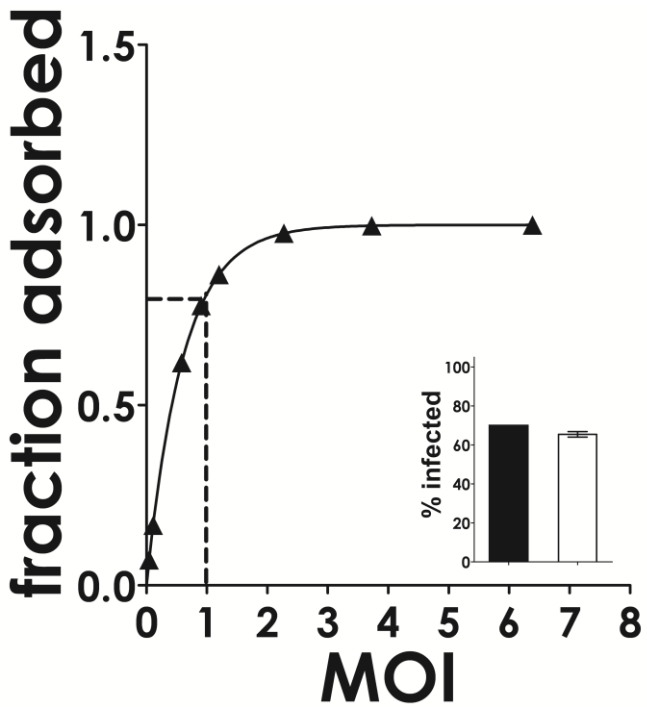

Figure S1. Theoretical and experimental estimation of the fraction of cells expected to be infected at a given MOI. The fraction of cells that are infected at a particular MOI can be estimated from the Poisson's equation.

$P(n) = e^{-m}m^n/n!$,

Where P(n) is the probability of finding cells bound by 'n' phage(s) and 'm' is the average number of phage per cell (MOI). Putting n=0 the expected number of uninfected cells can be computed. The equation then simplifies to:

$f_u = e^{-m}$ *and* $f_a = (1- e^{-m})$,

where f_u and f_a represent fraction of bacteria that have either not adsorbed (unadsorbed) even a single phage (f_u) or adsorbed at least one phage (f_a), if not more. The plot of the theoretically determined fraction of cells that adsorb phage against MOI is a rectangular hyperbola that tends to approach, the maximum value of 1. Since 100% adsorption is not possible to achieve we used an MOI at which near about 50% of the cells adsorb phage. Theoretically it can be shown that if MOI (m) = 1 then f_a = 66% (Fig. S1). Experimentally this was verified by incubating cells with phage (MOI of 1) for 1 h in triplicate and measuring the fraction of cells (mean of three experiments ±SD) that adsorbed phage. The experimentally determined value was found to tally with the theoretically determined one (Figure S1 inset, white bar for experimental finding and black for theoretical). Beyond 1 h it is not possible to monitor adsorption efficiency as the infected cells begin to lyse. However it is clear that within 1 h maximum possible adsorption, as predicted from the Poisson's principle, is achieved. Considering that at MOI of 1 more than 50% cells get infected therefore, unless stated otherwise, the working MOI used was always 1.

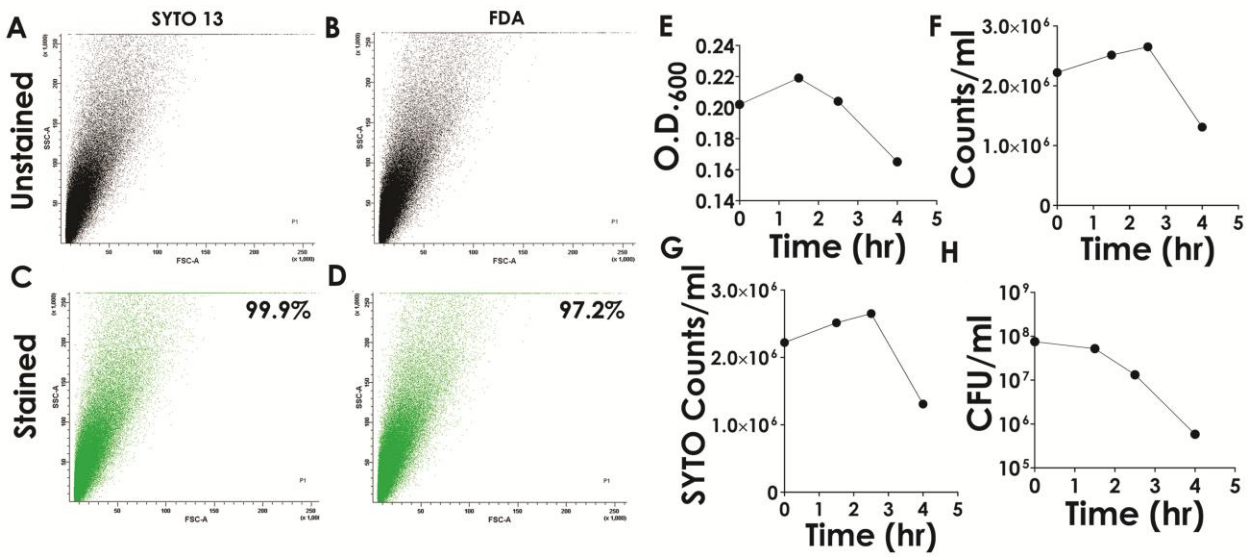

Figure S2: Fidelity of the FACS based cell counting procedure. Cell density (counts ml^{-1}) of a given bacterial suspension was derived by counting the number of dots that appeared on a scatter plot (FSC Vs SSC) following the injection of a known volume of the bacterial suspension into the FACS instrument (A and B). That the dots represent cellular entities was verified by staining the cells with the nucleic acid staining dye SYTO 13 (live and dead) as well as FDA (Live). Comparison of the scatter diagrams obtained with or without staining indicates that almost all the dots (99% for SYTO 13 and 97% for FDA) appear as stained units in the corresponding scatter plots (Compare C and D with A and B respectively). Hence we conclude that all the dots registered in the unstained scatter plots (A and B) represent cellular entities. The cell decay pattern (refer Fig. 1 D-F in text) observed following phage infection remained same irrespective of whether or not staining by SYTO 13 was done prior to cell counting (E-H). Hence, for the sake of operational simplicity we have omitted the staining step in those experiments where the counts alone mattered. However in the experiments where live – dead differentiation had to be made the necessary staining procedures were included.

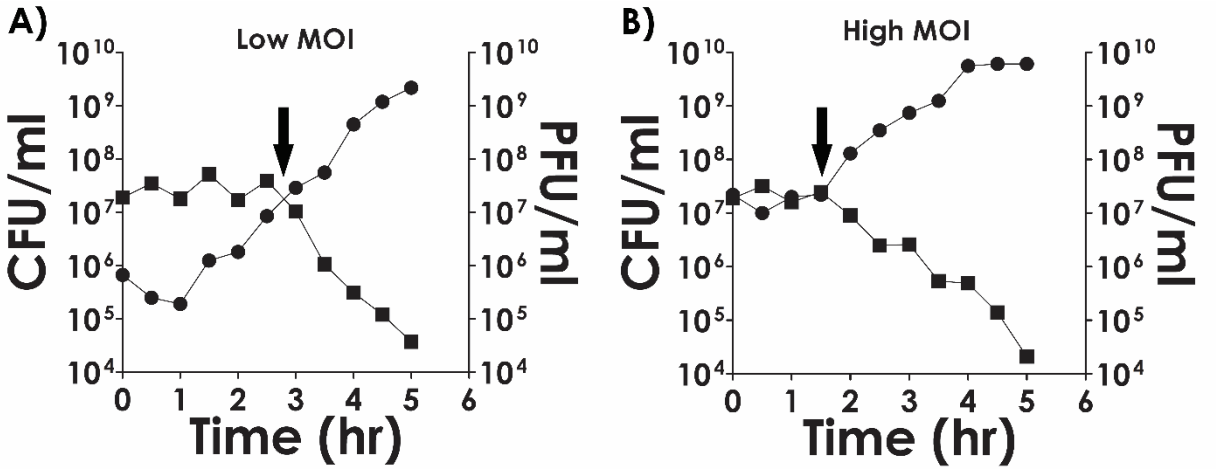

Figure S3: Simultaneous monitoring of cell death and phage growth. The kinetics of phage release and loss of viability were monitored in parallel at two different MOIs, 0.1 (low) and 1

(high) (A and B) respectively. The results show that cell death increases as phage titers increase. In the case where MOI was low, a time delay in the onset of cell death was observed. The time delay can be explained by considering that the level of a secondary lethal factor released from cells lysed due to phage infection, must reach a critical level for it to be effective (refer to the results of mathematical modelling and simulation attempts, as well proposed model, Fig. 7 and 8 in the main text).

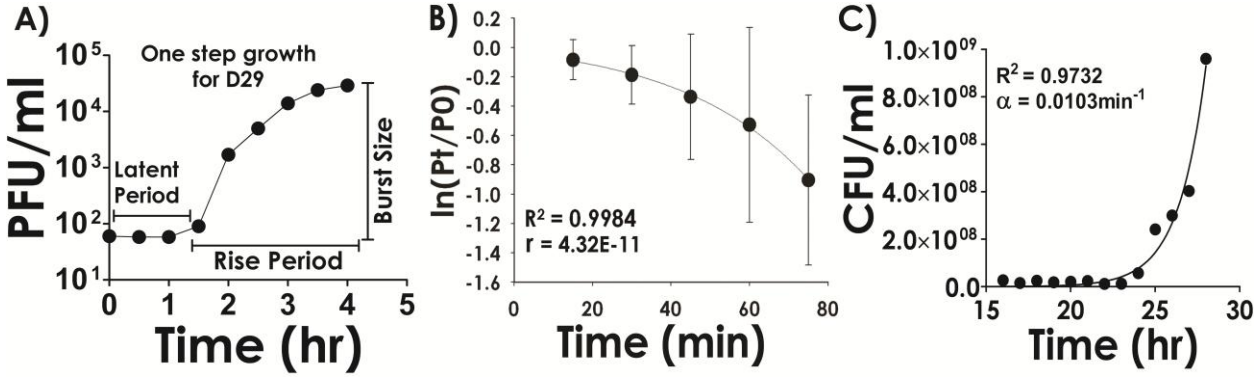

Figure S4. Deriving rate constants for bacterial and phage growth. (A) The latent phase and burst sizes were determined by performing a one-step growth experiment. For one step growth experiment, host cells were mixed with phage (MOI of 0.1). Adsorption was allowed for a limited period of time (20 min) after which the cells were diluted 10^4 times, to prevent further adsorption. The MOI of 0.1 and not 1 was used in these experiments to ensure that following dilution the free phage concentration is as low as possible. This ensures that multiple rounds of adsorption do not take place. The time period was restricted to only 20 mins to ensure that adsorption events take place as far as possible synchronously. This is necessary to ensure that phage development in all the infected cells start at approximately the same time.

The diluted cells were incubated at 37°C and samples withdrawn at regular intervals. Time course of phage release was monitored by estimating the titers at regular intervals. The period during which no change in phage titer was observed was considered to be the latent period. The PFUs obtained at the saturation point of a one step growth experiment was divided by the number of infectious centers present initially to obtain the burst size.

(B) The adsorption rate constant:

The phage adsorption rate constant was derived using the equation $\ln\frac{P(t)}{P(0)} = -\frac{rS(0)}{\alpha}(e^{\alpha t} - 1)$ which was derived in an earlier study (1). This equation considers that a certain number of phage units or Pfus designated as *P(0)* are allowed to infect a certain number of susceptible cells, *S(0).* At any time point 't' we may consider the phage titer to be *P(t*). The ratio *P(t)/P(0)* therefore represents the fraction of the phage that has adsorbed. The constants 'α' and 'r' represent bacterial growth and phage adsorption rate constants respectively.The equation is valid only if phage concentration is sufficiently low (negligible) as compared to the bacteria. The experiment was done as follows: phage was mixed with host at an MOI of (0.1). At regular intervals, the cells were centrifuged and the phage titer in the supernatant determined. The data points generated after plotting $\ln\frac{P(t)}{P(0)}$ against 't' was be fitted to an equation of the type $y = y(0) +$

$\frac{a}{b}$ $(e^{bt} - 1)$ which yields the values of the rate constants 'r' and 'α'. Triplicate experiments were performed. Each data point represents mean of three determinations performed independently ± SD.

The value of 'r' turned out to be about 4.32E-11 $cell^{-1}$ $phage^{-1}$ $min^{-1}ml^{-1}$ whereas α was found to be 0.0307 min^{-1}. The value of α in this experiment was about 3 times more than what was determined directly (refer C). This indicates that in the presence of phage cells grows faster. Alternatively this may be due to day to day variations in culture conditions.

(C) Bacterial growth rate constant:

The instantaneous rate of bacterial growth can be described by the equation $\frac{ds}{dt} = \alpha S$ where S represents the number of bacteria present at any instant. Upon integration we get $S(t) = S(0)e^{\alpha t}$ where 'α' is the bacterial growth rate constant and S(t) and S(0) represent the number of bacteria at time 't' and '0' min respectively. The growth rate constant 'α' was determined by inoculating an overnight grown culture of M. *smegmatis* into fresh medium and monitoring the CFUs present at various time points during the exponential phase of growth. The data points were then fitted to an exponential growth equation $y = y(0)e^{x}$, from which 'α' was derived . The value of 'α' turned out to be 0.0103 min^{-1} (Table S2) For mathematical modelling this value was used.

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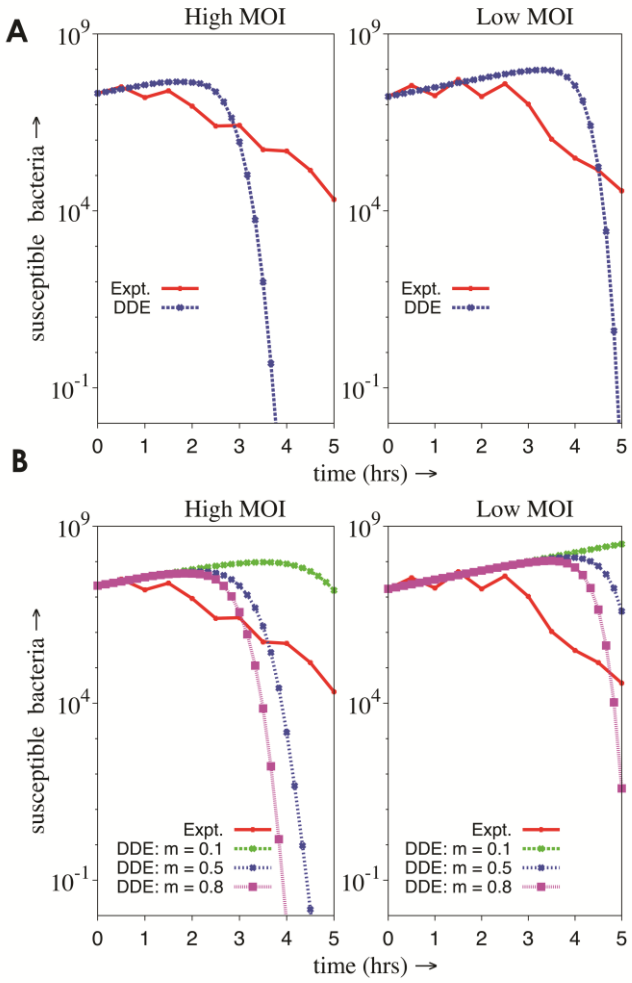

Figure S5. Mathematical modeling of host phage interactions. Bacteria-phage dynamics models include essential characteristics that rule the bacteria-phage behavior. In this model we consider a multistep process in which phage will infect the host cells. Following infection the phage will propagate inside the cell. After a latent period the infected cells will get lysed and progeny phages are released. The released phages will again infect other cells resulting in the initiation of the next round of infection (secondary infection). At each stage cells which lyse will lose viability and thus the host count will decrease. The parameters controlling the interaction dynamics, the phage adsorption rate and bacterial growth rate have been determined experimentally (Table. S2). Using the experimentally determined values it is possible to predict the number of bacteria that would survive phage attack after time 't' by using the following equations;

$$\frac{dS}{dt} = \alpha S(t) - rS(t)P(t) \quad (1)$$

$$\frac{dI}{dt} = rS(t)P(t) - rS(t-\tau)P(t-\tau) * Heavi(t-\tau) \quad (2)$$

$$\frac{dP}{dt} = brS(t-\tau)P(t-\tau) * Heavi(t-\tau) - rS(t)P(t) \quad (3)$$

Heavi (t – τ) is Heaviside step function (not a delay variable) and is defined as

$$Heavi\,(t-\tau) = 0,\ t < \tau \ and\ Heavi\,(t-\tau) = 1,\ t \geq \tau \quad (4)$$

As is evident from Fig. S5A, this basic system of equations shows a rather huge decay in bacterial cells (compare red trace against blue). This is not consistent with the experimental data, which shows that the cell death is less than predicted. To overcome this discrepancy it was assumed that not all infected cells will lyse following infection. Only a fraction designated as '*m'* will get lysed. Thus, upon varying '*m'*, the number of decayed bacterial cells changes. Lower the value of *'m'* lesser will be the decay in bacterial cell counts. When *'m'* is equal to one cell death will be the maximum. Therefore, the system of equations reduces to the following:

$$\frac{dS}{dt} = \alpha S(t) - rS(t)P(t) \quad (5)$$

$$\frac{dS}{dt} = rS(t)P(t) - rmS(t-\tau)P(t-\tau) * Heavi(t-\tau) \quad (6)$$

$$\frac{dP}{dt} = brmS(t-\tau)P(t-\tau) * Heavi(t-\tau) - rS(t)P(t) \quad (7)$$

The solution of (5) – (7), when compared to experimental results indicates that when m = 0.1 cell lysis is delayed significantly. Although this appears to explain why the observed killing is less than that anticipated, the rate of decay predicted through this approach (green trace), does not correspond to the rate observed. Apparently the bacterial counts decay at a faster rate than expected.

Thus, repeated cycles of phage infection is not the only phenomenon involved in bacterial decay after the latent period. Therefore, a "secondary killing factor", may be responsible for the secondary cell death, namely, 'q', which must be introduced into the equation. 'q' represents the amount of a hypothetical factor that is released in the environment exclusively due to phage infection. With increase in time, the ratio of the number of phages to the number of bacterial cells also increases. This in-turn leads to very low cell concentration in comparison to phage concentration and hence, active role of secondary decay due to superoxide radicals will decrease with time. Thus, the secondary killing is a decreasing function of time and modeled akin to eqn. 15 of Ref.(2) mentioned below. Secondary decay of susceptible cell population due to radicals depends on the susceptible bacterial cell population as,

$D_{secondary} \propto S(t)e^{-(\frac{t}{a\tau})}$ Where, 'a' is a constant.

By taking into account these factors it was possible to apply the system of equations (5-7) to predict the rate of phage killing and bacterial decay (Fig. 7 in text). The results show that the predicted curves are in fair agreement with the experimentally determined ones indicating that the assumption that a secondary factor is involved is correct.

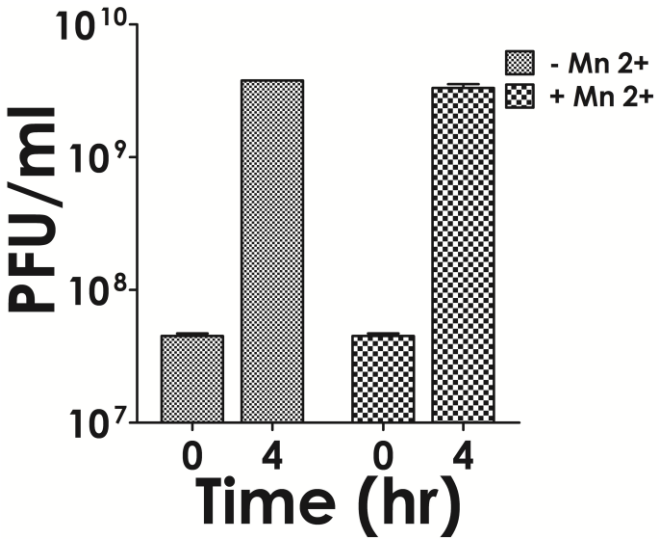

Figure S6. Effect of Mn 2+ on phage growth. Mycobacterial cells were infected with Mycobacteriophage D29 at an MOI of 1. After two hrs of incubation, $MnCl_2$ was added at a final concentration of 5 mM. The PFU counts taken after 4 h of incubation was compared to that at time zero, (immediately after phage addition). Phage titers were determined in triplicate and the average values ± standard error of mean was plotted. The results show that there is no significant difference in the phage titers after 4 h between experimental sets performed in the presence (+) and absence (-) of Mn^{2+}.

Table S1*: Calculation of the counts ml^{-1} using FACS aria system.

Relative conc.	Number of counts recorded in 2.4 µl	Counts/ml
0.0010	856	356000
0.0100	3331	1387000
0.0625	10000	4166000
0.1000	15776	6573000
0.1250	15861	6608000
0.2500	21190	10204000
0.5000	53041	22100000
1.0000	121334	50550000

* Values for Fig. 1 in the MS.

Table S2: Evaluation of all the parameters considered both theoretical and experimental

DDE parameters	Experimentally/Theoretically determined values	
	High MOI	Low MOI
α (bacterial growth rate constant)	0.01 min^{-1}	0.01 min^{-1}
r (phage-adsorption rate)	4.32E-11$cell^{-1}phage^{-1}min^{-1}ml^{-1}$	4.32E-11 $cell^{-1}phage^{-1}min^{-1}ml^{-1}$
τ (latent period)	60 min	60 min
b (average burst size)	219	219
m (lysed fraction of infected cells)*	0.4	0.7
q (secondary killing factor)*	0.000009 lysed $cell^{-1}$	0.00003 lysed $cell^{-1}$
a (DDE parameter)*	1	2

* Values determined theoretically.

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2. **Santos SB, Carvalho C, Azeredo J, Ferreira EC.** 2014. Population dynamics of a Salmonella lytic phage and its host: implications of the host bacterial growth rate in modelling. PloS one **9:**e102507.