REVIEW

Bacteriophage secondary infection

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Phages are credited with having been first described in what we now, officially, are commemorating as the 100th anniversary of their discovery. Those one-hundred years of phage history have not been lacking in excitement, controversy, and occasional convolution. One such complication is the concept of secondary infection, which can take on multiple forms with myriad consequences. The terms secondary infection and secondary adsorption, for example, can be used almost synonymously to describe virion interaction with already phage-infected bacteria, and which can result in what are described as superinfection exclusion or superinfection immunity. The phrase secondary infection also may be used equivalently to superinfection or coinfection, with each of these terms borrowed from medical microbiology, and can result in genetic exchange between phages, phage-on-phage parasitism, and various partial reductions in phage productivity that have been termed mutual exclusion, partial exclusion, or the depressor effect. Alternatively, and drawing from epidemiology, secondary infection has been used to describe phage population growth as that can occur during active phage therapy as well as upon phage contamination of industrial ferments. Here primary infections represent initial bacterial *population* exposure to phages while consequent phage replication can lead to additional, that is, *secondary* infections of what otherwise are not yet phage-infected bacteria. Here I explore the varying meanings and resultant ambiguity that has been associated with the term secondary infection. I suggest in particular that secondary infection, as distinctly different phenomena, can in multiple ways influence the success of phage-mediated biocontrol of bacteria, also known as, phage therapy.

KEYWORDS lysis from without; lysis inhibition; coinfection; parallel secondary infection; phage therapy; pharmacology; serial secondary infection; superinfection

INTRODUCTION

Phages interact with bacteria in the course of adsorption, infection, and virion release. Phage-phage interactions also can take place and may be categorized as occurring among phage virions (such as their aggregation), between phage genomes (particularly in terms of genetic recombination but also physiologically), or between virions and phage-infected bacteria. In this commentary I initially consider the latter, which involves virion adsorption to already phage-infected bacteria—over the course of which a number of interesting phage-phage interactions can occur, e.g., (Abedon, 1994). Though these interactions all involve secondary adsorptions, they usually are referred to instead as either secondary infections or superinfections.

Alternatively, the term secondary infection has been used in the phage literature with a distinctly different meaning, that is, to describe sequential infections that occur during phage population growth, such as in the course of phage therapy or within the context of phage contamination of industrial fermentations. Secondary infections thus can occur either in "parallel", as equivalent to secondary adsorptions or superinfections (one cell simultaneously interacting with more than one phage), or instead in "serial" as occurs during phage propagation through a bacterial culture (one phage lineage interacting

sequentially with a series of cells). Here I consider these differing uses along with their distinct impacts on the pharmacology of phage therapy.

Parallel secondary infection and its ambiguity

The first use of the phrase "secondary infection" within the context of virion interaction with already phage-infected individual bacteria was, to my knowledge, by Doermann (1948), who was studying the then newly appreciated lysis inhibition phenomenon, and this usage is reflected in Benzer et al. (1950). Specifically, with lysis inhibition an already phage-infected bacterium– that is, a primary infection–can be induced to delay its lysis if a second phage should adsorb (Abedon, 1990; 1994; 2009a; 2012c; Moussa et al., 2012; 2014; Tran et al., 2005; 2007). In 1948, when Doermann published his lysis-inhibition study, the mechanism by which lysis inhibition was induced was little understood. Indeed, the phenomenon of superinfection exclusion had not yet been discovered (see French et al., 1952, for overview of the earliest descriptions of superinfection exclusion-related phenomena in bacteriophage); superinfection exclusion itself is a phage-expressed means by which progression from phage attachment to an already phage-infected bacterium can be prevented from progressing to coinfection of same bacterium (Abedon, 1994). There thus was little reason to distinguish between "secondary infection" and "secondary adsorption" in describing the action of subsequently adsorbing phages on already phage-infected bacteria since the secondarily adsorbing phages in the absence of superinfection exclusion could be assumed to reach the cytoplasms of their target bacteria. Indeed, Doermann employed both "secondary adsorption" and "secondary infection" in his 1948 study, seemingly interchangeably.

The term secondary *infection*, as used to describe interactions between virions and phage-infected cells, subsequently has been much more frequently used than secondary *adsorption*. This stems, perhaps, from continuing uncertainty about the extent to which secondary adsorption in fact results in coinfection by a secondarily adsorbing phage. Alternatively, it is often the case that the terms "adsorption" and "infection" are used interchangeable, such as to describe a phage-bacterium interaction in which knowledge of successful infection also is uncertain; witness for example the use of multiplicity of infection which in many cases instead more correctly describes multiplicity of phage *adsorption* (Abedon, 2008; 2011a; 2012b; 2014; Abedon et al., 2010; Adams, 1959; Bigwood et al., 2009). It is possible, however, to define the concept of adsorption narrowly, that is, as a means of describing solely virion attachment, with any subsequent phage-genome uptake considered instead to represent infection. An interchange of the use of infection and

adsorption, and also secondary infection vs. secondary adsorption, nonetheless is neither uncommon nor necessarily incorrect.

The term adsorption, if defined more broadly, can describe aspects of phage-bacterium interaction that follow mere virion attachment to a bacterial cell. From this latter perspective, it thus may be possible to infer a time course of secondary phage interaction with a phage-infected bacterium, here with superinfection defined as equivalent to coinfection by a secondary phage:

$$\frac{\text{secondary}}{\text{attachment}} \rightarrow \frac{\text{secondary}}{\text{adsorption}} \rightarrow \frac{\text{secondary}}{\text{infection}} \rightarrow \frac{\text{superinfection}}{\text{(coinfection)}}$$

In this time course the concepts of secondary attachment and secondary adsorption, secondary adsorption and secondary infection, and also secondary infection and superinfection are assumed to *not* be synonymous, three assertions which are *not* necessarily valid. To more legitimately reflect this time course, I therefore replace arrows with the lesser-than-or-equal signs to indicate ambiguous progression by secondary phages towards successful coinfection:

$$\frac{\text{secondary}}{\text{attachment}} \leq \frac{\text{secondary}}{\text{adsorption}} \leq \frac{\text{secondary}}{\text{infection}} \leq \frac{\text{superinfection}}{\text{(coinfection)}}$$

That is, secondary adsorption may or may not represent further progression ("greater than") towards secondary infection than secondary attachment, secondary infection may or may not represent further progression towards coinfection than secondary adsorption, and secondary infection may or may not be synonymous with superinfection or coinfection.

Contrasting this lesser-than-or-equal-to progression from secondary phage attachment through coinfection we can state with much less ambiguity the following claim:

$$\frac{\text{secondary}}{\text{attachment}} < \frac{\text{superinfection}}{\text{(coinfection)}}$$

Namely, and particularly if we define infection as beginning with phage-genome entrance into the adsorbed bacterium's cytoplasm, there is no overlap between the two extremes of virion attachment to an already phage-infected bacterium, on the one hand, and coinfection on the other. Though with less certainty, it also may be argued that the following series of inequalities might hold as well,

$$\frac{\text{secondary}}{\text{attachment}} \leq \frac{\text{secondary}}{\text{adsorption}} < \frac{\text{secondary}}{\text{infection}} \leq \frac{\text{superinfection}}{\text{(coinfection)}}$$

where a lack of overlap is being claimed to exist between the concepts secondary adsorption and that of secondary infection. This perspective is simply a means of stating that phage infection follows phage adsorption.

This contention of a distinction between adsorption

and infection is not without precedent. The phenomenon known as lysis from without, for example, is a mechanism of phage-induced bacterial lysis that results from substantial amounts of phage adsorption but little phage infection (Abedon, 1992; 1994; 1999; 2011b). Furthermore, unless high multiplicities of adsorbing phages attach truly simultaneously to individual bacteria, then it is substantial *secondary adsorption* that must be inducing lysis from without rather than substantial secondary infection. Distinctions made between secondary attachment, secondary adsorption, secondary infection, and even superinfection are not necessarily consistent within the phage literature, however, nor unambiguously clear even within specific studies. Secondary infection nevertheless is a term that often has been used to mean the exposure of phage-infected bacteria to additional free phages; see Abedon (1994) for numerous older references and see also its usage by Adams (1959) and Stent (1963).

One distinction that can be made with little ambiguity is that between infections that are productive versus infections that instead display lysogenic cycles, with both infection types at least potentially serving as primary infections, that is, infections of otherwise not phage-infected bacteria. Logically, therefore, the *primary* infection of a bacterial lysogen can be viewed as a *secondary* infection of a lysogenic infection, see for example, (Davis et al., 1999; Espeland et al., 2004; Fogg et al., 2007; Mann , 2003; Slavcev and Hayes, 2002; Smith et al., 2012; Weinfeld and Paigen, 1964; Werner and Christensen, 1969; Yamada et al., 2007). One can also describe such secondary infections within this context as superinfections (Sturino and Klaenhammer, 2006) and indeed the term "superinfection" generally appears to be more common within the phage literature than "secondary infection", including within the context of the concept of superinfection immunity as well as that of superinfection exclusion.

Parallel secondary infection-associated phenomena

Phenomena that are directly associated with phage secondary infection or adsorption of already phage-infection bacteria include the above-noted lysis from without plus mechanisms of resistance to lysis from without (Abedon, 1994; Abedon, 1999). Both phenomena possibly play roles in the life history of certain phages, most notably the T-even phages, allowing for resistance to the premature lysis of cultures as mediated by secondary adsorption (i.e., resistance to lysis from without) along with a means of eventually assuring the lysis of those cultures, i.e., so-called lysis-inhibition collapse (Abedon, 1992; Abedon, 1999; Abedon, 2009a). Secondary infection is also seen in the parasitism of phage infections by satellite phages, such as phage P4's parasitism of phage P2 infec-

tions. See Turner and Duffy (2008) for discussion of the evolutionary ecology of phage-on-phage parasitism and Hyman and Abedon (2012) for its consideration in viral systems more generally. In addition, there is the also above-noted superinfection exclusion and superinfection immunity.

These latter two phenomena operate by distinctly, conceptually different mechanisms (Hyman and Abedon, 2010), with superinfection exclusion a blockage as expressed by primary phages especially on the successful phage genome translocation into the adsorbed bacterium (Abedon, 1994) whereas superinfection immunity is a post genome-translocation mechanism by which subsequent secondary phage genetic contribution to infections is curtailed, though not always successfully (Fogg, et al., 2010). From the terms employed, we can view these phenomena literally as prevention (or exclusion) of secondarily adsorbing phages from superinfecting (superinfection exclusion) versus resistance (i.e., immunity) of a primary infection to the continuation of infection by secondary phages that nonetheless have successfully initiated superinfection (superinfection immunity).

Note that Berngruber et al. (2010) collectively describe these two otherwise mechanistically distinct phenomena of blockage on successful secondary infection as superinfection *inhibition*. To the extent that secondary infection gives rise to some degree of coinfection then we can also consider what have been described as mutual exclusion, partial exclusion, or the depressor effect. These respectively are the prevention of replication of one of two lytically coinfecting but not closely related phages, essentially mutually exclusion but between closely related phages, and reductions in burst sizes given coinfection between phages that are not clonally related (Abedon, 1994). That is, to the extent that they act against phages that are secondarily infecting, then these mechanisms might be viewed as ones of partial or incomplete superinfection inhibition.

In practical terms, the consequence of a failure of secondary infection is loss of the secondarily adsorbing phage. This loss occurs because the secondary phage must commit to adsorbing–that is, irreversible adsorption–prior to testing the infection status of the now adsorbed bacterium. The result inevitably is an ecological loss of phages, one which can be considered to be a potential mechanism of virion inactivation that is in addition to nucleic acid or virion-protein damage. This loss of secondary phages is of issue when phages are being employed as antibacterial agents, e.g., (Abedon et al., 2011), since phages display one-hit killing kinetics of bacteria (Bull and Regoes, 2006) and thus any more than one adsorbing phage to a single bacterium represents a loss of antibacterial agent. This loss of secondary phages as killing agents occurs regardless of whether superinfection inhibition is expressed

by the primary infection since a single bacterial cell at most can only support a single phage burst. In addition, partial interference between coinfecting phages–i.e., mutual exclusion, partial exclusion, or depressor effect– can result in reductions of the productivity of resulting infections that in turn could result, for example, in less effective *in situ* phage amplification in numbers and/or poor phage penetration into bacterial biofilms (Abedon and Thomas-Abedon, 2010).

We would expect partial interference between coinfecting phages to occur particularly given phage therapy treatments that employ cocktails of otherwise not full coinfection-compatible phage strains. Resulting reductions in infection productivity during phage therapy, however, may be less of a concern given the formulation of infection-incompatible phages into cocktails (Chan et al., 2013; Chan and Abedon, 2012) so long as coinfection does not occur until late in phage population growth (i.e., until after most or all bacteria have already been bactericidally adsorbed), if passive rather than active treatments are employed (see below), or instead if phages are applied in multiple doses to infections rather than in just a single application (e.g., see arguments for multiple versus single dosing made in Abedon, 2012a; Abedon, 2014).

From a non-applied perspective the superinfecting phage, even if otherwise "wasted" in terms of bacterial killing, nevertheless may still survive to some degree genetically. This survival may be measured in terms of the proportion of a burst that is associated with the secondary phage's genome (Abedon, 1994), though it remains an open question whether the associated proportional loss of primary phage genetic contribution to such bursts in fact evolutionarily selects for mechanisms of superinfection inhibition (Abedon, 1999). Of far greater relevance, however, is the contribution of secondary phage genetic survival to phage genome evolution, that is, as can result phage genomic mosaicism (Hatfull and Hendrix, 2011; Hendrix et al., 1999; Hendri et al., 2008). Whether the associated recombination between phages is a consequence of coinfection during lytic infections, the superinfection of lysogenic infections, or in some manner occurring between prophages, unless two phages have initially adsorbed simultaneously then coinfections are a consequence of secondary infections, and particularly *parallel* secondary infections.

Parallel vs. serial secondary infection plus somewhere in between

Whether or not phage attachment, adsorption, and genome entry into a bacterium's cytoplasm occur, or indeed whether a phage contributes to an infection's burst, if the bacterium is already infected (primary infection) then these secondary phenomena can be viewed, as noted,

as occurring in *parallel* with the primary infection. That is, they occur essentially at the same time in association with the same bacterial cell. Furthermore, and as equivalent to superinfection, this usage of secondary infection is borrowed from medical microbiology, that is, the acquisition by an already infected individual of an additional infectious agent: Two different infection processes by potentially independently originating parasites occur in association with same host at the same time, that is, in parallel.

Within an individual host, secondary infection with a more serial connotation can refer to infections that are spatially removed from a primary focal infection, though which nonetheless are caused by the same lineages of pathogens. In a search of PubMed on "secondary infection" (in quotes), the oldest hit, dating from the late 19th Century (Pratt, 1899), appears to employ just that meaning. Here secondary can be viewed as temporally as well as spatially subsequent and therefore representing infections or at least "sub" infections that occur in serial. As the subsequent infection also occurs within the same host, the process can be viewed in addition as having tendencies towards parallel secondary infection. I described this form of secondary infection therefore as mixed parallel-serial.

This concept of mixed parallel-serial secondary infection would not appear to be applicable to the phage infection of individual bacteria. It is also not applicable to phage penetration into clonal bacterial clumps (Abedon, 2011a; Abedon, 2012b; Abedon, 2012c; Abedon, et al., 2010), that is, unless phage spread is discontinuous such that multiple foci of phage infection (secondary infections) both become established within and are seeded by infections–focal-infection equivalents–of the same, clonal bacterial formation. Hence,
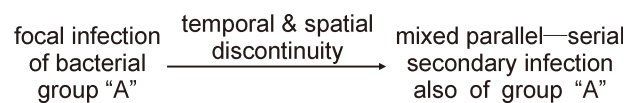
Alternatively, Iyer and James (1978) speak of using anti-phage serum to *prevent* the "secondary reinfection"–and perhaps synonymously "secondary infection"– of cells that perhaps had been phage infected but in fact are no longer phage infected. These phage and bacterial hosts otherwise display what the authors describe as a carrier state (Abedon, 2009b).

Thus, at least in principle a phage could be latently infecting a given bacterial lineage. As these bacteria replicate, at least one daughter cell could host a lytic infection while at least one other daughter cell could become spontaneously cured of its phage infection. The cured bacterium or its daughters could then become reinfected by a phage produced by the previously mentioned lytic infection. The result is secondary infection of a

not-currently phage infected bacterium but where that bacterium nonetheless is a member of the same bacterial population that otherwise is still hosting the same phage population, but in a different cell/location. This is a convoluted though not necessarily unrealistic scenario and is at least suggestive that this mixed parallel-serial concept of secondary infection could be applied to more than one phage-associated circumstance. For the general scenario to hold, however, then bacteria as host organisms must in some sense be viewed as multi-celled, which provides the parallel aspect, and also some form of temporal as well as spatial discontinuity separating infection foci, infections by what otherwise is a single lineage of phages, must exist as well to supply the serial aspect.

Secondary infections can be more purely serial by not involving subsequent infections in different locations of the same host but instead infections of entirely different hosts. Distinct from the phage literature, this usage is seen in epidemiology, e.g., (May and Anderson (1983): "R_0 is the average number of secondary infections produced when one infected individual is introduced into a wholly susceptible host population" (p. 197), but comparable wording is found in the phage literature as well (Payne et al., 2000; Payne et al., 2001; Wei and Krone, 2005). This meaning, as applied to phages, appears to be equivalent to that of Sanders (1987) (p. 215), who describe how during "Cheesemaking protocols… effectively disperse phage throughout the product, encouraging secondary infections." Similarly, sequential phage infections described as "secondary infections" can be viewed as occurring within individual bacterial arrangements, e.g., such as streptococci (Barron et al, 1970), or within biofilms (Hughes et al, 1998). Equivalent usage is seen also with descriptions of the prevention of the secondary infection of clonally related bacteria as a consequence of the action of bacterial abortive infection systems (Fukuda et al, 2008).

In short, serial secondary infections are sequential infections of different hosts, so thereby are not equivalent to parallel secondary infections which instead are of the same host. In addition, and also because infections are of different hosts, temporal and spatial discontinuities between infections occur automatically rather than requiring complicated scenarios, i.e., as was the case when considering instead mixed parallel-serial secondary infections. See Table 1 for further comparison of these varying forms of secondary infection.

Serial secondary infection and phage therapy

The epidemiological or serial perspective on secondary infection has entered the phage therapy literature as a means of characterizing what has been described as "active" treatment (Payne et al, 2000; Payne and Jansen, 2001; Payne and Jansen, 2003); see also for example (Golshahi et al, 2008; Hudson et al, 2005; Ryan et al, 2011). From Payne and Jansen (2003) (p316): "It is conceptually useful to distinguish primary infection (infection of bacteria by phage from the inoculum) and secondary infection (infection of bacteria by phage that have been released by lysis of already infected cells). If the inoculated phage are numerous enough then the first round of primary infection and lysis can by itself remove significant numbers of bacteria, which we refer to as passive phage therapy. When therapy is based on secondary infection then there is an increase in phage numbers via viral self-replication, which we refer to as active phage therapy." That is, at a minimum phage-mediated biocontrol of bacteria, such as phage therapy (Abedon, 2009c), can be differentiated into that which does not require *in situ* phage replication (described as passive or inundative treatment) versus that which does require *in situ* phage population growth, i.e., active treatment (Abedon et al., 2010). With passive treatment, phages are supplied to target bacteria at high multiplicities (>>1) since bacterial eradication, for statistical reasons, requires multiple phage adsorptions of individual bacteria (Abedon, 2011).
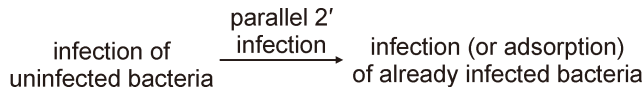
These multiple adsorptions, unless they all occur simultaneously, presumably involve secondary (2') infections, but of a parallel nature, that is, phage adsorption of already phage-infected bacteria:

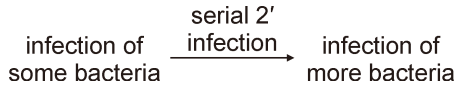Table 1. Distinguishing among secondary infection varieties

Secondary infection type	Parallel	Mixed parallel-serial	Serial
Involves single host organism?	Yes	Yes	No
Host must be relatively large?[a]	No	Yes	No
Spatially separated infections?	No[b]	Yes	Yes
Potentially multiple infecting lineages?	Yes[c]	No	No
Initial infection is called?	Primary	Focal	Primary or Index
Historically used in phage literature?	Yes	No[d]	Yes

[a]: For example, multicellular; [b]: Or at least parallel secondary infection is not defined in terms of spatial separation; [c]: For example, two distinct pathogen types or two distinct phage types, though for phages especially this is not an essential criterion; [d]: See, however, discussion in main text.
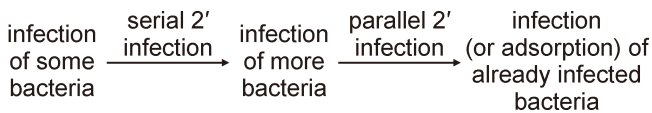
$$\text{infection of uninfected bacteria} \xrightarrow{\substack{\text{parallel 2}' \\ \text{infection}}} \text{infection (or adsorption) of already infected bacteria}$$

Payne R J H and Jansen V A A (2000) nevertheless refer to passive treatment as "by primary infection alone". They are not incorrect in this usage, however, but instead are using the term secondary infection to describe infections that occur serially rather than in parallel, i.e., in the course of phage population growth:

$$\text{infection of some bacteria} \xrightarrow{\substack{\text{serial 2}' \\ \text{infection}}} \text{infection of more bacteria}$$

Thus, initially supplied phages provide primary infections which, through phage infection and virion release, give rise to subsequent, that is, *secondary* infections. Indeed, the following progression more or less occurs as phage densities increase *in situ* in the course of phage population growth,

$$\text{infection of some bacteria} \xrightarrow{\substack{\text{serial 2}' \\ \text{infection}}} \text{infection of more bacteria} \xrightarrow{\substack{\text{parallel 2}' \\ \text{infection}}} \text{infection (or adsorption) of already infected bacteria}$$
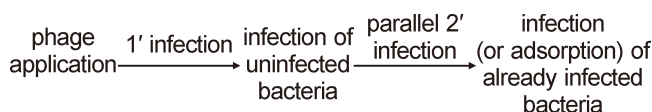
with the transition between serial to parallel secondary infections, on a population-wide basis, occurring gradually rather than abruptly. Thus, the proportion of adsorptions to non-yet phage infected bacteria (serial secondary adsorption) at first predominates but then declines as the proportion of phage adsorption to already phage-infected bacteria ascends (parallel secondary adsorption). I derive this latter point further in the following section.

## Secondary infection relevance to phage therapy

There is more to active phage therapy than simply phage population growth. Just as with passive treatment, ratios of adsorbing phages to target bacteria must substantially exceed one–due to the Poisson distribution of adsorbing phages to bacteria–if bacteria are to be eliminated (Abedon, 2011). This requirement that multiplicities ultimately exceed one leads to an interesting conflict in terms of use of the concepts of secondary infection.

First, as noted and just as one sees with lysis from without as considered above, the "primary infections" of passive treatment, unless truly occurring simultaneously in terms of their adsorption, involve both primary and secondary infections, the latter of the parallel kind:

$$\text{phage application} \xrightarrow{1' \text{ infection}} \text{infection of uninfected bacteria} \xrightarrow{\substack{\text{parallel 2}' \\ \text{infection}}} \text{infection (or adsorption) of already infected bacteria}$$
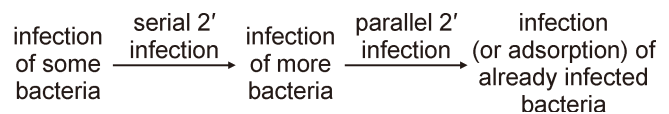
For active treatment to be successful, this same end point must be reached, i.e., where phage numbers are sufficiently high that bacterial numbers are substantially reduced, a process which inherently will be associated with phage adsorption and potentially also infection of already phage-infected bacteria.

Second, and contrasting passive treatment, with active treatment the original infections will tend to be primary in both a serial and parallel sense, with the latter due to the initially lower phage multiplicities that active treatment tends to imply. Therefore, there will be a relative lack of parallel secondary infections among these otherwise serial primary infections:

$$\text{phage application} \xrightarrow{1' \text{ infection}} \text{low multiplicity adsorption and infection of some bacteria}$$
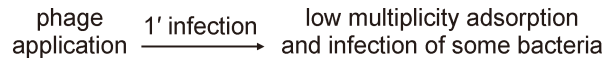
That is, the initially added phages, which are primary infections in a serial sense, will tend to give rise to bacteria that have been adsorbed at most by only a single phage (and which as a consequence will make the majority of these serial primary infections also primary infections in a parallel sense).

As noted, subsequent secondary infections, in an epidemiological/serial sense, ultimately must result in secondary infections in a per-target-cell/parallel sense if phage therapy is to be successful (multiple phage adsorptions of individual bacteria to assure multi-fold eradication of those target bacteria). These secondary infections that are associated with higher phage multiplicities, in other words (parallel secondary infections), represent a consequence of secondary infections that are associated with phage population growth (serial secondary infections). Thus, as presented also towards the end of the previous section,

$$\text{infection of some bacteria} \xrightarrow{\substack{\text{serial 2}' \\ \text{infection}}} \text{infection of more bacteria} \xrightarrow{\substack{\text{parallel 2}' \\ \text{infection}}} \text{infection (or adsorption) of already infected bacteria}$$

Secondary infection must occur for active treatment to result in substantial elimination of target bacteria. It must occur, however, as two wholly distinct phenomena: Infection of subsequent bacteria by phages produced *in situ* (secondary infection in a serial sense) in combination with what in most cases is a subsequent as well as staggered high multiplicity infection or adsorption of remaining target bacteria (secondary infection in a parallel sense).

CONCLUSION

Because of differing traditions, as well as numerous associated phenomena, the concept of secondary infection can be surprisingly complex. It is important nonethe-

less to distinguish meanings, perhaps particularly as they may be employed in the phage therapy literature. To help stem confusion, for authors it can be important to define intended meanings of secondary infection upon use. In particular, parallel secondary infection, as defined here, involves at a minimum multiple, distinct phages interacting with a single bacterial cell whereas serial secondary infection involves multiple phage interactions with multiple bacterial cells.

COMPLIANCE WITH ETHICS GUIDELINES

The author declares that they have no competing interests. This article does not contain any study with human or animal subjects performed by any of the authors.

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