10, \quad \beta = 1, \quad n = 10, \quad x_0 = 10^4, \quad \sigma_\epsilon = 0.2. \tag{12}$$

It turns out that this is a reasonable choice, since roughly we obtain these estimates for the azithromycin data, see the next section. The mean offspring function $m(c)$ is given on Fig. 2.

Choose $K = 3$ different concentrations such that $c_1 = (2^{-6}, 2^{-4}, 2^{-2})$. Then for the asymptotic covariances we obtain

$$\sigma_\alpha^2 = 8.63, \quad \sigma_{\alpha, \beta} = 0.25, \quad \sigma_\beta^2 = 0.00767, \quad \sigma_\varphi^2 = 0.00012. \tag{13}$$

However, as we see in Table 1 wrong choice of the concentrations might results much larger variances. For $c_2$ we only observe the process at large concentrations, killing all
the bacteria, while in case $c_3$ the concentration is small, the antibiotic does not have any effect. The combination of large and small values as in $c_4$ does not help either.

Choosing the values as in (12), $K = 3$ and $c_1 = (2^{-6}, 2^{-4}, 2^{-2})$ we simulated the process as follows. For a given concentration $c_k, k = 1, \ldots, K$, we calculate $m(c_k)$ from (1), and choose the offspring distribution

$$\epsilon_i, k = 1 - \frac{m(c_k)}{2}, \quad p_1; k = 0, \quad p_2; k = \frac{m(c_k)}{2}. $$

With this offspring distribution we simulate $n = 10$ generations of the two-type Galton–Watson process $(X_n, Y_n)$ described in Sect. 2. Therefore we obtain $Z_{10; c_k, x_0}$. Independently, we repeat the simulation $N$ times for each concentration $c_k$. Independently of the $Z$’s, let $\{\epsilon_i, c_k : i = 1, \ldots, N; k = 1, \ldots, K\}$ be an iid sequence of Gaussian random variables with mean zero and variance $\sigma^2$. Take $a = 0$ in (2). The resulting sequence $(C_i(c_k, x_0) : i = 1, \ldots, N; k = 1, \ldots, K)$ is one simulated measurement. From each measurement we calculate the estimator $(\hat{\alpha}, \hat{\beta})$ as described in (10). We simulated the measurement this way 1000 times. The resulting means and empirical variances of $\sqrt{N}(\hat{\alpha} - \alpha, \hat{\beta} - \beta)$ and $\sqrt{N}(\hat{\vartheta} - \vartheta)$ are given in Table 2. We see that the empirical values are very close to the theoretical ones in (13) even for $N = 3, 10$. It is somewhat surprising that the estimates work even for $N = 3$, which is the suggested number of measurements at each concentration in microbiology (see e.g. Yuan et al. 2006; Eszik et al. 2016).

Next we investigate our estimator with a steeper killing curve. Let $\alpha = 100$ and $\beta = 2$, and the other values as in (12). This is also a possible choice, see the estimates for the ciprofloxacin data in the next section. In Fig. 2 we see the mean offspring function $m(c)$ for $(\alpha, \beta) = (10, 1)$ and for $(\alpha, \beta) = (100, 2)$. Note that the MIC value is 0.1 in both cases. Therefore, we can compare two rather different and practically

### Table 1

| Concentrations | $\sigma^2_\alpha$ | $\sigma_{\alpha, \beta}$ | $\sigma_\beta^2$ | $\sigma_\vartheta^2$ |
|----------------|-------------------|--------------------------|------------------|---------------------|
| $c_1 = (2^{-6}, 2^{-4}, 2^{-2})$ | 8.63 | 0.25 | 0.00767 | 0.00012 |
| $c_2 = (2^{-2}, 2^{-1}, 1)$ | 112 | 9.41 | 0.833 | 0.012 |
| $c_3 = (2^{-9}, 2^{-8}, 2^{-7})$ | 967 | 18.7 | 0.364 | 0.0298 |
| $c_4 = (2^{-8}, 2^{-7}, 2^{-1}, 1)$ | 58 | 1.17 | 0.0257 | 0.00179 |

### Table 2

| $N$ | $\bar{\alpha}$ | $\bar{\beta}$ | $\bar{\vartheta}$ | $\hat{\sigma}^2_\alpha$ | $\hat{\sigma}_{\alpha, \beta}$ | $\hat{\sigma}^2_\beta$ | $\hat{\sigma}^2_\vartheta$ |
|-----|----------------|----------------|------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| 3   | 10.359         | 1.004          | 0.9998           | 12.95                    | 0.325                    | 0.00891                  | 0.000121                 |
| 10  | 10.106         | 1.002          | 0.1              | 9.27                     | 0.262                    | 0.00789                  | 0.000116                 |
| 50  | 10.03          | 1.0005         | 0.1              | 9.3                      | 0.265                    | 0.008                    | 0.000124                 |
| 100 | 9.999          | 0.9999         | 0.1              | 8.83                     | 0.258                    | 0.008                    | 0.000117                 |
| $\infty$ | 10               | 1              | 0.1              | 8.63                     | 0.25                     | 0.00767                  | 0.00012                  |
Table 3  Asymptotics variances for different choices of $c$ for $(\alpha, \beta) = (100, 2)$

| Concentrations                  | $\sigma^2_\alpha$ | $\sigma_{\alpha,\beta}$ | $\sigma^2_\beta$ | $\sigma^2_\vartheta$ |
|---------------------------------|--------------------|--------------------------|-------------------|-----------------------|
| $c_1 = (2^{-6}, 2^{-4}, 2^{-2})$| 11298              | 35.6                     | 0.0124            | 0.000364              |
| $c_5 = (2^{-5}, 2^{-4}, 2^{-3})$| 1431               | 5.49                     | 0.0216            | 0.0000126             |

Fig. 3  Measured (○) and simulated (×) Ct values for azithromycin

relevant scenarios. In the latter case the curve is much steeper, therefore there are less relevant concentrations, so we expect larger variances. In Table 3 we see that this is partly true, however the estimate of $\vartheta$ is good.

6 The Experiment

In the experiment 50,000 mother cells were infected by *Chlamydia trachomatis*. The multiplicity of infection (MOI) value, the ratio of the initial number of bacteria and number of mother cells is 0.2. That is $x_0 = 10,000$. The measurements correspond to 12 different antibiotic concentrations using twofold dilution technique, meaning that $c_i = 2^i c_0$, $i = 0, 1, \ldots, 11$. For each concentration 3 measurements were taken. For the technical details of the experiment we refer to Eszik et al. (2016).

We analyze two antibiotics: azithromycin and ciprofloxacin. These antibiotics have different antimicrobial effects: azithromycin is a bacteriostatic antibiotic, meaning that it does not necessarily kill the bacteria, only prevents growth, while ciprofloxacin is a bactericide antibiotic, which usually kills bacteria. In Figs. 3 and 4 we see the qPCR measurements as a function of $\log_2 c$.

If $c$ is large enough, i.e. at very high antibiotic concentration $m(c)$ is close to 0, that is $Z_{n; x_0, c} \approx x_0$, since all the bacteria die without offspring. Therefore, for $c$ large
Fig. 4 Measured (○) and simulated (×) Ct values for ciprofloxacin

enough we can estimate the constant $a$ in (2) as

$$\hat{a}_N = \frac{1}{N} \sum_{i=1}^{N} C_i(c, x_0) + \log_2 x_0.$$  \hfill (14)

Then $\hat{a}_N$ is normally distributed with mean $a$ and variance $\sigma_e^2 / N$. Furthermore, $\sigma_e$ can also be estimated from these data. For azithromycin we used those measurements for which $c \geq 2^{-1}$, while for ciprofloxacin those for which $c \geq 1$.

Chlamydiae cannot replicate indefinitely, because they propagate in a closed system, where the available nutrients are finite. A special feature of Chlamydia is that it has an infectious elementary form that is not capable to grow. After the infection of the host cell, it differentiates to a reticulate body which is capable to propagate by binary division, but the number of its divisions is limited. Then the reticulate body redifferentiates into the elementary body, exits the original host cell, infects a new one and its developmental cycle starts again in another host cell. Our wet-laboratory experiment followed one round of the developmental cycle, which is approximately 48 hours. Therefore, the number of generations $n$ is typically a fixed small number, in our experiments around 10. If $c$ is small then there is no antibiotical effect so the bacterial population grows freely, that is $Z_{n, x_0, 0} \approx 2^n x_0$. We can estimate $n$ as

$$\hat{n}_N = \hat{a}_N - \log_2 x_0 - \frac{1}{N} \sum_{i=1}^{N} C_i(c, x_0).$$

Then $\hat{n}_N$ is normally distributed with mean $n$ and variance $2\sigma_e^2 / N$. To estimate $\hat{n}_N$ we used the smallest possible concentration, $c = 2^{-7}$.  

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Using Proposition 2 we estimate $m(c)$. In Figs. 5 and 6 we see the estimated means and the corresponding fitted curve $m(c)$, where the parameters $\alpha, \beta$ are estimated as described in (10). In the previous section we showed that the best strategy is to choose few concentration where the mean offspring is not close to 0, nor to 2. For the azithromycin we chose $c = (2^{-5}, 2^{-4}, 2^{-2}, 2^{-1})$ and obtained $\hat{\alpha} = 9.1, \hat{\beta} = 1.12$, and $\hat{\vartheta} = 0.139$. (We obtain similar estimates for various reasonable choices.) For ciprofloxacin in Fig. 4 we see a rapid drastic change; for $c \geq 2^{-2}$ the population dies out, while for $c \leq 2^{-4}$ the population freely grows. We chose $c = (2^{-4}, 2^{-3}, 2^{-2})$ and obtained $\hat{\alpha} = 71.8, \hat{\beta} = 2.46, \hat{\vartheta} = 0.175$. (These values are less stable to the change in $c$.) Simulated measurements with the estimated values are given in Figs. 3 and 4, where the circles denote the values of the real measurements and the crosses denote the values of the simulated ones. In both cases we obtain a remarkably good fit.

7 Conclusion

To model the growth of a bacterial population and its dependence on the antibiotic concentration we proposed a simple Galton–Watson model, where the offspring distribution depends on the antibiotic concentration via (1). A stochastic model is more natural compared to the previous deterministic model in Liu et al. (2004), because we are able to estimate the parameters of the model and investigate the properties of the estimator. Taking into account the measurement error using qPCR technique, from the measurements at different antibiotic concentrations we obtained a weakly consistent asymptotically normal estimator for the unknown parameters $(\alpha, \beta)$ in (1).
The *minimal inhibitory concentration* (MIC), the smallest concentration of antibiotic that prevents bacterial growth, is a very important parameter in pharmacology. Its estimation is rather troublesome, since due to the usual twofold dilution technique one can observe only the bacterial growth under antibiotic concentrations $c_0, 2c_0, \ldots, 2^k c_0$. Therefore one can claim only that the MIC belongs to some interval $[c, 2c]$, or give an upper bound for it. The vast majority of the literature does not provide a proper mathematical model for the growth of the bacterial population, only determines the MIC value as the smallest concentration without visible bacterial growth. In our framework an explicit mathematical definition of the MIC is given, and we constructed an estimator for it.

Simulation study showed that the estimators work surprisingly well even if the number of measurements at different concentrations is 3, which is the suggested number in microbiology (see e.g. Yuan et al. (2006), Eszik et al. (2016)).

We applied the model to real measurements, where growth of *Chlamydia trachomatis* was analyzed by two different antibiotics. Although the mathematical model has only 2 parameters, we found extremely good fit to the real data for both the bactericide and the bacteriostatic antibiotic.

**Acknowledgements** We are grateful to the anonymous referees for the helpful comments and suggestions.

**Declaration**

**Conflict of interest** The authors declare that they have no conflict of interest.
Appendix

Proof of Lemma 1  Conditioning on $X_n$

$$\mathbb{E}[X_{n+1}|X_n] = \left( \begin{array}{cc} mX_n & pX_n + Y_n \\ p0 & 1 \end{array} \right) = X_nM,$$

thus

$$\mathbb{E}X_n = X_0M^n.$$

We have, by induction on $n$ that

$$M^n = \left( \begin{array}{c} m^n p0(1 + \cdots + m^{n-1}) \\ 0 \end{array} \right),$$

thus

$$\mathbb{E}Z_n = m^n + p0(1 + m + \cdots + m^{n-1})$$

$$= \begin{cases} m^n \left( 1 + \frac{p0}{m-1} \right) - \frac{p0}{m-1}, & \text{if } m \neq 1, \\ 1 + np0, & \text{if } m = 1, \end{cases}$$

as claimed. $\Box$

Proof of Proposition 3  In what follows all the iterated limits are meant as first $x_0 \to \infty$ and then $N \to \infty$. By Proposition 2 and the delta method

$$\frac{\sqrt{N}m(c_i)(2 - m(c_i))\mu'(m(c_i))}{2\mu_{\mu_n}(m(c_i)) \log 2} \left( h_i - \log \left( \frac{2}{m(c_i)} - 1 \right) \right)$$

$$= \frac{\sqrt{N}}{k_i} \left( h_i - \log \left( \frac{2}{m(c_i)} - 1 \right) \right) \overset{D}{\to} N(0, 1),$$

for $i = 1, 2, \ldots, K$. Recall the notation in (11). Then using the independence of $h_i$’s

$$\sqrt{N} \sum_{i=1}^{K} \left( h_i - \log \left( \frac{2}{m(c_i)} - 1 \right) \right) (K \ell_i - L_1) \overset{D}{\to} N(0, s_n^2),$$

with $s_n^2 = \sum_{i=1}^{K} \ell_i^2 (K \ell_i - L_1)^2$. Substituting back into (10)

$$\sqrt{N}(\hat{\beta} - \beta) \overset{D}{\to} N(0, \sigma_\beta^2).$$

Similarly

$$\sqrt{N}(\log \hat{\alpha} - \log \alpha) \overset{D}{\to} N(0, z_n^2),$$

(15)
with \( z_n^2 = \sum_{i=1}^{K} k_i (L_2 - L_1 \ell_i)^2 / (K L_2 - L_1)^2 \), which implies

\[
\sqrt{N} (\hat{\alpha} - \alpha) \xrightarrow{D} N(0, \sigma_\alpha^2).
\]

The statement for the covariance follows the same way. From (15) and (16) we obtain

\[
\sqrt{N} (\hat{\theta} - \theta) \xrightarrow{D} N(0, \sigma_\theta^2),
\]

as claimed. \( \square \)

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