Stochastic risk assessment of *Listeria monocytogenes*

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Abstract

The availability of information on the population's exposure to the biological agents is crucial for characterising the risks of associated food-borne pathogens. Unfortunately, the available exposure data are insufficient to assess the public health impact of pathogens. Effective dose-response models are required to evaluate the risks. The growth of the micro-organisms in the food is also important. The prediction of the microbial population in food plays important role in finding the risk of certain adverse effects on human population. This study combines the stochastic growth models with the dose-response models to find the risk of illness in consumers due to the consumption of contaminated food.
Keywords: stochastic model, listeria, probability of illness.

1 Introduction

The consumption of chilled and frozen food products is growing continuously due to the changes in society, whereas food safety and control are of great concern for consumers. The reinforcement of confidence in chilled and frozen food products is of high priority for all involved in food manufacturing, trade, logistic and distribution.

A quantitative microbiological risk assessment (QMRA) is necessary to assure high quality food in the society. It is also crucial to become more knowledgeable about the consequences of infectious food-borne diseases. *Listeria monocytogenes* (Lm) is one of the most virulent food-borne pathogen affecting the health of many consumers every year [1].

The emergence of listeriosis can be attributed to many factors, chief among them being the change in food production and consumption. The tendency to preserve

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foods for longer time through chilling or freezing is one of them. Also, the ready-to-eat, fresh-cooked-taste foods that require no or little cooking is a major factor.

L. monocytogenes has a higher case fatality rate (20–30%) [2], compared to the other pathogens, even though other pathogens account for higher morbidity. The increasing number and cost of food-borne outbreaks and illnesses justifies the quantitative approach to assess the risk of infection from food-borne pathogens.

2 Mathematical model

The mathematical modelling of microbial growth is a three step process. Firstly, in the primary model one needs to fit experimental data into the mathematical formulae or equations. This gives us the values of the parameters in the model for the specific species and food type. In the second step, the dependency of model parameters with temperature with the help of a secondary model are determined. Finally, the model is tested by comparing to the experimental data taken from a dynamic temperature condition.

2.1 Primary model

Our model extends from the robust deterministic model of Baranyi and Roberts [3]. This model could be used for different types of micro-organisms in different food types [4]. The model is described by the following set of differential equations with appropriate initial conditions [5–7].

$$\frac{dq(t)}{dt} = \nu q(t), \tag{1}$$

$$\frac{dN(t)}{dt} = \mu_0(t)\alpha(t)\left(1 - \frac{N(t)}{N_{max}}\right)N(t); \tag{2}$$

where $\alpha(t) = \frac{q(t)}{1+q(t)}$, and the initial conditions are $q(0) = q_0$, $N(0) = N_0$.

Here q_0 and $q(t)$ are the quantities which are related to the critical substance necessary for growth and characterize the physiological state of the culture in the moment of inoculation and later time, respectively. $\mu_0(t)$ is the specific growth rate, expressed in $[h^{-1}]$ (per hour), dependant on the temperature. The concentrations for the initial, maximal and actual cells are denoted by N_0, N_{max} and $N(t)$ respectively. These values are expressed in colony forming units per gram (cfu/g).

The adjustment function, denoted by $\alpha(t)$, takes into account the lag phase of the culture during which the population adapts to the new environment. The relative growth rate, denoted by ν, determines the quickness of the transition from the lag phase to the exponential phase. The growth of the bacterial culture is a result of production of the critical substances by certain enzymatic reactions and it has been assumed that after inoculation, the critical substance increases at the same specific rate as the cells in the exponential phase [6]. It also suggests that the specific growth rate for physiological quantity $q(t)$ is equal to the relative growth rate of the cells. i.e., $\nu = \mu_0$.

The specific growth rate depends on certain environmental conditions. viz., temperature, pH, salt content, water activity, etc. This paper only considers the dependance of the temperature on specific growth rate. The other parameters are taken as steady throughout the process. The dependance of temperature for the specific growth rate ensures the inclusion of the dynamical temperature conditions in equations (1) and (2). The equation parameters $N_{\max}$ and q_0 are constant.

Taken into account the temperature variation in time, described by the temperature profile $T(t)$, equation (1) after integration becomes [8],

$$q(t) = q_0 \exp\left(\int_0^t \mu_0(T(t_1))dt_1\right); \tag{3}$$

and equation (2) after integration becomes,

$$y(t) = y_0 + A(t) - \ln\left(1 + \frac{\exp(A(t)) - 1}{\exp(y_{max} - y_0)}\right) \tag{4}$$

where $A(t) = \int_0^t \mu_0(T(t_1))\alpha(t_1)dt_1$, $y(t) = \ln(N(t))$, $y_0 = \ln(N_0)$, $y_{\max} = \ln(N_{\max})$.

The quantity $y(t)$ is the natural logarithm of the cell concentration $N(t)$ as seen in the above equation (4). The function $A(t)$ expresses a delay in growth during the transitions from lag phase to exponential growth phase. The increment of the critical substance given by $q(t)$ in (3) determines these transitions of the growth phases.

The quantity h, defined by $h = \ln\left(1 + \frac{1}{q_0}\right)$, has been used for more numerical stability purpose in the practical calculations, instead of q_0.

Using the new relation between h and q_0, Baranyi and Roberts model can be expressed by the following equation:

$$y(t) = y_{\max} + \ln\left(\frac{1 - \exp(-h) + \exp(\mu_0 t - h)}{\exp(y_{\max} - y_0) + \exp(\mu_0 t - h) - \exp(-h)}\right). \tag{5}$$

2.2 Secondary model

The dynamic temperature conditions are achieved through the temperature dependance of the growth rate. There are a large number of models which exist for the growth-temperature relation. The most appropriate model is provided by [9]. The empirical formula is as follows:

$$\sqrt{\mu_{\max}} = b(T - T_{\min}), \tag{6}$$

where b is a model parameter, expressed in $[h^{-\frac{1}{2}} {}^{\circ}C^{-1}]$. $T_{\min}$ is the theoretical minimum temperature that is required for the micro-organisms to grow. Note that, this model is only valid for the temperature range $[T_{\min}, T_{opt}]$ where T_{opt} is the optimal temperature for microbial growth. It is also possible to include other environmental parameters, such as pH, water activity, salt content, etc., in equation (6).

2.3 Stochastic model

The microbial growth and concentrations of the population are largely affected by many environmental, biological and other parameters during the time. These parameters are also very much intrinsic for each supply chain and the type of food the micro-organism has to grow. The mathematical model, therefore, need to include the uncertainty in measurement data and variability in the microbial population.

The stochastic model includes the probability component in the model parameters and results in a probability, or probability distribution function (PDF), at a time instant. The probabilistic components, uncertainty in measurement and the variability in the population, could be considered as overall stochastic component or could be individual stochastic components in the model [10–12].

The random fluctuation in the growth rate can be introduced to the model by adding a white noise on the deterministic expression for the specific growth rate. The expression is as follows:

$$\mu_s(T(t)) = \mu_0(T(t)) + \sigma\zeta(t), \tag{7}$$

where σ is a noise coefficient which describes the noise influence of the stochastic growth rate, $\zeta(t)$ is the white noise, μ_s and μ_0 are the stochastic and deterministic growth rates respectively, which of course depend on the temperature variation in time.

Substituting μ_0 by μ_s in the deterministic model differential equation (2), we get the following stochastic differential equation:

$$\begin{aligned}
\frac{dN(t)}{dt} &= \mu_s(t)\alpha(t)\left(1-\frac{N(t)}{N_{\max}}\right)N(t);\\
\frac{dN(t)}{dt} &= \mu_0(t)\alpha(t)\left(1-\frac{N(t)}{N_{\max}}\right)N(t) + \sigma\zeta(t)\alpha(t)\left(1-\frac{N(t)}{N_{\max}}\right)N(t);\\
dN(t) &= \mu_0(t)\alpha(t)\left(1-\frac{N(t)}{N_{\max}}\right)N(t)dt + \sigma\alpha(t)\left(1-\frac{N(t)}{N_{\max}}\right)N(t)dW,
\end{aligned} \tag{8}$$

where $W(t)$ is the Wiener process. Here, our assumption for σ is that it is independent of temperature throughout the process.

The solution of the stochastic differential equation (8) could be expressed by using the Itó integral formula. Applying the Itó integration on the equation we get the following:

$$N(t) = N_0 + \int_0^t \mu_0 A(N(s), s)ds + \int_0^t B(N(s), s)dW \tag{9}$$

where

$$A(N(t),t) = \alpha(t)\left(1 - \frac{N(t)}{N_{\max}}\right)N(t), \tag{10}$$

$$B(N(t),t) = \sigma A(N(t),t). \tag{11}$$

The Itó integral property also ensures us the mean value of the Wiener process term, rightmost in the right hand side of equation (9), is equal to 0 (zero). Hence the expected value of the microbial cells will be:

$$E(N(t)) = N_0 + \int_0^t E(A(N(s),s))ds, \tag{12}$$

$$E\left(\int_0^t B(N(s),s)\right)dW = 0. \tag{13}$$

This implies that the mean value of the cell concentration in stochastic model (8) is exactly the same value as the mean value of the concentration in deterministic model (2).

We know that the initial concentration of the microbes can also be noisy. It is also known that the pathogenic micro-organisms are found in the food very rarely. The non-frequent presence of the pathogens in the food leads us to use a probabilistic initial concentration in the model.

Hence N_0 is substituted by the probability distribution function $P(N_{\min})$, where $N_{\min}$ is the minimum threshold for the culture population that can be detected. Thus the equation (9) becomes

$$N(t) = P(N_{\min}) + \int_0^t \mu_0 A(N(s),s)ds + \int_0^t B(N(s),s)dW. \tag{14}$$

3 Dose-response model

A dose-response model is defined by a mathematical function that takes the measure of dose and yields the probability of a particular adverse effect. Of course, this function is bounded by $[0,1]$. In our case, we are interested in the probability of illness after a product is consumed. There are several dose-response models for microbial risk assessment, see [13, 14].

Some dose-response models are summarised in the following equations:

$$P_i(d) = 1 - \exp(-rd), \tag{15}$$

$$P_i(d) = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha}, \tag{16}$$

$$P_i(d) = 1 - \left(1 + \frac{d^b}{\beta}\right)^{-\alpha}, \tag{17}$$

$$P_i(d) = 1 - \exp(-\exp(a + bf(d))). \tag{18}$$

These formulae are also known as Exponential, Beta-Poisson, Weibull-Gamma and Gompertz models respectively.

In this study we have chosen Beta-Poisson model since the model parameters are known from the available literature [2].

4 Numerical results

The procedures for the modelling are taken by following steps. Firstly, laboratory experiment data were fitted with the primary model to find the model parameters for Baranyi-Roberts model, viz., y_0, $y_{\max}$, $\mu_{\max}$ and h shown in the equation (5). The non-linear regression was performed by using the free software Qtiplot.

The model parameters have to be obtained for the standardised initial physiological state also. i.e., the value of h must be same for all of the different temperature measurements. For this purpose, the average value $\langle h \rangle$ is obtained for different temperatures [4]. This assumption is followed from the measurement procedures which should be standard for initial physiological state of the cells for different temperatures during the static measurements [4].

Therefore, the model parameters have been recalculated by fitting the experimental data into the model keeping the quantity $\langle h \rangle$ fixed. This step provides the required parameters for the model more accurately.

The specific growth rate $\mu_{\max}$ found from this fitting is next used in the secondary model (6) to find the temperature dependance.

After all the model parameters are found by the regressions, the model runs for dynamic environmental conditions. The temperature variations are taken into account as a vector of temperature profile in time.

The second order Milnstein Algorithm is used in the Monte Carlo simulation to solve the stochastic equation (14).

The number of simulations was fixed to 10,000 iterations in order to obtain the probability distribution function for cell numbers at different time instants. By minimising the mean square root error of standard deviation between measurements data and the stochastic model, the noise parameter σ is obtained, while the Poisson distribution functions are used for the initial bacterial count.

4.1 Data collection

The experimental laboratory data used in this paper were obtained in Atlantic cod (*Gadus morhua*) samples prepared from fresh fillets. The *L. monocytogenes* strain used for spiking originated from an Icelandic fish product and it was pre-cultured twice in Nutrient broth (Difco, BD, USA) at $20°C$ following a two-step procedure (24 h and 48 h). Cod pieces of 50 g were aseptically transferred to plastic bags (PET12/LLDPE50, Plasprent, Iceland), spiked under laboratory conditions (400–1000 cells/g) and stored aerobically. Listeria enumeration was done on Modified Oxford agar (Difco), following incubation at 35–37°C (48 h). The growth of *Listeria m.* was followed at several static temperatures i.e., -2°C, 0°C, 2°C, 4°C, 5.3°C, 6°C, 8°C, 9.8°C and 14.6°C.

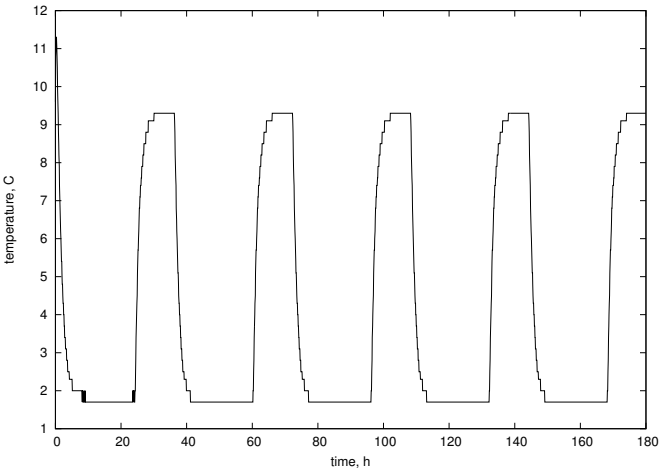

Figure 1: The temperature profile.

4.2 Figures

The variable temperature profile that is used in this study is shown in Figure 1.

Figure 2 shows the stochastic growth of *Listeria monocytogenes* when growth rate is noisy. i.e., the initial population is known and has not been considered by probability distribution function. Figure 3 shows the stochastic growth when only initial count is taken as stochastic, keeping the growth rate deterministic. Figure 4 shows the stochastic growth when both initial population and growth rate are taken as stochastic values. Just few curves are shown here rather than the 10,000 obtained in the experiments.

Figures 5–7 show the probability distribution at different times.

It can be easily seen that the spread of the distribution function increases with time.

4.3 Illness risk

The probability of illness has been calculated from the models and is shown in table 1. It has been found that probability of getting illness increases along with time, as expected. Note that, when initial population is not noisy, a pre-defined value for N_0 has been used. In this study, the value of N_0 in this case is defined as $\ln(N_0) = 5.3$.

5 Conclusion

A stochastic mathematical model for microbial growth under dynamic temperature conditions is considered. The Baranyi and Roberts model is used as primary

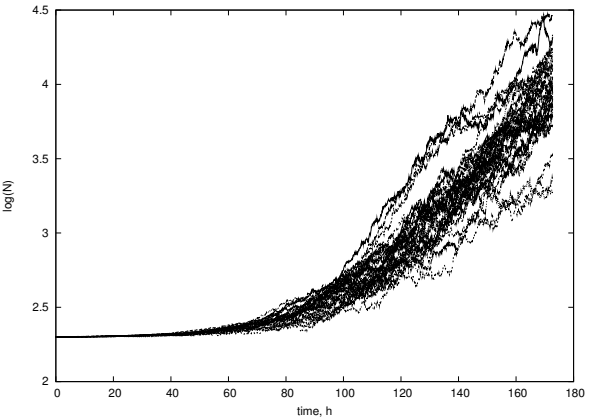

Figure 2: Growth curves of *Lm* while growth rate is noisy.

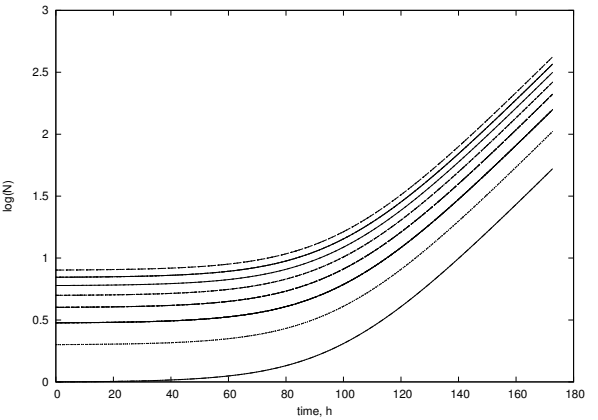

Figure 3: Growth curves of *Lm* while initial population is noisy.

Table 1: Probability of illness after different times.

	Probability of Illness %		
time, h	stochastic μ only	stochastic N_0 only	combined stochastic
60	0.07761	0.00203	0.00205
90	0.09705	0.00279	0.00281
119	0.15704	0.00595	0.00589
180	0.41977	0.07787	0.07172

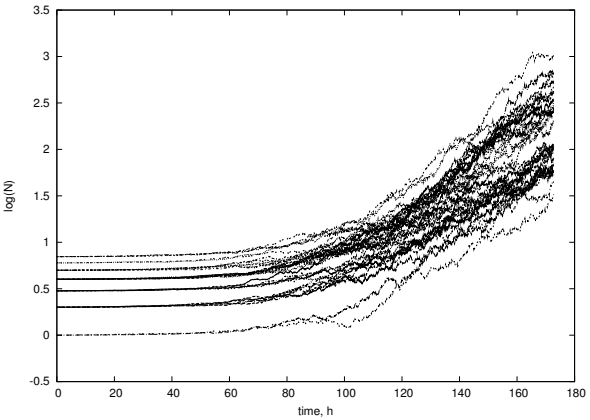

Figure 4: Growth curves of *Lm* while both initial population and growth rate are noisy.

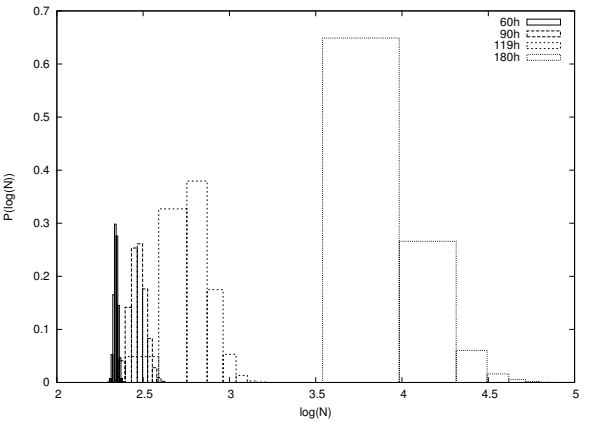

Figure 5: Probability distribution function at different times, when only growth rate is considered noisy.

model. The square root model is used as a secondary model to describe the dependence of the specific growth rate on the temperature. The stochastic fluctuations of the specific growth rate are included using the white noise and corresponding stochastic differential equation is obtained. Only overall stochastic variation is considered without distinguishing between uncertainty, which is due to the inhomogeneity in cell population, and variability. However, the initial population variation is taken into account and included in the stochastic model. By applying the developed stochastic model the possible different growth paths under dynamic temperature conditions were obtained.

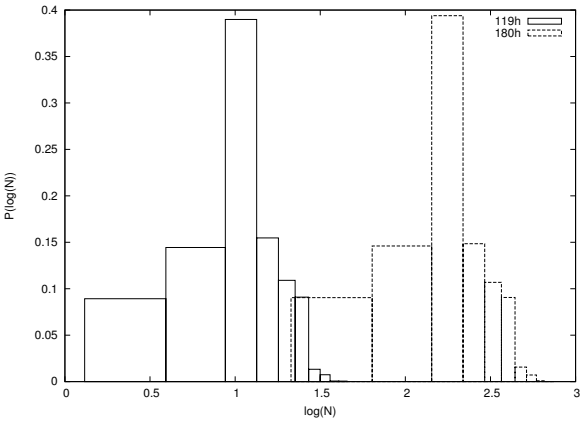

Figure 6: Probability distribution function at different times, when only initial population is considered noisy.

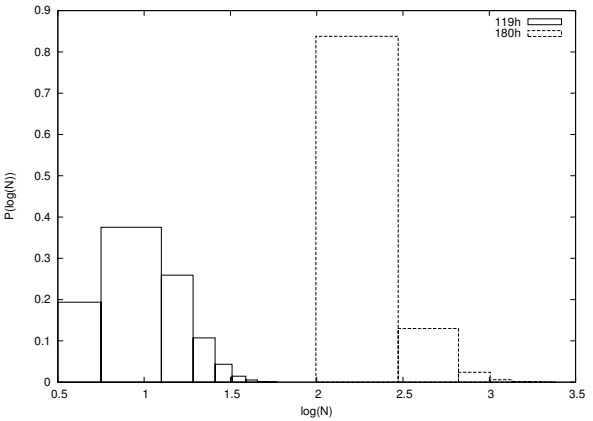

Figure 7: Probability distribution function at different times, when both initial population and growth rate are considered noisy.

The second order Milstein Algorithm is used in the Monte Carlo (MC) simulation to solve the considered stochastic differential equations. The histograms for microbial concentration at different times under constant temperatures as well as under dynamical temperature were obtained. It is shown that by using the developed stochastic model, the possible infection probability can be predicted. The developed model can be used in the QMRA of possible contamination of food products.

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