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Research Article

In Silico Simulation of Lytic Bacteriophage Population Dynamics and Its Bacterial Host *Salmonella typhi*

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Abstract

Introduction: Simulation of bacteriophages' efficiency is the integration of their lytic cycle in destroying bacteria with those found in the disciplines of mathematics, statistics and computational systems in order to find the exact numbers of phages that can lysate bacteria.

Aims: This work was conducted to create a simulative model for the development of dynamic interactions between phages and their host.

Methods and Results: The model was developed from calculations of bacteriophage replication to solve the distinct mechanisms of their efficiency including changes in lifecycles, therapeutic dose and mortality rates. Simulated data are compared with data obtained *in vitro* to assess the suitability of the model for multiplicity of infection. *In vitro* observations showed that the strength and mechanisms of bacteriophage can alter the determination of *Salmonella typhi* as antimicrobial therapy. The exponential growth curves solved the interactions of bacteriophage with their host in certain time decay, the changes in concentrations over time was solved by differential equations used to determine the therapeutic outcome.

Conclusion: *In silico* predicting of the potentiality of phages in lysing *Salmonella typhi* was estimated by using this model due the experiments conditions (*in vitro*). For more accurate estimations the model was programmed MS Excel sheet and simulated as a simple computer program.

Significance and impact: The predicting of the potentiality of lytic phages in lysing *Salmonella typhi* can be estimated by using this mathematical model due the experiments environment conditions. Therefore, it is likely that the mathematical model could be made to work computationally by changing their values according to the laboratorial experiment conditions.

Keywords: *Bacteriophage; Model; Salmonella Typhi; Simulation; Therapy*

Introduction

A bacteriophage can be considered as a virus or a parasite that attacks bacteria, multiplies in the host cell causing the bacteria's death (lysis) [1]. *Salmonella typhi* has about 29 different phages [2]. A large number of different antimicrobial treatments have been developed and applied mostly under experimental conditions only and, comparably, rarely to a commercial application level, [3-5]. Several species of bacteria have become resistant to most antibiotics, with some strains being resistant to all antibiotics. Unfortunately, it has been demonstrated that some hospital strains of methicillin-resistant *S. aureus* (MRSA) that are widespread [6,7]. There should be an alternative solution for this spreading crisis and one of the most promising drugs is the bacteriophage therapy. Phage therapy is the application of bacteriophages to bacterial infections of humans (or other animals) with the goal of reducing bacterial load. Phage therapy is complicated by the self-replicating nature of phage, [8]. The success of phage therapy is depended on the knowledge of the phage replication kinetic properties in the presence of the host as well as on the simulation and prediction, controlling and optimization of phage production for future application. Other methods which have been used to investigate the structure of phage lysins include nuclear magnetic resonance

[9]. *In silico* modelling based on homologous sequences [10] and various biophysical techniques [11]. While bacteria have developed special bacteriophage-defense mechanisms, phages also continuously adapt to these altered host systems [12]. Spontaneous mutations conferring bacteriophage resistance may actually have deleterious effects on these bacteria, and not necessarily confer an evolutionary advantage in the absence of phages Laboratory experiments have shown that a mutation in the bacterial host can make it resistant to phage infection, and occasionally the phage can mutate to overcome this resistance, [13]. The reasons for this are being the difficulty of carrying out controlled experiments in vivo and the novelty of a self-replicating therapeutic agent, [12]. At a mathematical level, whenever the mobility of the different biological actors is high enough, bacteriophage systems can be modeled by a kind of predator-prey equation. Mathematical modeling has long played a significant role in the study of phage-bacterial interactions for ecological reasons [14] as well as for medical ones, [15]. The reasons for this are obvious among them being the difficulty of carrying out controlled experiments *in vivo* and the novelty of a self-replicating therapeutic agent. Mathematical models have been used for studying the population and evolutionary dynamics of bacteriophage [16,17], and, more recently, have been used to consider how these dynamics apply to phage therapy, [18]. In these models, the rate at which a bacterial population declines due to phage predation, the rate at which the phage population increases and the levels at which they are maintained depends primarily on five parameters, the infectivity of the phage, as determined by the adsorption rate of the burst size, or number of phage progeny that are produced from a single cell, β; the latent period, λ, which is the time between adsorption and burst; the rate at which the phage are killed or removed from the site of the infection, ρ; the maximum rate bacterial growth, ψ and (two variables) the density of susceptible bacteria, S; and the density of phage, P. The full dynamics of the interactions between bacteria and phage depend on the details of the models, how the populations are maintained over time, the contributions of latent periods, and the distribution of variation in these parameters and the existence of retreats, [16,17].

Objectives

The aim of this work was to create a simple *in silico* simulation program from *in vitro* tests in order to obtain a global model describing the potentiality of bacteriophages in lysing bacteria and to point out the importance of these simulated models as required for clinical decision.

Materials and Methods

The isolation of *Salmonella typhi*

To diminish the risk of obtaining false negative results, the (Standard ISO 6579, 2002) was applied; a non-selective pre-enrichment (buffered peptone water) was prepared for wastewater samples 1:10 v/v. Samples were incubated for 12 hrs, the bacteria growth was indicated by culture turbidity. One ml from each wastewater sample was added to 10 ml pre-enrichment Rapapport vassiliadis and Tetra Thionate broth. Samples were incubated for 12 hrs, bacteria growth was indicated by media color changes. Selective agars Xylose Lysine Desoxycholate (XLD) and Salmonella Shiegella (SS) were cultured. White colonies with black centers and total black colonies were indicated to be the most dominant bacteria growth on the media. Systemic biochemical and serotyping tests were done to confirm the isolated strain results. These include; in order, growth on Triple sugar iron (TSI) agar and urea agar, Methyl Red (MR)-Vogues Proskauer (VP) Indole reaction for biochemical, and polyvalent O-anti sera A-G and H (flagellar)-anti sera for serotyping. The isolates were subjected for *in vitro* bacteriophage reactions.

The isolation and inoculation of bacteriophages

Five ml of crude sewage were taken, and then 1 ml of chloroform was added, mixed and centrifuged at 5000 rpm for 15 minutes. One ml of the supernatant liquid was transferred to 5 ml of a broth culture of bacteria in the exponential phase (this was prepared by adding 1 ml of an overnight culture to 4 ml of broth and then incubated at 37oC for 24hrs [19].

Bacteriophage titer determination

The (standard ISO 10705, 1995), double-layer agar technique, using Salmonella typhi, was used to isolate the corresponding phages. The host culture was grown up to the log phase; the bacteriophage containing solution was serially diluted in Tryptone Soy Broth (TSB).

Each dilution was subjected to plaque assay using the double layer method. Plaques were counted in the plate containing 50-300 plaques and expressed as plaque forming unit per milliliter (PFU/ml), the following formula was used to determine the titer (pfu/ml) of viral stock.

$$\frac{PFU}{ml} = \frac{Number\ of\ plaques}{dvx} \quad \text{----------(1)}$$

Where, d = dilution, and v = volume of diluted virus added to the plate.

Model constructions

The descriptive data sets of this model were obtained from the growth of bacteria and replication of bacteriophages follows the lag phase, the exponential phase and the stationary phase over incubation period. For the replication coefficient of bacteriophage, spectrophotometer was used to take readings of a tube of 5 ml of freshly grown overnight bacterial culture with 1 ml of extracted bacteriophage twice for each replication. Bacteriophage growth curve was obtained from the spectrophotometer readings and gave the burst size, the latent period and the burst time specific to the bacteriophage. Then the Transmission coefficient was set by determining the concentrations of lytic bacteria and free phage at different times to obtain an estimate value of the transmission coefficient [18]. The bacterial dynamics undergo intrinsic growth and decay cycles due to the interaction with the phage and both populations oscillate in time and give an estimate value of the decay rate coefficient of free phage. To determine effects of varying inputs the analysis of the mathematical equations has been done. The model was simulated in a programmed MS Excel to simulate and predict alternative management options.

Results

***Salmonella typhi* and its associated phage dynamics in liquid media**

In broth media the effect of the Salmonella typhi and its associated bacteriophages extraction was recognized by culture turbidity in the first and second inoculated tubes. Salmonella typhi cell numbers per ml in (log and stationary phase) were estimated by optical density readings of the culture before the addition of bacteriophage for the positive control and after the inoculation of bacteriophage for the other samples.

Sample	First replication			
	log phase (OD_{600nm})	Salmonella number ml^{-1} (log phase)	Stationary phase (OD_{600nm})	Salmonella number ml^{-1} (stationary)
Control (-)	0	0	0	0
Control (+)	0.6	5.41×10^8	0.1	1.67×10^8
Inlet	0.125	1.13×10^8	0.133	2.22×10^8
Outlet	0.206	1.86×10^8	0.29	4.83×10^8
Surface	0.403	3.63×10^8	0.48	8.00×10^8
Bottom	0.393	3.54×10^8	0.388	6.33×10^8

Table 1: *First replication of bacteriophages in Salmonella broth culture.*

First optical density readings of replicated bacteriophage and Salmonella typhi decay (lysing) rate at log phase is given by [y = 1 x108ln(x) + 1 x108]. Second optical density readings of replicated bacteriophage and Salmonella typhi decay (lysing) rate at stationary phase is given by [y = 4 x108ln(x) - 8 x107]as shown in Figure 1.

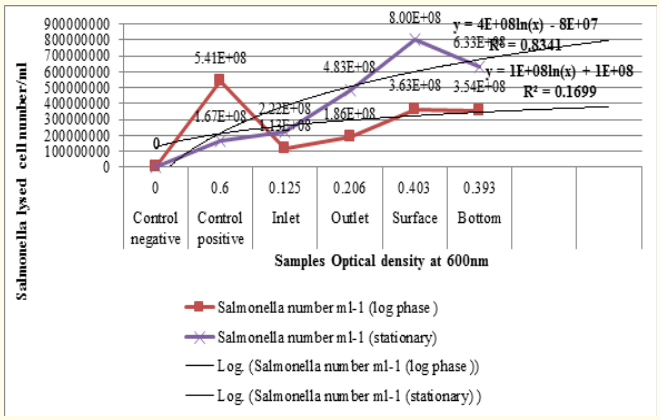

Figure 1: *Bacteriophage growth efficiency 1st replication in broth media*

Second replication was done by inoculating Salmonella typhi at log and stationary phase with stock of bacteriophage obtained from first replication. The optical density of the culture was monitored after the addition of bacteriophage, and incubation was continued until the density of the culture was stabled.

Sample	Second replication			
	log phase (OD_{600nm})	Salmonella number ml^{-1} (log phase)	Stationary phase (OD_{600nm})	Salmonella number ml^{-1} (stationary)
Control (-)	0	0	0	0
Control (+)	0.1	9.02×10^7	0.9	1.50×10^9
Inlet	0.25	2.25×10^8	0.26	4.33×10^8
Outlet	0.23	2.07×10^8	0.24	4.00×10^8
Surface	0.42	3.79×10^8	0.42	7.00×10^8
Bottom	0.61	5.50×10^8	0.61	1.02×10^9

Table 2: *Second replication of bacteriophages in Salmonella broth culture.*

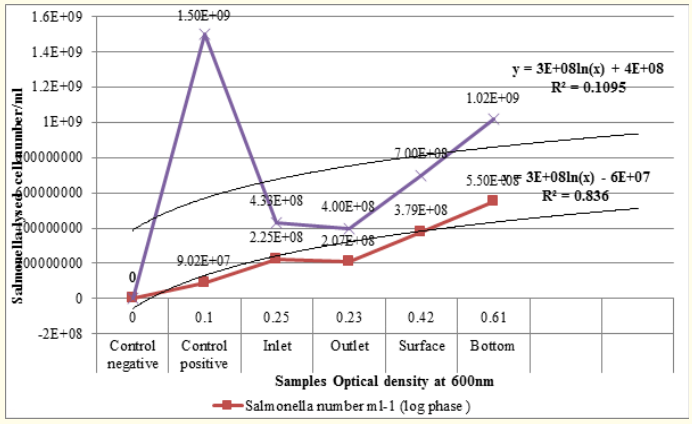

Figure 2: *Bacteriophage growth efficiency 2nd replication in broth media.*

First optical density readings of replicated bacteriophage and Salmonella typhi decay (lysing) rate at log phase is given by [y = 3 x108ln(x) - 6 x107]. Second optical density readings of replicated bacteriophage and Salmonella typhi decay (lysing) rate at stationary phase is given by [y = 3 x108ln(x) + 4 x108]as shown in Figure 2.

Salmonella typhi and its associated phage dynamics on solid media

Plaques are clear zones formed in a lawn of cells due to lysis by phage. At a low multiplicity of infection (MOI) a cell is infected with a single phage and lysed, releasing progeny phage which can diffuse to neighboring cells and infect them, lysing these cells then infecting the neighboring cells and lysing them, ultimately resulting in a circular area of cell lysis in a turbid lawn of cells. Countable plaque assay was performed when clear plaques that occurred on the plate ranged between 30 and 300.

The bacteriophage growth was done by the count of plaques on Salmonella typhi loans replicated serial dilutions PFU ml^{-1}. The replication growth rate and/or bacteria decay coefficient was obtained from [$y = 5 \times 10^6 \ln(x) - 5 \times 10^6$] as shown in Figure 3.

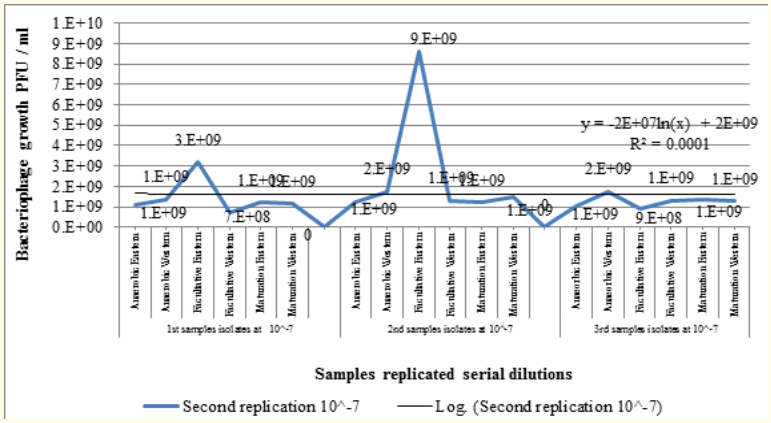

Figure 3: *Bacteriophage growth efficiency 1st replication 10^{-4} and 10^{-5}on solid media.*

The bacteriophage growth was done by the count of plaques on *Salmonella typhi* loans replicated serial dilutions PFU ml^{-1}. The replication growth rate and/or bacteria decay coefficient was obtained from [$y = -2 \times 10^7 \ln(x) + 2 \times 10^9$] as shown in Figure 4.

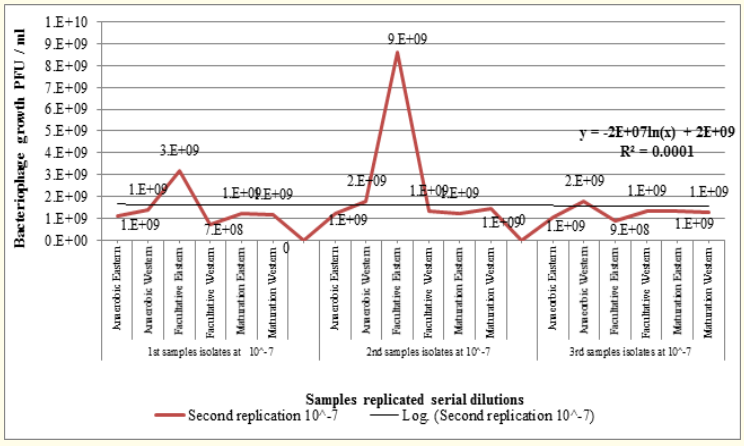

Figure 4: *Bacteriophage growth efficiency 2nd replication 10^{-7} on solid media.*

The multiplicity of infection (MOI) for physical phage tittering and amplification, the absorbance peak at 1 optical unit (A600) First optical density readings of replicated bacteriophage and *Salmonella typhi* decay (lysing) rate at log phase is given by

$$\left[y = a .10^{n} ln\left(x\right) + a .10^{n} \right] \text{--------(2)}$$

Second optical density readings of replicated bacteriophage and Salmonella typhi decay (lysing) rate at stationary phase is given by

$$[y = b.10^{n} ln\left(x\right) - b .10^{n}] \text{--------(3)}$$

where a, b = optical reading, n = titer rate and (x) = phage/ml.

Replication coefficient of bacteria: a

The standard logarithmic equation obtained from each curve was used to calculate replication coefficient from the samples collected from wastewater.

$$y = a\ ln\ \left(x\right) - b \quad \text{--------(4)}$$

The data obtained from equation (2) was used to fit the approximate replicated number of bacteriophage from each growth curve. The exponential functions for the four replicates in broth media are almost found to be similar (equations 3):

$$\begin{aligned} Replication_1 y &= 1 \times 10^{8} ln\left(x\right) + 1\, x10^{8} \\ Replication_2 y &= 4 \times 10^{8} ln\left(x\right) - 8 \times 10^{7} \\ Replication_3 y &= 3 \times 10^{8} ln\left(x\right) - 6 \times 10^{7} \\ Replication_4 y &= 3 \times 10^{8} ln\left(x\right) + 4 \times 10^{8} \end{aligned} \quad \text{----------(5)}$$

(in broth media)

Decay rate coefficient of free phage: m

The obtained replicates of bacteriophage (a) and/or decay rate of bacteria coefficient (m) from broth and solid media was done by using initial volumes of phage dilution (v) that were extracted from wastewater and titred against *Salmonella typhi* bacterium at an interval of incubation period (t_0-t_1). Countable plaque assay can be performed when clear plaques that occurred on the plate ranged between 30 and 300 regarding the replicates 10^{-4}, 10^{-5} and 10^{-7} given by:

$$\begin{aligned} Replication_5 y &= 5 \times 10^{6} ln\left(x\right) - 5 \times 10^{6} \\ Replication_6 y &= -2 \times 10^{7} ln\left(x\right) + 2 \times 10^{9} \end{aligned} \quad \text{----------(6)}$$

(on solid media)

Where: y = absorbance, (x) replicated phage number and m = Decay rate coefficient, therefore:

$$In\, x = \frac{y+b}{a} = m_{t1-t0} \quad \text{---------(7)}$$

The numerator in the equation can be made for accurate statistical estimate of phage titer to determine any concentration from known absorbance value, the changes in concentrations over time could be described by the following differential equations.

$$\frac{dx}{dt} = ax - bvx \quad \text{--------(8)}$$

Here (a) is the replication coefficient of the bacteriophage; (b) is the transmission coefficient; and (v) initial volume of phages added to infect the bacteria.

Burst size: L

The duration of these steps together define the phage generation time and help to define rates of phage population growth. Also controlling rates of phage population growth is the number of phage progeny produced per infection: the phage burst size = (L). Finally, on

average we have for:

The burst time: T = (t_1- t_0) and the burst size = L

Transmission coefficient: b

To obtain the phage transmission coefficient, the lytic phage was titred with Salmonella typhi, the uninfected bacteria and the free phage at incubation time points: at t_0 (before the burst time), and t_1 (after the burst time). Therefore, during such a clearance the role of secondary infection is negligible, so the behavior of phage and the behavior of the uninfected bacteria is dominated by the transmission term, so that

$$\frac{dy}{dt} = ay + bvx - ky \qquad \text{---------(9)}$$

Lytic rate coefficient: k

The lysing (decay) rate is determined by the time bacteria need in order to produce phages after infection. If (k) represents the lytic rate it means that for a burst time = (t_1-t_0), and the burst size = L, therefore:

$$\frac{dv}{dt} = kLy - bvx - mv \text{ -----(8) becomes } \frac{dv}{dt} = -mv \text{ -----(10)}$$

Where x (t) represents the number of uninfected bacteria, y (t) the infected bacteria and v (t) the free phage, then: From equations 6, 7 and 8 we got

$$\begin{bmatrix} \frac{dx}{dt} = ax - bvx \\ \frac{dy}{dt} = ay + bvx - ky \\ \frac{dv}{dt} = kLy - bvx - mv \end{bmatrix} \qquad \text{----------(11)}$$

Bacteriophage potentiality model simulation:

Here (a) is the replication coefficient of the bacteria, (b) is the transmission coefficient, (k) the lysis rate, (L) the burst size, and (m) the decay rate of free phage. The initial input of bacteria is of size x0 at time zero; inoculation of an amount (Vø) of phage is given at time (T), *in vitro* experimental values of model parameters are shown in (Table 3) for designing the *in silico* simulation. The relative magnitudes of the expected time of onset of host defenses (TH) and the projected time of initiation of active phage replication (TP) will be critical in determining the therapeutic outcome.

In silico solving of model equations:

The model was applied in MS Excel sheet as a simple computer program (Appendices S1). The equations where solved automatically as following:

Replication per infection is

$$R_0 = \frac{LK}{(k-a)} \cdot \frac{bx(t)}{\{bx(t)+m\}} = 0.019303595642245 \qquad \text{--------(1)}$$

The proliferation threshold XP, the phage can increase in number only when x (t) > XP, where

$$Xp = \frac{m(k-a)}{bkl} = 139.847814838713 \qquad \text{--------(2)}$$

Bacteria increase exponentially with x (t) = x0. Proliferation onset time TP found from the condition x (TP) = XP

$$T_p = \frac{1}{a} In \frac{\{m(k-a)\}}{(bkx_0)} = 0.39310148338682 \quad \text{---------(3)}$$

Approximation for the condition t > TF

$$T_f = T_p - \frac{1}{m} In\, v_{\varnothing} - \frac{1}{a} - 4.21142504230835 \quad \text{---------(4)}$$

Where the minimum dose is given by

$$V_1 = \frac{a}{b} = 3108\ phase / dose \quad \text{---------(5)}$$

Discussion

Once bacteriophages infected the bacteria, they assembled inside their hosts, and then cell lysis occurs causing a release of new phages. The number of released phages of one infected cell is the burst size and the time spent between adsorption and release of phages is called the latent period. Viral lysis is a major cause of bacterial mortality. On average 10 to 20 percent of the bacterial production is lysed daily by viruses, [20]. The *in vitro* measurements during bacteriophage replication in *Salmonella typhi* were taken by spectrophotometer at 600 nm to calculate the phage potentiality in lysing bacteria according to Godfrey and Santhosh [21] who stated that the growth was known from the increase in turbidity and increase in number of cells visible under microscope. Consequently, turbidity measurement was taken using a spectrophotometer at 600 nm. An absorbance of 0.6 to 0.8 at 600 nm is a good indication of bacteria cells that are in exponential or logarithmic growth (log phase) [22]. Spectrophotometer can be used to determine the cell number per ml, as each cell scatters light when it is in solution (broth culture), data indicates that bacterial culture absorbance at log phase OD_{600} = 5.41x10^8 cells/mL and at stationary phase bacteria reach concentrations of 10^9 cells/mL, [23,24]. There was a reversible relationship between the increasing of bacteriophages and declining of bacteria due to culture clearance which indicates the phages replication and bacteria mortality is occurred in the same time this was confirmed by [25]. The mathematical parameters of the model were achieved from the growth curve of bacteria follows the lag phase, the log phase and the stationary phase over incubation period. The first replicates were obtained from extracted wastewater, and the second replications were inoculated from the first replication in a freshly grown overnight culture which explained the burst size for bacteriophages and lysing of bacteria (Figures 1, 2, 3 and 4). The data was fitted to estimate the growth curves and exponential functions for the six replicates are not very similar. The charts give the values of collected samples data replicates, to explain these data, the burst time of phage progeny is related to the exponential phase of bacteria growth, the burst size corresponds to the division of the average number of phage after the burst time and the average number of phage before the burst time, and the latent period corresponds to the period before the exponential curve starts and after the stationary phase ends. It has been reported that phage burst sizes are significantly smaller when host cells grow slower, [26-28]. The standard logarithmic equation obtained from each curve was used to calculate replication coefficient from the samples collected from wastewater. Using the equation of the curve to solve simple equations involving logarithms and exponentials was recommended by [29]. Thus, modelling the phage life cycle, particularly for lytic phages, consists of an adsorption period, virion attachment and/or nucleic acid uptake, a latent period of bacteria lysing and virion release is known as burst size, [30-32]. The concentration of uninfected bacteria, lytic bacteria and phage are given in pfu/ml. The bacteria will be cleared if the inoculum of phage is greater than a certain threshold [33]. The value can be obtained from one step growth experiments and corresponds to the latent period, which spans from the point of phage adsorption to the point at which host lysis occurs. The latent period is about 45 minutes for *E. coli* bacteria infected with lambda phages [34]. Bacteriophages dynamically go through lysis and migrate between compartments. In addition to simulate active and inactive bacterial populations, mathematical models can be computed to describe the phage population. When designing the *in silico* model, we assume the replication rate of infected bacteria to be the same as for uninfected bacteria, but it would be easy to adapt the equations for situations where this assumption does not hold, by using a suitable redefinition of the parameter (k), [18]. The actual time of recovery cannot be precisely predicted, but in most cases will follow rapidly after whichever occurs first out of T_H or T_p. In principle, these two time-scales could be comparable, leading to a degree of interaction,

but for the present paper we restrict attention to when either one term or the other dominates, [18]. This finding also helps in deciding when to use a single phage dose and when multiple doses are necessary. If the target bacterial population is replicating rapidly, then 1 phage dose is sufficient, if bacteria replicate slowly as in the abscesses then multiple doses are definitely more effective than 1 dose, [34]. Obviously, this deterministic computational model breaks down at low densities of bacteria but aside from this, therapy can be successful if, even with large initial bacterial populations, there are feasible phage doses that will result in a bacteria levels decreasing to a small fraction of pre-treatment levels [32].

This model is an appropriate *in silico* program for simulating the potentiality of the smallest number of lytic phages in lysing *Salmonella typhi*. The basic reproductive number of phage depends on the density of bacteria and the density of extracted phages. The achievement of the model can be attributed to the correct assumption about the interactions between bacteria and bacteriophage and to the estimates of *in vitro* parameter values. Therefore, with more accurate parameter estimates that were solved in a programmed MS Excel sheet, it is likely that mathematical modelling could be made to work by changing their values according to the laboratorial experiment conditions.

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Conflicts of Interest

We do not have any conflict of interest (financial or other) other than those declared.

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