1 # Combatting intracellular pathogens using bacteriophage delivery

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8 ## Abstract

9 Intracellular pathogens reside in specialized compartments within the host cells
10 restricting the access of antibiotics. Insufficient intracellular delivery of antibiotics
11 along with several other resistance mechanisms weaken the efficacy of current
12 therapies. An alternative to antibiotic therapy could be bacteriophage (phage)
13 therapy. Although phage therapy has been in practice for a century against various
14 bacterial infections, the efficacy of phages against intracellular bacteria is still being
15 explored. In this review, we will discuss the advancement and challenges in phage
16 therapy, particularly against intracellular bacterial pathogens. Finally, we will highlight
17 the uptake mechanisms and approaches to overcome the challenges to phage
18 therapy against intracellular bacteria.

19 **Keywords:** Antibiotic resistance, Phage uptake, Intracellular phage delivery, Particle
20 engineering

21 **Abbreviations:** MRSA: methicillin-resistant *Staphylococcus aureus*, PLGA: poly
22 (lactic-co-glycolic) acid, CPP: cell-penetrating peptides

1. Introduction

Several bacterial pathogens have developed strategies to survive within a mammalian host cell and are known as intracellular bacteria. These pathogens modulate the intracellular environment, create a suitable niche, and bypass the harsh consequences of encountering the host immune system. Intracellular bacteria can be classified as obligate, which are unable to grow outside a host cell like *Chlamydia trachomatis (C. trachomatis)* and *Coxiella burnetii*, or facultative, which can grow outside and inside a host cell for e.g. *Salmonella enterica (S. enterica), Mycobacterium tuberculosis (M. tuberculosis) and Listeria monocytogenes (L. monocytogenes)* [1-4]. These pathogens account for high mortality in humans. About 1.4 million patients succumbed to tuberculosis in 2019 [5]. In 2015, 90,300 deaths occurred from non-typhoidal salmonellosis and about 178,000 people died of typhoidal salmonellosis [6]. Additionally, other pathogens like *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli),* and *Pseudomonas aeruginosa* (*P. aeruginosa)* can also invade and survive within host cells [7-9].

A major challenge for treatment intracellular bacteria is the inability of many antibacterial agents to cross the mammalian cell membrane barrier [10]. The rising concern over antibiotic resistance demands novel treatment approaches. Phages are viruses that infect and lyse specific host bacteria and their application as therapeutics is termed as phage therapy. There is a large reservoir of around 10^{31}-10^{32} phages in the biosphere, which could be tapped in, to solve the challenge of antimicrobial resistance [11,12]. Multiple review articles on phage therapy have been published recently [13-16], however, to our knowledge, only a few review articles have exclusively focused on developing new strategies to deliver phages for targeting intracellular pathogens [17,18]. In this review, we have primarily discussed the

48 advancement in phage therapy to control intracellular pathogens. We have also
49 highlighted pertinent challenges and possible solutions for phage therapy to tackle
50 intracellular bacterial infections.

51 2. Intracellular bacteria

52 Intracellular bacterial pathogens are classified in facultative if able to grow either
53 inside or outside a host cell or obligate if unable to grow outside a host cell.
54 Intracellular pathogens have the inherent ability to cross the mammalian cell
55 membrane via multiple mechanisms [19,20]. These pathogens can penetrate and
56 localize in both phagocytic (for instance, macrophages) and non-phagocytic (for
57 instance, endothelial/epithelial cells) hosts [21]. Organisms like *Mycobacterium*
58 utilizes the natural phagocytic ability of the macrophages to invade via receptor
59 mediated endocytosis and reside within them [22,23]. On the other hand, pathogens
60 like *C. trachomatis, S. aureus,* and *Shigella* have specialized mechanisms to induce
61 cytoskeletal reorientation that generates phagocytic activity in non-phagocytic
62 epithelial cells [24-27]. Unlike these pathogens which can invade specific cell type,
63 *Salmonella* has the ability to infect both the macrophages and the non-phagocytic
64 epithelial cells. These pathogens utilize several mechanisms of cellular entry **(Table
65 1)**. Some pathogens like *Listeria* and *Yersinia* have surface proteins that binds to
66 specific host cell receptor and induces a signalling cascade event that facilitates
67 clathrin mediated endocytosis (Zipper mode) [28,29]. Alternatively, pathogens like
68 *Salmonella* and *Shigella* utilizes its type 3 secretion system to translocate effector
69 molecules into the host cell that triggers actin rich membrane ruffles necessary for
70 endocytosis (Trigger mode) [30,31]. Both mechanisms are extremely crucial to
71 infiltrate non-phagocytic epithelial cells.

72 Upon internalization, these pathogens reside within specialized intracellular
73 compartments known as phagosomes and/or endosomes (**Fig 1**). In phagocytes,
74 these phagosomes are destined to lysosomal fusion, where the low pH and
75 degradative enzymes destroy the pathogens. However, most of these intracellular
76 pathogens have evolved strategies to block phago-lysosomal fusion and subvert the
77 harsh bactericidal environment within the lysosomes. Interestingly, some pathogens
78 can escape from the phagosomes and survive in the cytoplasm [8,32] (**Fig 1**). For
79 instance, *M. tuberculosis* arrests phago-lysosomal fusion and even survives in the
80 cytoplasm and lysosomes, thus creating a diverse niche for its survival within
81 macrophages [4,33]. *S. aureus* can survive and replicate within the phago-lysosomes
82 in a low pH condition [34]. These diverse mechanisms employed by the pathogens to
83 reside within the target cell create additional limitations for the current treatment
84 strategies.

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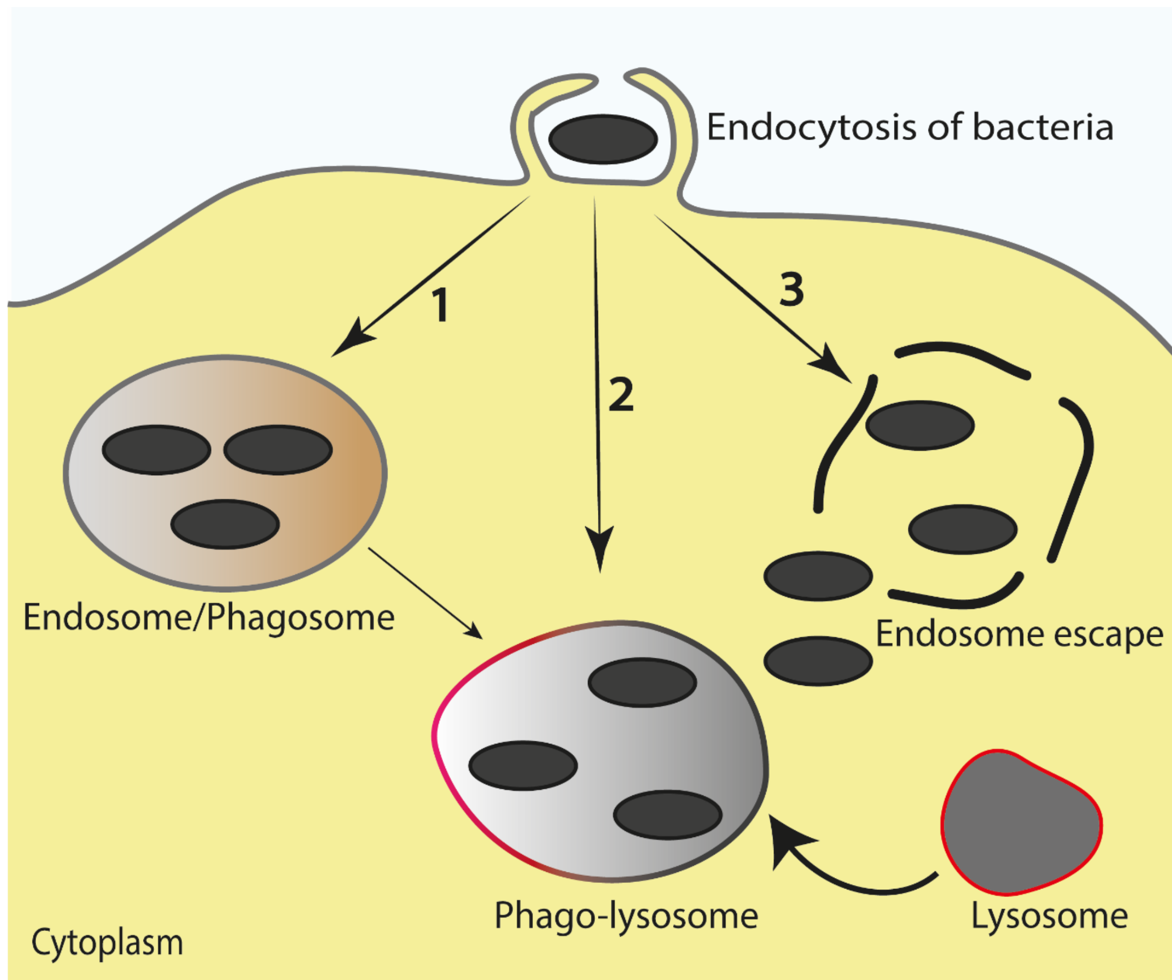


86 **Fig 1: Intracellular sites where the pathogens reside.** Intracellular bacteria reside at
87 specialized locations within the host mammalian cell. These sites include (1) the
88 endosome/phagosome, (2) the phago-lysosomal compartment, or (3) the cytoplasm.

89 ## 3. Drawbacks of antibiotics to curtail intracellular bacterial infection

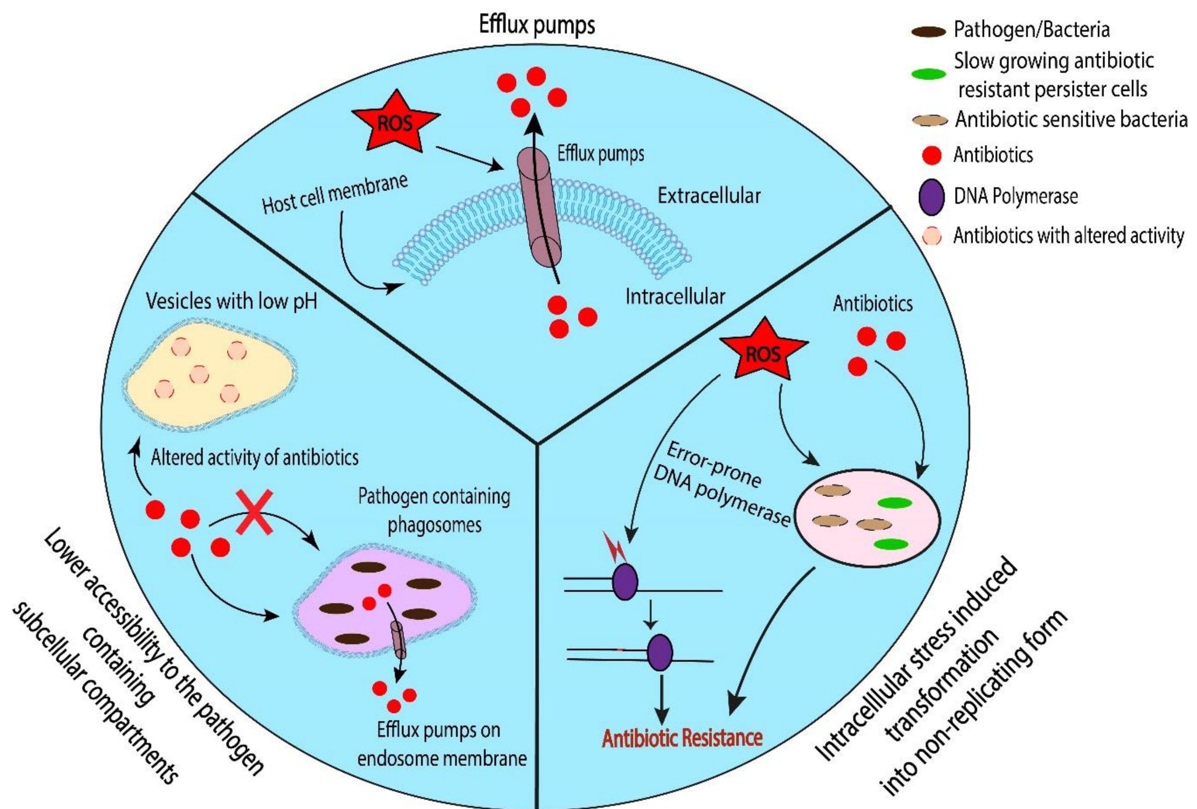
90 The effectiveness of an antibacterial agent depends on its local concentration, which
91 should be sustained at a therapeutic level to eliminate the pathogen. Exposure to a
92 suboptimal dose of antibiotics leads to the selection of drug-resistant bacteria or the
93 development of a tolerant phenotype [35]. Some of the hurdles that encountered
94 during antibiotic treatment while targeting intracellular pathogens are illustrated in
95 **figure 2**.

Drugs like macrolides and quinolones are readily taken up by phagocytic cells. Despite the intracellular accumulation of antibiotics, efflux pumps present on mammalian surface and endosomal membranes, like P-glycoproteins and multidrug resistance proteins [36], lower the intracellular drug concentration within a host cell [37,38]. This hampers the local intracellular concentration required for killing the pathogen. For example, reduced intracellular activity of several antibiotics like fluoroquinolones, macrolides, streptogramins, lincosamides and rifampicin was observed against *L. monocytogenes* in multidrug resistant protein 1 overexpressed carcinoma cell line [39]. P-glycoprotein inhibitors (verapamil, ciclosporin, and GF 120918) and multidrug resistance protein inhibitors (gemfibrozil or probenecid) were shown to be effective in increasing the intracellular accumulation of the antibiotics in mammalian cells; however, disparate effects of various inhibitors are observed and their efficacy is dependent on the antibiotics used [40-42].

Apart from intracellular drug concentration, subcellular localisation of bacteria also governs the efficacy of antibiotics [43-45]. Some pathogens like *S. aureus* and *M. tuberculosis* have an inherent ability to survive within the phago-lysosomal compartment with a low pH condition of 4.5-5.0. Maintaining the activity of antibacterial agents at such a low pH is another challenge [46,47].

Stress induced by antibiotics and host oxidative response can cause the transformation of bacteria into a non-replicating metabolic state, known as persisters [48,49]. Numerous bacteria like *M. tuberculosis, P. aeruginosa, Salmonella* and *S. aureus* can persist in a growth arrested state with reduced metabolism which results in high tolerance to antibiotics over a long period of time [50]. For example, internalization of *S. typhimurium* within the macrophage vacuolar environment induces phenotypic heterogeneity that results in the formation of non-replicating

121 persister cells [51]. Wayne and Sohaskey had extensively discussed the non-
122 replicating nature of *M. tuberculosis* within the host that causes latent infections that
123 are often resistant to conventional treatment [52]. Although there are alternative
124 approaches like intermittent drug doses to target persisters, these have not been
125 widely explored in clinics [53] (**Fig 2**). Phagocytes produce reactive nitrogen and
126 oxygen species as a response to invading pathogens [54]. The antimicrobial function
127 of reactive oxygen species (ROS) has been well documented in killing intracellular
128 pathogens and inducing signaling events to trigger an inflammatory response [55].
129 Interestingly, recent studies have reported that the consequence of respiratory burst
130 leading to ROS production failed to kill intracellular pathogens like *S. aureus* and was
131 shown to induce tolerance to multiple antibiotics [56]. Similarly, connection between
132 ROS and antibiotic tolerance has been established in *M. marinum* where intracellular
133 ROS resulted in increased expression of drug efflux pump [57,58]. Although not
134 experimentally proven, another explanation could be DNA mutagenesis caused by
135 ROS exposure leading to physical damage to the genetic material [59,60]. Error-
136 prone polymerase activity in response to the stress induced by respiratory burst might
137 lead to random mutations causing antibiotic resistance or tolerance [61,62]. (**Fig 3**).



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139 **Fig 2: Drawbacks of antibiotics against intracellular pathogens.** A schematic
140 representation that depicts the multidimensional shortcomings of antibiotics against
141 intracellular infection.

142 4. Advances in phage therapy to combat intracellular pathogens

143 Phage therapy is emerging as a last-resort treatment for patients with antibiotic-
144 resistant infections. In a recent report, Dedrick et al. showed that phage therapy could
145 be used for treating disseminated drug-resistant *M. abscessus* infection in a young
146 cystic fibrosis patient [63]. A cocktail of genetically engineered phage effectively
147 cleared *M. abscessus* infection when delivered intravenously and led to wound closure
148 and resolution of infected skin nodules [63].

149 For phages to be effective against intracellular infections, they need to reach the
150 intracellular site where the bacteria reside. The studies on intracellular phage delivery

can be grouped into two categories - free phage delivery and carrier-mediated delivery of phages.

4.1 Free Phage delivery

Within their host, intracellular pathogens are shielded from numerous effector molecules and cells due to the protective semi-permeable plasma membrane. Phages are high molecular weight molecules (> 10 megadaltons) that are unable to passively diffuse across the plasma membrane. However, there are several mechanisms that can be utilized by phages to overcome this biological barrier. The possibility of phage interaction and transport through mammalian cells became evident in the early 1970s when high doses of phages against *V. cholerae* were administered orally for the treatment of diarrhea in humans and about 10^2 pfu/mL phages were observed in the blood throughout treatment duration [64]. Similarly, phages have been found in different organs after intravenous delivery. Recently, a phage mixture was administered intravenously for the treatment of a patient with disseminated drug resistant *M. abscessus,* and a substantially high titer of phages was detected in sputum and feces, in addition to serum [63]. Phages have also been detected in the brain tissue after intranasal delivery [65] and even fetal tissue after systemic administration [66]. Nguyen et al. demonstrated rapid transcytosis of phage T4 from apical to basolateral chambers in a variety of cells like Madin-Darby Canine Kidney (MDCK) cells, T84 cells (colon epithelial), Caco-2 cells (colon epithelial), A549 cells (lung epithelial), Huh7 cells (hepatocyte epithelial cell-like), and hBMec cells (brain endothelial) [67]. It was suggested that phages traffic through the Golgi apparatus. Phage UAB_Phi20 was also shown to be transcytosed across Caco-2 cells and human colon tumorigenic (HT-29) cells [68]. In addition to transcytosis, phages have been reported to utilize phagocytosis, endocytosis and pinocytosis to enter

176 mammalian cells **(Fig 3)**. Phagocytosis is carried out by professional phagocytes,
177 usually immune cells such as monocytes, macrophages, and dendritic cells. These
178 cells can engulf invading bacteria, particles, or cell debris and eventually degrade them
179 in lysosomes. Endocytosis is a constitutive process carried out by all mammalian cells
180 for the uptake of nutrients and small molecules. Clathrin and caveolae-mediated
181 endocytosis are two major types of endocytosis, among others, observed in
182 mammalian cells. Pinocytosis involves uptake of large amounts of extracellular fluid,
183 which is also a constitutive process in mammalian cells. For instance, phages can be
184 engineered to enable penetration through the gut. The peptide YPRLLTP (identified
185 by *in vivo* bio-panning protocol) displayed on the capsid of M13 phages facilitated
186 translocation across the intestinal lining [69].
187 Phage interaction with mammalian cells for therapeutic implications has been
188 extensively reviewed [70,71]. Tian et al. showed that in HeLa cells and MCF-7 breast
189 cancer cells, M13 enters through clathrin-mediated endocytosis and micropinocytosis
190 [72], while it uses caveolae-mediated endocytosis for human dermal microvascular
191 endothelial cells. The internalization efficiency of M13 phage was enhanced several
192 log folds by genetically modifying the phages to display cell-penetrating domains 3D8
193 VL transbody or TAT peptide [73]. These modifications resulted in different modes of
194 phage internalization and fate as they interact with distinct cell surface
195 glycosaminoglycans. 3D8 VL-M13 utilized the caveolae-mediated endocytosis and
196 remained stable after internalization for more than 18 h in the cytosol. TAT modified
197 phage was mainly internalized via clathrin and caveolae-mediated endocytosis and
198 were found in multiple subcellular compartments and were degraded in lysosomes
199 within 2 h of internalization. Overall, this report focuses on the efficiency rates at which

200 engineered M13 displaying various peptides can be delivered into the mammalian
201 cells [73].
202 Phage opsonisation by serum proteins can trigger phagocytosis [74]. Møller-Olsen et
203 al. showed internalization of fluorescently labelled phage K1F-GFP into human urinary
204 bladder epithelial cells via phagocytosis [75]. These phages were successful in killing
205 both extracellular and intracellular *E. coli* EV36-RFP infection in T24 urinary epithelial
206 cells. The authors used a SYTOX dead cell stain and estimated the co-localization of
207 SYTOX with *E. coli* RFP using confocal microscopy. Phage treatment showed 77%
208 co-localization compared to 29% in untreated samples [75].

209 Zhang et al. showed endocytosis and accumulation of *S. aureus* phage
210 vB_SauM_JS25 in non-phagocytic MAC-T bovine epithelial cells over time [76].
211 Microscopic evaluation showed that about 12% of cells were positive for phages. A
212 time-dependent intracellular killing (1-1.5 log order reduction in 12 h) of *S. aureus* by
213 phage vB_SauM_JS25 was observed in MAC-T bovine epithelial cells. The
214 extracellular bacteria was eliminated by adding lysostaphin (20 μg/ml) 1h after
215 infection [76].
216 Lehti et al. described that endocytic uptake of *E. coli* PK1A2 phage into live eukaryotic
217 neuroblastoma cells is dependent on the presence of polysialic acid residues on the
218 mammalian cell surface [77]. The phages show initial adsorption to the polysaccharide
219 receptor which enabled uptake. Phage adsorption and internalization were lower when
220 there was less sialic acid on the cell surface or when sialic acid was added separately
221 as a competitive binding site. Many internalized phages (~30%) were found to be
222 active even after 24 h. The phages eventually localized to the lysosome and became
223 undetectable in 48 h [77].

224 Peng et al. showed that free mycobacteriophage D29, a lytic phage, significantly
225 reduced the intracellular *M. tuberculosis* count in peritoneal macrophage cells [78].
226 Mouse peritoneal macrophages were infected with *M. tuberculosis* H37Rv for 4 h
227 followed by washing for three times with Hank's buffered salt solution (HBSS) to
228 remove all extracellular bacteria. Phages treatment were given for 24 h and 48h post-
229 infection. Compared to the control group (no phage treatment), a high dose of phages
230 (2.0 x 10^7 pfu/well) effectively lowered the viable intracellular bacterial count by 76%
231 after 24 h and 92% after 48 h [78]. Additionally, Lapenkova et al. investigated the effect
232 of D29 on the mouse macrophages infected with virulent mycobacterial strain H37Rv
233 [79]. Phage D29 (10^8 pfu) was incubated with infected macrophages for 24 h, followed
234 by washing and re-incubation with fresh D29 for another 24 h. Intracellular bacteria
235 (H37Rv) were plated after disrupting the infected macrophage membrane by two
236 freeze/thaw cycle and allowed to grow from 3 weeks. Results depicted a 10-fold
237 reduction in CFU counts in phage treated samples compared to control samples [79].
238 Promising results were also observed against other opportunistic intracellular
239 pathogens like *E. coli* and *S. aureus*. Capparelli and group documented that *in vitro*
240 phage (M^{Sa}) treatment of intracellular *S. aureus* infected peritoneal macrophages
241 resulted in 70% reduction in CFU [80]. To check for efficacy of M^{Sa} against local
242 infection, *S. aureus* was administered subcutaneously followed by M^{Sa} treatment 4
243 days later. Phage treatment resulted in a 2-log fold reduction in bacterial CFUs
244 compared to untreated group and led to 97% survival of mice infected with lethal doses
245 of *S. aureus* A170 strain. Although in *in vivo* studies, there was no direct evidence
246 about whether the phages were effective against extracellular or intracellular bacteria
247 [80]. The authors further showed a significant role of the phage in controlling

248 Methicillin-resistant *S. aureus* (MRSA) infection (100% survival of mice treated with
249 10^9 pfu/mice compared to 20% survival in the untreated group) [80].

250 Phage therapy against facultative intracellular pathogen *Burkholderia pseudomallei*
251 was successful both *in vitro* and *in vivo* [81]. When phage C34 was added prior to
252 infection, the survival rate of *B. pseudomallei* infected lung epithelial cells (A549 cell
253 line) increased by 2-fold *in vitro*. However, no significant effect was observed when
254 the phage was delivered post-infection. The authors further showed that pre-treatment
255 (24 h before infection) and post-treatment (2 h after infection) also protected (33%
256 survival compared to no survival in untreated) the intranasally infected (*B.*
257 *pseudomallei*) mice which highlights the prophylactic and therapeutic potential of
258 phages. Nevertheless, no direct evidence of reducing intracellular bacterial load was
259 documented *in vivo* [81]. Similar study in support of prophylactic ability of phage
260 treatment has been reported for *M. tuberculosis* infection using D29 phage [82]. C57
261 black mice were exposed to aerosolized phages (7.7 ± 0.3 $\log_{10}$ PFU/mouse) 30 mins
262 prior to 50-100 CFU of *M. tuberculosis* H37Rv. Compared to untreated mice, 70%
263 reduction in CFU count was observed in phage treated animals after 24 h of infection
264 [82]. Kolenda et al. reported that in uninfected cells, only ~100 pfu/mL were recovered
265 from the osteoblasts after 24 h of incubation with free phages (10^7-10^9 pfu/mL) while
266 in *S. aureus* infected osteoblasts, 10^6-10^7 pfu/mL of phages were recovered indicating
267 that phage entry into the osteoblasts was dependent on re-infecting bacteria and the
268 phages were proliferating within the bacteria. This was supported by co-incubation
269 with vancomycin and rifampin that reduced the extracellular re-infecting bacteria and
270 resulted in only ~10^3 pfu/mL present intracellularly [83]. Detailed investigation in this
271 study revealed that phages could get internalized only after adsorbing to the re-
272 infecting bacteria however they were ineffective in reducing intracellular CFU despite

internalization. This lack of efficacy could be either loss of the phage activity in the intracellular environment or induction of bacterial dormancy within osteoblasts which may have inhibited propagation of phages. However, no evidence was provided in the study to clarify these speculations. Studies which have discussed free phage delivery for intracellular pathogens have been summarized in Table 2.

To improve therapeutic outcomes for intracellular infections, it is essential to enhance phage uptake by infected cells and target the intracellular bacteria.

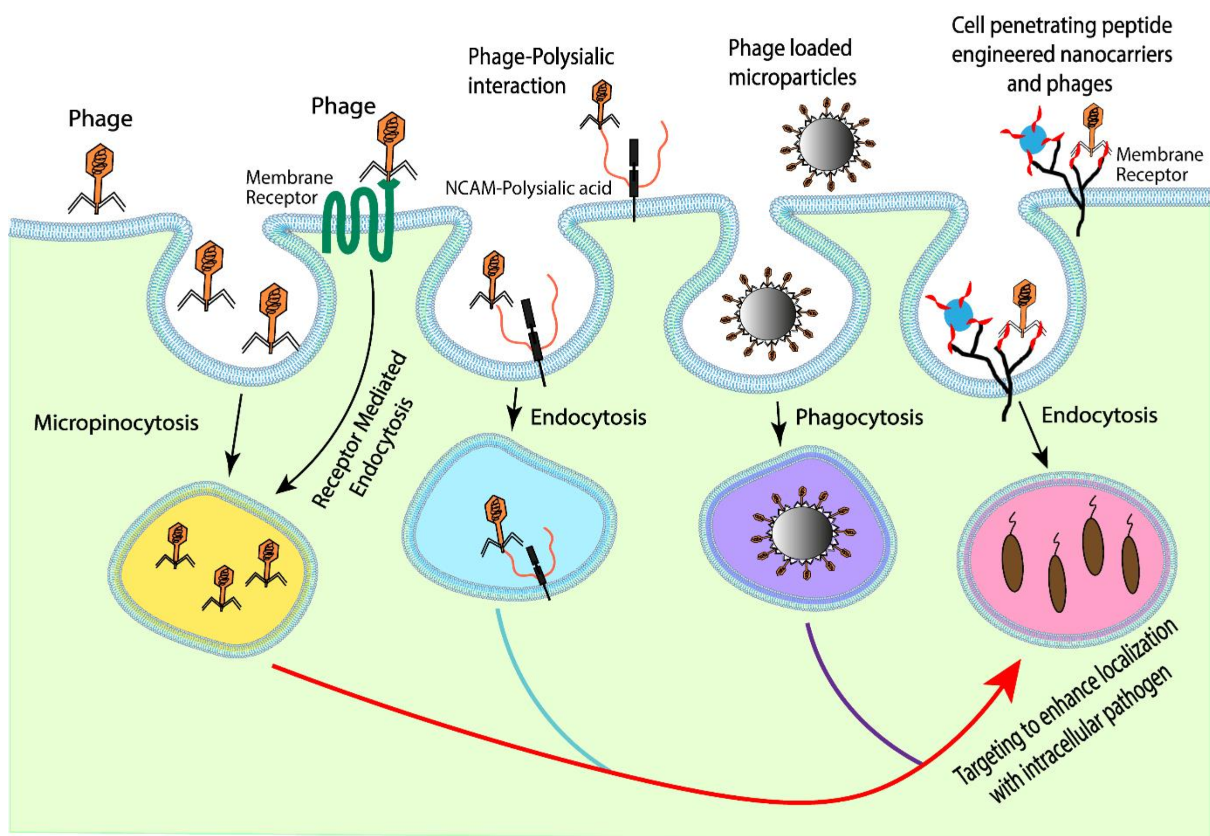


Fig 3: Mechanism of phage uptake. The illustration depicts various modes of intracellular uptake of free phages or carrier mediated phages.

4.2 Carrier Mediated Phage delivery

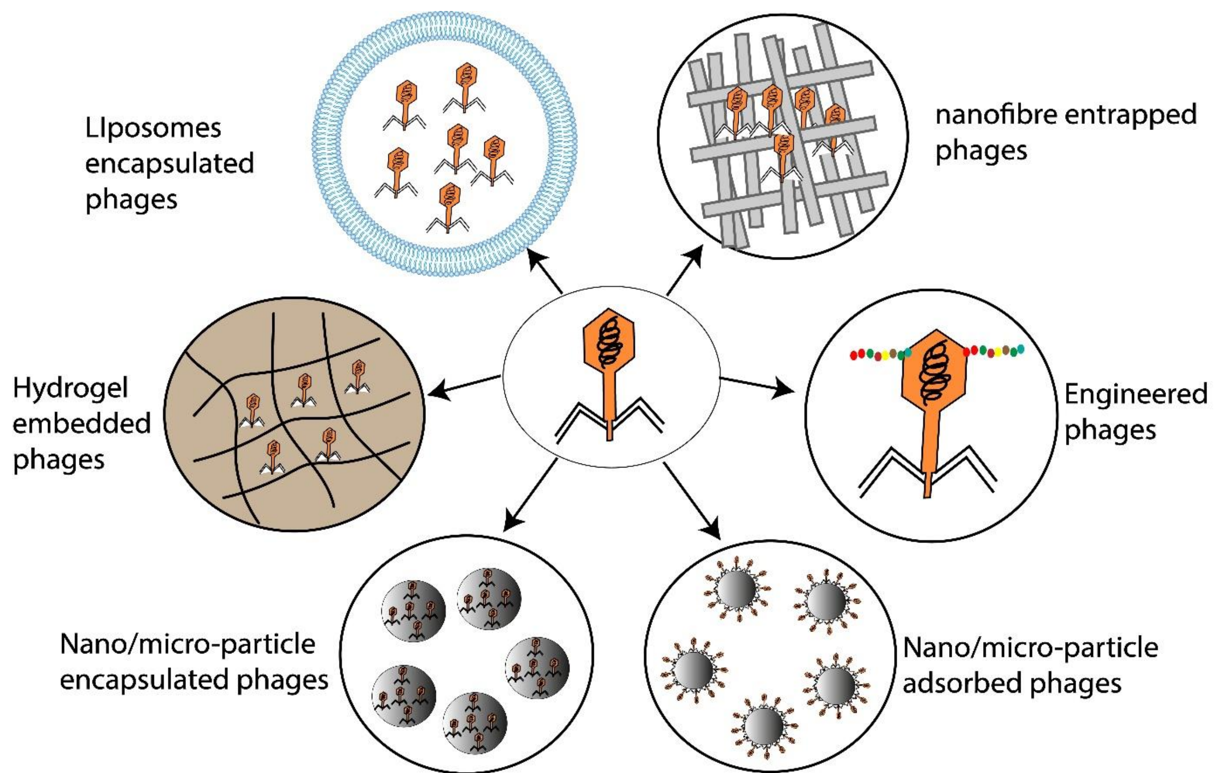
Although free phage therapy is effective in some instances, as mentioned above, there is a need to improve the delivery of the phages intracellularly. This can be achieved

286 by a “Trojan horse” approach that involves encapsulation or loading of phages onto
287 carriers. Studies on carrier mediated phage therapy have been summarized in Table
288 3. Broxmeyer et al. reported a novel strategy of phage delivery via non-pathogenic
289 strain *M. smegmatis* infected with phage TM4 [84]. Neither *M. smegmatis* alone nor
290 phage TM4 treatment alone affected intracellular *M. tuberculosis* and *M. avium* count
291 within RAW 264.7 cells. Interestingly, TM4 infected *M. smegmatis* was phagocytosed
292 and colocalized with *M. avium* containing vacuole after internalization. This resulted in
293 an approximately 100-fold reduction in the bacterial load after 48 h of treatment *in vitro*
294 [84]. The results were further validated *in vivo* by Danelishvili et al. to treat
295 disseminated *M. avium* infection using phage TM4 adsorbed on *M. smegmatis* that
296 accounted for significant reduction in bacterial load in the spleen. Although significant,
297 direct evidence of phage mediated intracellular bacterial killing was not presented in
298 animal model [85]. The idea of vectorization proposed by Broxmeyer and group [84]
299 ensured the delivery of active phage to the target site after subcutaneous
300 administration. While innovative, the administration of live bacteria in patients is risky
301 for patients. The use of biomaterials such as liposomes and polymeric particles can
302 provide a suitable alternative for clinical translation as several such systems are
303 already clinically approved (such as AmBisome and Doxil) [86,87]. A few approaches
304 have been developed to encapsulate phages in liposomes and polymers [88,89]. For
305 instance, 100 nm cationic liposome-encapsulated phages, KPO1K2, (average phage
306 size of ~ 50nm) formulated by conventional approach (lipid thin film hydration) ensured
307 effective intracellular delivery inside *Klebsiella pneumonia* (*K. pneumoniae*) infected
308 macrophages [90]. Gentamicin was added to the culture media to kill any extracellular
309 bacteria and only focus on intracellular bacteria. The authors reported that cationic
310 liposomes carrying phages caused 94.6% killing of intracellular *K. pneumoniae*

311 compared to free phages which accounted for 21% killing after 24 h [90]. In addition,
312 liposome-encapsulated phages were also shown to be protected against neutralizing
313 antibodies compared to free phages [90]. Another group reported that intraperitoneal
314 delivery of liposome-encapsulated phage cocktails was effective against *K.*
315 *pneumoniae* infected burn wounds in BALB/c mice model [91]. In this study, phage
316 entrapped liposomes with an average diameter of 229 nm showed an encapsulation
317 efficiency of 79.2 ± 5.6%. After 72 h of treatment, liposome entrapped phage cocktail
318 showed 1-2 log order reduction in bacterial counts compared to free phages in skin,
319 blood, and liver. Although the phage activity on killing intracellular bacteria couldn't be
320 established from the *in vivo* experiment [91].

321 Along with liposomes, nanocrystals and polymers can also serve as an efficient tool
322 for phage encapsulation and delivery [92-94] **(Fig 4)**. Fulgione et al. reported that
323 biomimetic hydroxyapatite (HA) nanocrystals effectively delivered *Salmonella* phage
324 (SR φ1) intracellularly [95]. HepG2 cells were infected with 10^8 CFU/mL of *S. enterica*
325 serovar Rissen followed by treatment with free SR φ1 (10^7 PFU/mL), only HA
326 nanocrystals or equivalent phage-loaded HA nanocrystals for 24 h. Intracellular CFU
327 count revealed reduced count of 10^5 CFU/ml for HA-SR φ1 compared to 10^8 CFU/ml
328 in free phage and only HA group. Extracellular bacteria was eliminated by using
329 gentamicin for 3 h post infection. The authors also confirmed enhanced stability of
330 HA-SR φ1 at pH 4.0 compared to free phages suggesting a potential use of such
331 mineral crystals at low pH conditions like phagolysosomes [95].

332 Overall, these studies suggest that the use of carrier-mediated delivery systems in the
333 form of liposomes or polymers can be utilized for efficient intracellular phage delivery.



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335 **Figure 4: Various phage delivery technologies**. Multiple encapsulation and delivery
336 techniques have been implemented to increase the stability and intracellular delivery
337 of phages.

338 5. Challenges towards intracellular phage therapy

339 While phage therapy holds the potential to tackle intracellular bacterial infections,
340 several challenges constrain their translation into clinics. Apart from the usual
341 challenges for translation of phage therapy for extracellular bacteria such as bacterial
342 defence mechanisms (CRISPR/CAS machinery, restriction-modification system) [96-
343 99], bacterial accessibility [100] and phage stability in in-vivo conditions [101], there
344 are several other challenges for intracellular phage therapy. Intracellular bacteria can
345 develop resistance against phage infection by multiple mechanisms. When these
346 pathogens establish infection intracellularly, they encounter multiple environmental
347 stresses such as nutrition deprivation, hypoxia, or changes in pH [102]. Such

conditions can induce a state of dormancy and render the bacteria metabolically inactive, thus preventing phage amplification and cell lysis [103]. Studies on phage infection on bacteria under dormant, acidic, and hypoxic growth conditions are limited. It was shown by Swift et al. that phage D29 was not effective in lysing the bacterial host *M. smegmatis* in a hypoxic environment [104]. On the contrary, some studies have reported successful phage infection in stationary phase host bacteria [105,106]. A recent study has reported that a cocktail of phages was effective in inhibiting *M. smegmatis* growth under acidic, hypoxic and stationary phase growth conditions [107]. Such investigations are critical for designing phage formulations that will be effective in lysing bacteria in their intracellular niche. In addition, stress conditions can trigger the production of outer membrane vesicles that carry phage receptors and can act as a decoy and bind phage while the host bacteria remains unaffected [108,109]. Intracellular pathogens may also be sequestered in vacuoles and phages may not be able to target such infections.

It is evident that the occurrence of phage-resistance is inevitable and thus bacterial susceptibility testing is critical before initiation of therapy. Unfortunately, resistance can also emerge during treatment. The application of a phage cocktail is suggested to mitigate the problem. Unlike antibiotics, there are numerous lytic phages for most bacterial species that can be used to form phage mixtures and overcome resistance. Phage resistance can emerge if the bacteria modify its cell surface receptor and blocks attachment of phages. It is expected that similar to extracellular bacteria, use of phage cocktails would decrease the probability that bacteria would develop resistance against all the phages at once. Hence, rational designing of a phage cocktail, identification of bacterial receptors targeted by each phage becomes imperative to design phage cocktails that target diverse bacterial receptors. This would ensure that

373 the evolving phage-resistant population will still be susceptible to other phages in the
374 cocktail [110-112]. A phage cocktail was shown to reduce development of phage
375 tolerance in *M. smegmatis* and *M. tuberculosis* compared to treatment with single
376 phages [107]. Broad-range lytic phages need to be identified or developed and tested
377 against clinical strains and demographically predominant strains in addition to lab
378 strains [113-115]. Synergy between phages and antibiotics is a widely reported for
379 extracellular bacteria [116]. The combinatorial effect of phages along with antibiotics
380 has been investigated in multiple opportunistic pathogens like *E. coli, S. aureus, K.*
381 *pneumoniae, Acinetobacter baumannii and Enterococcus faecalis* (extensively
382 reviewed in [14,117]). However, there is need to study phage-antibiotic synergy in
383 context of intracellular bacteria as it could pave way for combinatorial treatment to
384 prevent rapid rise in resistance against phages and antibiotics.

385 The host defence mechanism can exert additional barrier to the circulating phages
386 and influence the pharmacokinetics depending on the route of administration. Immune
387 response can act on phages both by innate and adaptive response. Innate responses
388 include complement mediated lysis or opsonization of phages, and eventual
389 phagocytosis and degradation by mononuclear phagocyte system (MPS) [118].
390 Phages are mostly found in liver or spleen after intravenous delivery which harbours
391 most of the MPS cells [119]. Antibody production against phages has also been
392 reported that can reduce efficacy of phage therapy [118,119]. Kim et al. showed that
393 attachment of hydrophilic polymers, such as polyethylene glycol, to phages increases
394 the half-life while in circulation and decreases susceptibility to innate and adaptive
395 immune response [120]. Carrier mediated delivery of phages as discussed above
396 could also modulate the adaptive immune response but needs to be further tested
397 [90,121]

398 In addition, engineering approaches such as synthetic biology-based phage
399 modification, development of chimeric phages that exhibit strong lytic activity [122,123]
400 and biomaterial-based delivery can significantly improve the performance of the phage
401 formulation by enhancing uptake by infected cells and targeting the intracellular niche
402 as discussed before [124-126].

403 Several phase 1 trials with phages have been conducted which validate the safety of
404 the formulation in healthy volunteers. A list of clinical trials utilizing phages for therapy
405 has been tabulated (**Table 4**). It is expected that the results of these trials would pave
406 the way for bringing phage therapy into regular clinical practice.

407 6. Future strategies for phage targeting to intracellular bacteria

408 Despite the advances in the application of phage as a potential therapeutic agent
409 against bacterial pathogens, additional refinements are necessary to ensure
410 sustainability. Some of the major questions that need to be addressed are in terms of
411 the delivery of phages at the site of infection. Since intracellular pathogens are
412 generally present inside specialized vesicles within a host cell, new strategies must
413 be designed for intracellular trafficking of phages/particles to various subcellular sites
414 like phagosomes, phagolysosomes, or some escaping from these vacuoles to the
415 cytoplasm. Chemical conjugation of peptides or administration of small molecules
416 along with phage carrying particles for enhanced uptake can be an innovative
417 strategy to deliver them into the specific subcellular sites. Effective uptake of phage
418 particles can be facilitated by cell-penetrating peptides (CPP). Apart from
419 endocytosis, CPPs are directly translocated by toroidal pore and barrel stave pore
420 formation (detailed uptake mechanism reviewed elsewhere) [127,128]. These
421 features of CPPs evade endosomal or vesicular trapping and ensure proper targeting
422 of cargos to specific organelles. Hussain et al. conjugated vancomycin-carrying

423 nanoparticles with cyclic 9-amino-acid peptide CARGGLKSC (CARG) that
424 specifically accumulated in staphylococcal infected lung and skin but not in normal
425 uninfected tissues [129]. The peptide targets bacterial surface components as was
426 observed by *in vitro* labelling. Infected mice treated with intravenous injections of the
427 CARG-conjugated vancomycin particles (one-day post-infection) showed 100%
428 recovery and long-term survival [129]. Engineered nanoparticles with specific homing
429 peptides ensured targeted delivery into the intracellular niche harboring the pathogen.
430 Additionally, genetically modified phages expressing certain peptide sequences on
431 their outer surface can facilitate the uptake process and organellar targeting. By the
432 virtue of protein-ligand interactions, particles can be engineered for targeted delivery
433 using the specific receptors present on the cell surface. For instance, a 2.5-fold
434 increase in cellular uptake was observed with liposomes comprised of 7.5%
435 mannosylated cholesterol compared to bare liposomes in alveolar macrophages
436 [124]. In another report, Yang et al. demonstrated that mannose-functionalized star-
437 shaped antimicrobial polycarbonates were effective compared to control polymer in
438 causing a 3-fold reduction in intracellular *M. bovis* BCG CFU/mL count after 72 h
439 treatment in THP-1 monocytic cells [130]. Use of such ligand functionalized polymer
440 could increase the efficiency of intracellular delivery of phage and should be explored
441 further.

442 Carrier mediated delivery was shown to be more effective in reducing intracellular
443 pathogen as phage internalization by infected cells was enhanced [84,90,131]. There
444 are several methods of achieving high phage encapsulation within various carriers.
445 The cost of the polymers and lipids is low compared to biomolecules and generally
446 encapsulation and purification processes are rapid and can be done in a few hours.
447 Cinquerrui et al. proposed a microfluidics-based nano-encapsulation of phages in

448 sub-micron sized liposomes using phospholipid 1,2-distearoyl-sn-glycero-3-
449 phosphocholine (DSPC) and cholesterol [88]. The size of liposomes could be
450 modulated by varying the concentration of cholesterol and regulating the
451 hydrodynamic conditions. Phage T3 with a diameter of around 65 nm and Phage K
452 with a head of around 80 nm and a tail length of approximately 200nm were used in
453 the study. Interestingly, the compact tail-free head of phage T3 showed higher
454 encapsulation yield compared to phage K [88]. Considering the large size of the
455 mycobacteriophages, Neith et al. bypassed the conventional liposome formulation
456 with alternative approaches by performing rehydration of lipid films by gel-assisted
457 giant unilamellar vesicle formation and inverse emulsion [131]. In gel-assisted vesicle
458 formation, lipid film was rehydrated on dried polyvinyl alcohol (PVA) gel using 10%
459 sucrose solution. In inverse emulsion technique, a step-wise lipid bilayer formation
460 occurs where the inner layer was created by water-in-oil emulsion and was placed on
461 top of another oily lipid solution (outer layer) which was emulsified by titration with a
462 blunt end syringe. In both these techniques, a moderate to high encapsulation
463 efficiency was observed (approx. 50% phage positive vesicles in case of gel-assisted
464 technique and almost 100% phage positive vesicles in case of inverse emulsion
465 technique). Both the techniques resulted in large vesicles of approximately 15-20 μm.
466 Conversely, conventional liposome formulation resulted in particle size of around 5
467 μm but had low encapsulation efficiency compared to the gel-assisted technique. This
468 could be due to large size of phages and low phage concentration used at the time
469 of synthesis. When cell uptake experiments were performed with 5 μm size
470 liposomes, a 4-fold higher uptake was observed in THP-1 macrophage cells
471 compared to the free phages. However, *in vitro* efficacy of the phages in reducing
472 intracellular bacteria was not reported [131].

473 Several studies have successfully shown the use of polymers to develop nanocarriers
474 for intracellular antibiotic delivery which can be adapted towards delivery of phages
475 [132-134]. For instance, polymers like poly (lactic-co-glycolic acid) (PLGA)
476 encapsulated azithromycin enhanced the efficacy of the drug to reduce intracellular
477 *Chlamydia* infection by decreasing the area of inclusion (proportional to infection load)
478 by 50% in lung epithelial cells compared to the free drug [132]. Similarly, particle
479 engineering approaches for intracellular delivery of vancomycin showed specific
480 release at lower pH and enhanced MRSA killing by 5-fold compared to the free drug
481 in J774A.1 macrophages [133]. Such mechanisms will ensure minimal loss of drug
482 while in circulation, thus maintaining an optimal drug concentration only inside
483 pathogen containing vesicles with low pH compared to free drug [113,134,135].
484 Phages can also be encapsulated within PLGA microparticles using a water/oil/water
485 double emulsion process [89]. Phages against *S. aureus* and *P. aeruginosa* were
486 encapsulated with 18% and 27% efficiency respectively within 10 μm polymeric
487 microparticles. Modifications were made to the standard double emulsion process to
488 minimize the interaction of phages with the organic solvent which can cause
489 denaturation. The resultant dry powder formulation of phages had good aerosol
490 properties but a low shelf life [89]. Phage inactivation due to exposure to organic
491 solvents like ethanol, acetonitrile, dimethylsulfoxide, and dimethylformamide hamper
492 the efficacy of the phage-loaded microparticles [136]. Alternative surfactants like
493 polyvinyl alcohol can be employed to improve formulation stability [113]. Additionally,
494 phages can be adsorbed on the particle surface to alleviate concerns of denaturation
495 during the fabrication process. Agarwal et al. showed that a mixture of three to five
496 phages can be adsorbed on polymeric (PLGA) particles [121]. The authors were able
497 to load ~10^6 phages/mg of particles and phage-particles were effective against

498 *Pseudomonas aeruginosa* cystic fibrosis mice. Phage adsorbed microparticles
499 significantly reduced *P. aeruginosa* infection by 1.5 log fold in cystic fibrosis mice
500 compared free phages [121]. Although these studies have not focused on intracellular
501 phage therapy, they provide technologies that can be adapted and explored against
502 intracellular bacteria.

503 Use of engineered phages to broaden the host range [137], conversion from
504 lysogenic phages to lytic forms [138,139], expressing cell-penetrating peptides [95]
505 and incorporate enzybiotics such as endolysins [140] and phage encoded cell wall
506 degrading enzymes into their genomes have been shown to be effective *in vitro*,
507 which have paved a way for future opportunities . For instance, Xu et al reported
508 enhanced internalization of T7 phage by mammalian cells when human
509 immunodeficiency virus type 1 TAT peptide was present on its surface [141]. The
510 authors reported 2-log orders higher uptake of modified phage in mammalian cells
511 (kidney epithelial cells) compared to T7 not modified with TAT peptide [141]. In
512 another instance, increased internalization of engineered M13 was reported by HeLa
513 cells [142]. M13 was genetically engineered to express an integrin binding peptide
514 (RGD) on the major viral coat proteins. The engineered phages demonstrated a 4-
515 fold increase in uptake by HeLa cells compared to wild-type phages [142]. Dedrick et
516 al. engineered a lytic derivative of phage ZoeJ by precisely removing the repressor
517 gene identified as gene 45 which can efficiently kill *M. abscessus* (GD01) [63]. Using
518 a cocktail of engineered phages (Muddy, BPs33ΔHTH-HRM10, and ZoeJΔ45) the
519 authors reported effective killing of infectious *M. abscessus* strain [63]. Phages can
520 also be genetically engineered to endure acidic environments encountered *in vivo* by
521 displaying phospholipids on the phage capsid [143]. pH-responsive biopolymers like
522 Eudragit®S100 have been shown to protect phage activity from acid damage at pH

as low as 2.0 compared to free phages [144]. Interestingly, phages were conditioned for maximum release (70%) in a simulated intestinal fluid with pH 7.0 compared to 40% at pH 5.0 [144]. The use of pH-sensitive biopolymers as a carrier for intracellular phage delivery may result in specific release of phages within specialized organelles and facilitate the killing of bacteria residing within these organelles. Endocytosis mediated uptake of particles sometimes results in endosomal compartmentalization and renders it inaccessible to the pathogen residing in the cytoplasm. Cationic polymers containing several secondary and tertiary amines are known to induce osmotic stress by entrapment of protons in the endosome membrane, a phenomenon known as “proton sponge effect”. Trapped protons increase the membrane potential that causes an influx of chloride ions into the endosome. This raises the osmotic pressure which eventually ruptures the endosome and facilitates cytosolic delivery [145,146]. These mechanisms could be utilized for efficient delivery of phage-loaded particles directly into a specific intracellular site of infection.

7. Concluding remarks

Phages are natural predators of bacteria and serve as excellent therapeutic agents against various bacterial infections. Although phage therapy has been well practiced against several bacterial infections, studies focusing on the therapeutic efficacy of phages against intracellular infection have remained largely unexplored. By the virtue of residing within a specific intracellular compartment, these intracellular pathogens are challenging to treat. With rising concern over antibiotic resistance, phage therapy could be an alternative approach to reduce such bacterial infections. In this review, we have focussed on discussing the efficacy of phage delivery against intracellular infection. An overview of studies that have used phages in free and encapsulated form has been highlighted. Liposomes and biopolymers could serve as an efficient carrier

to deliver phages into the intracellular milieu. Mechanisms to increase the uptake of phages by the infected mammalian cells are needed to enhance the therapeutic efficacy of phages against intracellular infection. Additionally, the challenges and future strategies pertaining to intracellular phage therapy has also been discussed. These challenges provide ample scope for research to develop phages as a therapeutic approach towards combating intracellular bacterial infection.

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Disclosure statement

The authors declare no conflict of interest

568 **Table 1: List of intracellular bacteria, their mechanism of entry, target cell and phages**
569 **available in literature**

Sr. No	Intracellular Bacteria	Mechanism of entry	Target Cell	Phages available	Reference
1	*Mycobacterium abscessus*	Receptor mediated endocytosis	Macrophages	phiT46-1	[147]
2	*Mycobacterium tuberculosis*	Receptor mediated endocytosis	Macrophages	D29, TM4, DS6A	[148-150]
3	*Shigella flexneri*	Trigger mode*	Epithelial cells	Sfin-1, Sf11-Sf25	[151,152]
4	*Shigella dysenteriae*	Trigger mode*	Epithelial cells	SF-9	[153]
5	*Listeria monocytogenes*	Zipper Mode	Epithelial cells	A511, P100, LMP1, LMP7	[154-156]
6	*Salmonella typhimurium*	Trigger mode*	Epithelial cells/Macrophages	P22-B1, P22, PBST10, PBST13, PBST32, and PBST 35	[157]
7	*Yersinia Pestis*	Zipper Mode**	Epithelial cells	PhiA1122, Yep-Phi	[158,159]
	Yersinia enterocolitica	Zipper Mode**	Epithelial cells	Yersinia Phage X1	[160]
8	*Staphylococcus aureus*	Zipper Mode**	Epithelial cells/Macrophages	vB_SauS-phiIPLA35 (phiIPLA35),vB_SauS-phiIPLA88 (phiIPLA88)	[161]
9	*Chlamydia*	Trigger mode*	Epithelial cells	Chp2, Chp3, φCPG1 φCPAR39 (φCpn1) and Chp4	[162]
10	*S. enterica*	Trigger mode*	Epithelial cells/Macrophages	ZCSE2	[163]
11	*E coli*	Trigger mode*	Epithelial cells	K1F	[164]
12	*Mycobacterium leprae*	Receptor mediated endocytosis	Epithelial cells	No	
13	*Coxiella burnetii*	Trigger mode*	Epithelial cells	No	

570 *Trigger mode- A Macropinocytosis- related Process- involves type 3/4 secretion system
571 **Zipper mode- A Clathrin- and Actin- mediated Internalization Process
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582 **Table 2: Free phages against intracellular bacterial infection**

Sr No.	Intracellular pathogen targeted	Phage used for the study	Experimental Model/Cell type	Reference
1	*Chlamydia psittaci*	phiCPG1	HeLa cell	[165]
2	*M. tuberculosis*	Mycobacteriophage D29	Primary cells: mouse peritoneal macrophages	[78]
3	*S. aureus*	M^{Sa}	Peritoneal mouse macrophages-has in vivo results also	[80]
4	*M. ulcerans*	D29	Murine footpad model	[166]
5	*S. aureus*	MR-5	Peritoneal mouse macrophages	[167]
6	*S. aureus*	vB_SauM_JS25	Bovine Mammary Epithelial Cells (MAC-T)	[76]
7	*B. pseudomallei*	C34	BALB/c mice	[81]
8	*E coli P5-AmpR*	Uncharacterized (from sewage)	MAC-T	[168]
9	*S. typhimurium KCCM40253 ATCC19585 ATCC19585 CCARM8009*	P22-B1,P22,PBST10 PBST13,PBST32,PB ST35	*in vitro* effect on bacteria	[169]
10	H37Rv (virulent strain of mycobacteria)	D29	Peritoneal mouse macrophages (RAW 264.7)	[79]
11	*E. coli*	K1F	Urinary bladder epithelial cell line, T24 (HTB-4)	[75]
12	*M. abscessus*	Muddy, BPs, ZoeJ (genetically engineered)	A 15-year-old patient	[63]
13	*E. coli* strain EV36	K1F	human cerebral microvascular endothelial cells (hCMEC)	[170]
14	*S. aureus*	PP1493, PP1815, and PP1957	MG63 osteoblastic Cells	[83]
15	*Salmonella spp*	SR ϕ1	HepG2 cells	[95]

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585 **Table 3: Carrier mediated intracellular phage delivery**

Sr No.	Intracellular pathogen targeted	Phage used for the study	Experimental Model/Cell type	Reference
1	*M. tuberculosis/ M. avium*	Mycobacteriophage TM4 (Adsorbed on *M. smegmatis*)	Mouse peritoneal macrophage cell line, RAW 264.7	[84]
2	*M. avium*	TM4 (Adsorbed on *M. smegmatis*)	Female C57BL/6 black mice	[85]
3	*M. tuberculosis*	Phage λeyfp + Mycobacteriophage TM4 (Liposomes)	Human macrophage cell line (THP-1)	[131]
4	*K. pneumoniae*	KPO1K2 (Liposomes)	Peritoneal mouse macrophages	[90]
5	*K. pneumoniae*	KØ1, KØ2, KØ3, KØ4 and KØ5 Isolated from sewage (Liposomes)	Male BALB/C mice	[91]
6	*Methicillin Resistant Staphylococcus aureus*	MR-5 MR-10 (Liposomes)	Female BALB/C mice	[171]

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601 **Table 4 Recent phage therapy clinical trials**

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Sr No.	NCT number	Pathogen/Infection	Phage/ Cocktail	Phase of the study	Reference
1	NCT02664740 not yet recruiting	*S. aureus* and MRSA/ Diabetic Foot Ulcer mono-infections	Not mentioned	Phase 1,2	[172]
2	NCT04287478 not yet recruiting	*E. coli* and *K. pneumoniae*/ Urinary Tract Infections	Personalized cocktail	Phase 1,2	[172]
3	NCT04323475 not yet recruiting	*S. aureus*, *P. aeruginosa*, or *K. pneumonia* / Second degree burn wounds	Phage Cocktail-SPK	Phase 1	[172]
4	NCT03808103 recruiting	Adherent Invasive *E. coli* /Patients with Crohn's disease	EcoActive	Phase 1,2	[172]
5	NCT04191148 recruiting	*E. coli* / Lower urinary tract infections	LBP-Ec01	Phase 1	[172]
6	NCT02116010	*E. coli* and *P. aeruginosa*/ Burn wound infections	Described in [173]	Phase 1,2	[174]
7	NCT03140085	Uropathogens/ Urinary tract infections	PYO phage	Phase 2/3	[175]
8	ACTRN12616000002482	*S. aureus*/ Chronic Rhinosinusitis	AB-SA01	Phase 1	[176]
9	NCT02757755	Healthy volunteers	AB-SA01	Phase 1	[172]
10	NCT00937274	*E. coli* / Diarrhoea	T4 phage cocktail	Phase 1	[177]

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610 ## References

611 1. Bastidas RJ, Elwell CA, Engel JN, et al. Chlamydial intracellular survival strategies. Cold Spring
612 Harbor perspectives in medicine. 2013 May 1;3(5):a010256.
613 2. Malik-Kale P, Winfree S, Steele-Mortimer O. The bimodal lifestyle of intracellular Salmonella
614 in epithelial cells: replication in the cytosol obscures defects in vacuolar replication. PloS one.
615 2012;7(6):e38732.
616 3. Gaillard JL, Berche P, Mounier J, et al. In vitro model of penetration and intracellular growth
617 of Listeria monocytogenes in the human enterocyte-like cell line Caco-2. Infection and
618 immunity. 1987 Nov;55(11):2822-9.
619 4. Armstrong JA, Hart PD. Response of cultured macrophages to Mycobacterium tuberculosis,
620 with observations on fusion of lysosomes with phagosomes. The Journal of experimental
621 medicine. 1971 Sep 1;134(3 Pt 1):713-40.
622 5. Organization WH. Tuberculosis [Report]. WHO fact sheets. 14 October 2020;2019.
623 6. Mortality GBD, Causes of Death C. Global, regional, and national life expectancy, all-cause
624 mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic
625 analysis for the Global Burden of Disease Study 2015. Lancet. 2016 Oct 8;388(10053):1459-
626 1544.
627 7. Garai P, Berry L, Moussouni M, et al. Killing from the inside: Intracellular role of T3SS in the
628 fate of Pseudomonas aeruginosa within macrophages revealed by mgtC and oprF mutants.
629 PLoS pathogens. 2019 Jun;15(6):e1007812.
630 8. Fraunholz M, Sinha B. Intracellular Staphylococcus aureus: live-in and let die. Front Cell Infect
631 Microbiol. 2012;2:43.
632 9. Sukumaran SK, Shimada H, Prasadarao NV. Entry and intracellular replication of Escherichia
633 coli K1 in macrophages require expression of outer membrane protein A. Infection and
634 immunity. 2003 Oct;71(10):5951-61.
635 10. Kamaruzzaman NF, Kendall S, Good L. Targeting the hard to reach: challenges and novel
636 strategies in the treatment of intracellular bacterial infections. British journal of
637 pharmacology. 2017 Jul;174(14):2225-2236.
638 11. Kortright KE, Chan BK, Koff JL, et al. Phage Therapy: A Renewed Approach to Combat
639 Antibiotic-Resistant Bacteria. Cell host & microbe. 2019 Feb 13;25(2):219-232.
640 12. Wittebole X, De Roock S, Opal SM. A historical overview of bacteriophage therapy as an
641 alternative to antibiotics for the treatment of bacterial pathogens. Virulence. 2014 Jan
642 1;5(1):226-35.
643 13. Carvalho C, Costa AR, Silva F, et al. Bacteriophages and their derivatives for the treatment and
644 control of food-producing animal infections. Critical Reviews in Microbiology. 2017
645 2017/09/03;43(5):583-601.
646 14. Gordillo Altamirano FL, Barr JJ. Phage Therapy in the Postantibiotic Era. Clinical microbiology
647 reviews. 2019;32(2):e00066-18.
648 15. Mohan Raj JR, Karunasagar I. Phages amid antimicrobial resistance. Critical Reviews in
649 Microbiology. 2019 2019/11/02;45(5-6):701-711.
650 16. Melo LDR, Oliveira H, Pires DP, et al. Phage therapy efficacy: a review of the last 10 years of
651 preclinical studies. Critical Reviews in Microbiology. 2020 2020/01/02;46(1):78-99.
652 17. Nieth A, Verseux C, Römer W. A Question of Attire: Dressing Up Bacteriophage Therapy for
653 the Battle Against Antibiotic-Resistant Intracellular Bacteria. Springer Science Reviews. 2015
654 2015/06/01;3(1):1-11.
655 18. Azimi T, Mosadegh M, Nasiri MJ, et al. Phage therapy as a renewed therapeutic approach to
656 mycobacterial infections: a comprehensive review. Infect Drug Resist. 2019;12:2943-2959.
657 19. McClarty G. Chlamydiae and the biochemistry of intracellular parasitism. Trends in
658 microbiology. 1994 May;2(5):157-64.

659 20. Moulder JW. Comparative biology of intracellular parasitism. Microbiological reviews. 1985
660 Sep;49(3):298-337.
661 21. Galan JE. Interactions of bacteria with non-phagocytic cells. Current opinion in immunology.
662 1994 Aug;6(4):590-5.
663 22. Ibarra JA, Steele-Mortimer O. Salmonella--the ultimate insider. Salmonella virulence factors
664 that modulate intracellular survival. Cellular microbiology. 2009 Nov;11(11):1579-86.
665 23. Riley LW. Determinants of cell entry and intracellular survival of Mycobacterium tuberculosis.
666 Trends in microbiology. 1995 1995/01/01/;3(1):27-31.
667 24. Hybiske K, Stephens RS. Mechanisms of host cell exit by the intracellular bacterium Chlamydia.
668 Proceedings of the National Academy of Sciences of the United States of America. 2007 Jul
669 3;104(27):11430-5.
670 25. Kumar Y, Cocchiaro J, Valdivia RH. The obligate intracellular pathogen Chlamydia trachomatis
671 targets host lipid droplets. Current biology. 2006 Aug 22;16(16):1646-51.
672 26. Sinha B, Herrmann M. Mechanism and consequences of invasion of endothelial cells by
673 Staphylococcus aureus. Thrombosis and haemostasis. 2005 Aug;94(2):266-77.
674 27. Jensen VB, Harty JT, Jones BD. Interactions of the invasive pathogens Salmonella typhimurium,
675 Listeria monocytogenes, and Shigella flexneri with M cells and murine Peyer's patches.
676 Infection and immunity. 1998 Aug;66(8):3758-66.
677 28. Pizarro-Cerdá J, Kühbacher A, Cossart P. Entry of Listeria monocytogenes in mammalian
678 epithelial cells: an updated view. Cold Spring Harbor perspectives in medicine. 2012 Nov
679 1;2(11).
680 29. Van Nhieu GT, Isberg RR. The Yersinia pseudotuberculosis invasin protein and human
681 fibronectin bind to mutually exclusive sites on the alpha 5 beta 1 integrin receptor. The Journal
682 of biological chemistry. 1991 Dec 25;266(36):24367-75.
683 30. Tran Van Nhieu G, Bourdet-Sicard R, Duménil G, et al. Bacterial signals and cell responses
684 during Shigella entry into epithelial cells. Cellular microbiology. 2000 Jun;2(3):187-93.
685 31. Patel JC, Galán JE. Manipulation of the host actin cytoskeleton by Salmonella--all in the name
686 of entry. Current opinion in microbiology. 2005 Feb;8(1):10-5.
687 32. Peng X, Jiang G, Liu W, et al. Characterization of differential pore-forming activities of ESAT-6
688 proteins from Mycobacterium tuberculosis and Mycobacterium smegmatis. FEBS letters. 2016
689 Feb;590(4):509-19.
690 33. Jamwal SV, Mehrotra P, Singh A, et al. Mycobacterial escape from macrophage phagosomes
691 to the cytoplasm represents an alternate adaptation mechanism. Scientific reports. 2016 Mar
692 16;6:23089.
693 34. Brouillette E, Grondin G, Shkreta L, et al. In vivo and in vitro demonstration that
694 Staphylococcus aureus is an intracellular pathogen in the presence or absence of fibronectin-
695 binding proteins. Microbial pathogenesis. 2003 Oct;35(4):159-68.
696 35. Odenholt I, Gustafsson I, Lowdin E, et al. Suboptimal antibiotic dosage as a risk factor for
697 selection of penicillin-resistant Streptococcus pneumoniae: in vitro kinetic model.
698 Antimicrobial agents and chemotherapy. 2003 Feb;47(2):518-23.
699 36. Kim H, Barroso M, Samanta R, et al. Experimentally induced changes in the endocytic traffic
700 of P-glycoprotein alter drug resistance of cancer cells. American Journal of Physiology-Cell
701 Physiology. 1997 1997/08/01;273(2):C687-C702.
702 37. Poole K. Efflux-mediated antimicrobial resistance. The Journal of antimicrobial chemotherapy.
703 2005 Jul;56(1):20-51.
704 38. Fu D, Roufogalis BD. Actin disruption inhibits endosomal traffic of P-glycoprotein-EGFP and
705 resistance to daunorubicin accumulation. American journal of physiology Cell physiology.
706 2007 Apr;292(4):C1543-52.
707 39. Nichterlein T, Kretschmar M, Schadt A, et al. Reduced intracellular activity of antibiotics
708 against Listeria monocytogenes in multidrug resistant cells. International journal of
709 antimicrobial agents. 1998 1998/05/21/;10(2):119-125.

710 40. Michot JM, Seral C, Van Bambeke F, et al. Influence of efflux transporters on the accumulation
711 and efflux of four quinolones (ciprofloxacin, levofloxacin, garenoxacin, and moxifloxacin) in
712 J774 macrophages. Antimicrobial agents and chemotherapy. 2005 Jun;49(6):2429-37.
713 41. Seral C, Carryn S, Tulkens PM, et al. Influence of P-glycoprotein and MRP efflux pump
714 inhibitors on the intracellular activity of azithromycin and ciprofloxacin in macrophages
715 infected by Listeria monocytogenes or Staphylococcus aureus. The Journal of antimicrobial
716 chemotherapy. 2003 May;51(5):1167-73.
717 42. Seral C, Michot JM, Chanteux H, et al. Influence of P-glycoprotein inhibitors on accumulation
718 of macrolides in J774 murine macrophages. Antimicrobial agents and chemotherapy. 2003
719 Mar;47(3):1047-51.
720 43. Ong CT, Babalola CP, Nightingale CH, et al. Penetration, efflux and intracellular activity of
721 tigecycline in human polymorphonuclear neutrophils (PMNs). Journal of Antimicrobial
722 Chemotherapy. 2005;56(3):498-501.
723 44. Seral C, Barcia-Macay M, Mingeot-Leclercq MP, et al. Comparative activity of quinolones
724 (ciprofloxacin, levofloxacin, moxifloxacin and garenoxacin) against extracellular and
725 intracellular infection by Listeria monocytogenes and Staphylococcus aureus in J774
726 macrophages. The Journal of antimicrobial chemotherapy. 2005 Apr;55(4):511-7.
727 45. Greenwood DJ, Dos Santos MS, Huang S, et al. Subcellular antibiotic visualization reveals a
728 dynamic drug reservoir in infected macrophages. Science. 2019;364(6447):1279.
729 46. Lemaire S, Tulkens PM, Van Bambeke F. Contrasting effects of acidic pH on the extracellular
730 and intracellular activities of the anti-gram-positive fluoroquinolones moxifloxacin and
731 delafloxacin against Staphylococcus aureus. Antimicrobial agents and chemotherapy. 2011
732 Feb;55(2):649-58.
733 47. Baudoux P, Bles N, Lemaire S, et al. Combined effect of pH and concentration on the activities
734 of gentamicin and oxacillin against Staphylococcus aureus in pharmacodynamic models of
735 extracellular and intracellular infections. The Journal of antimicrobial chemotherapy. 2007
736 Feb;59(2):246-53.
737 48. Grant SS, Hung DT. Persistent bacterial infections, antibiotic tolerance, and the oxidative
738 stress response. Virulence. 2013 May 15;4(4):273-83.
739 49. Fisher RA, Gollan B, Helaine S. Persistent bacterial infections and persister cells. Nature
740 Reviews Microbiology. 2017 2017/08/01;15(8):453-464.
741 50. Grant SS, Hung DT. Persistent bacterial infections, antibiotic tolerance, and the oxidative
742 stress response. Virulence. 2013 2013/05/15;4(4):273-283.
743 51. Helaine S, Cheverton AM, Watson KG, et al. Internalization of Salmonella by macrophages
744 induces formation of nonreplicating persisters. Science. 2014 Jan 10;343(6167):204-8.
745 52. Wayne LG, Sohaskey CD. Nonreplicating persistence of mycobacterium tuberculosis. Annual
746 review of microbiology. 2001;55:139-63.
747 53. Cogan NG. Effects of persister formation on bacterial response to dosing. Journal of
748 theoretical biology. 2006 Feb 7;238(3):694-703.
749 54. Fang FC. Antimicrobial actions of reactive oxygen species. mBio. 2011;2(5):e00141-11.
750 55. Spooner R, Yilmaz O. The role of reactive-oxygen-species in microbial persistence and
751 inflammation. International journal of molecular sciences. 2011 Jan 13;12(1):334-52.
752 56. Rowe SE, Wagner NJ, Li L, et al. Reactive oxygen species induce antibiotic tolerance during
753 systemic Staphylococcus aureus infection. Nature microbiology. 2020 Feb;5(2):282-290.
754 57. Adams Kristin N, Takaki K, Connolly Lynn E, et al. Drug Tolerance in Replicating Mycobacteria
755 Mediated by a Macrophage-Induced Efflux Mechanism. Cell. 2011 2011/04/01/;145(1):39-53.
756 58. Ramón-García S, Martín C, Thompson CJ, et al. Role of the P55 Efflux Pump in Intrinsic Drug
757 Resistance, Oxidative Stress Responses, and Growth. Antimicrobial agents and chemotherapy.
758 2009;53(9):3675.
759 59. Bertram C, Hass R. Cellular responses to reactive oxygen species-induced DNA damage and
760 aging. Biological chemistry. 2008 Mar;389(3):211-20.

761 60. Beckman KB, Ames BN. Oxidative decay of DNA. The Journal of biological chemistry. 1997 Aug
762 8;272(32):19633-6.
763 61. Hori M, Yonekura S, Nohmi T, et al. Error-Prone Translesion DNA Synthesis by Escherichia coli
764 DNA Polymerase IV (DinB) on Templates Containing 1,2-dihydro-2-oxoadenine. Journal of
765 nucleic acids. 2010 Sep 26;2010:807579.
766 62. Tang M, Pham P, Shen X, et al. Roles of E. coli DNA polymerases IV and V in lesion-targeted
767 and untargeted SOS mutagenesis. Nature. 2000 Apr 27;404(6781):1014-8.
768 63. Dedrick RM, Guerrero-Bustamante CA, Garlena RA, et al. Engineered bacteriophages for
769 treatment of a patient with a disseminated drug-resistant Mycobacterium abscessus. Nat
770 Med. 2019 May;25(5):730-733.
771 64. Monsur KA, Rahman MA, Huq F, et al. Effect of massive doses of bacteriophage on excretion
772 of vibrios, duration of diarrhoea and output of stools in acute cases of cholera. Bulletin of the
773 World Health Organization. 1970;42(5):723-32.
774 65. Dor-On E, Solomon B. Targeting glioblastoma via intranasal administration of Ff
775 bacteriophages. Frontiers in microbiology. 2015;6:530.
776 66. Srivastava AS, Chauhan DP, Carrier E. In utero detection of T7 phage after systemic
777 administration to pregnant mice. BioTechniques. 2004 Jul;37(1):81-3.
778 67. Nguyen S, Baker K, Padman BS, et al. Bacteriophage Transcytosis Provides a Mechanism To
779 Cross Epithelial Cell Layers. mBio. 2017 Nov 21;8(6):01874-17.
780 68. Otero J, Garcia-Rodriguez A, Cano-Sarabia M, et al. Biodistribution of Liposome-Encapsulated
781 Bacteriophages and Their Transcytosis During Oral Phage Therapy. Frontiers in microbiology.
782 2019;10:689.
783 69. Duerr DM, White SJ, Schluesener HJ. Identification of peptide sequences that induce the
784 transport of phage across the gastrointestinal mucosal barrier. Journal of virological methods.
785 2004 Mar 15;116(2):177-80.
786 70. Żaczek M, Górski A, Skaradzińska A, et al. Phage penetration of eukaryotic cells: practical
787 implications [Research]. Future Virology. 2019;14(11):745-760.
788 71. Górski A, Borysowski J, Międzybrodzki R. Bacteriophage Interactions With Epithelial Cells:
789 Therapeutic Implications [Opinion]. Frontiers in microbiology. 2021 2021-January-
790 18;11(3580).
791 72. Tian Y, Wu M, Liu X, et al. Probing the endocytic pathways of the filamentous bacteriophage
792 in live cells using ratiometric pH fluorescent indicator. Advanced healthcare materials. 2015
793 Feb 18;4(3):413-9.
794 73. Kim A, Shin TH, Shin SM, et al. Cellular internalization mechanism and intracellular trafficking
795 of filamentous M13 phages displaying a cell-penetrating transbody and TAT peptide. PloS one.
796 2012;7(12):e51813.
797 74. Van Belleghem JD, Dabrowska K, Vaneechoutte M, et al. Interactions between Bacteriophage,
798 Bacteria, and the Mammalian Immune System. Viruses. 2018 Dec 25;11(1):10.
799 75. Moller-Olsen C, Ho SFS, Shukla RD, et al. Engineered K1F bacteriophages kill intracellular
800 Escherichia coli K1 in human epithelial cells. Scientific reports. 2018 Dec 3;8(1):17559.
801 76. Zhang L, Sun L, Wei R, et al. Intracellular Staphylococcus aureus Control by Virulent
802 Bacteriophages within MAC-T Bovine Mammary Epithelial Cells. Antimicrobial agents and
803 chemotherapy. 2017 Feb;61(2):e01990-16.
804 77. Lehti TA, Pajunen MI, Skog MS, et al. Internalization of a polysialic acid-binding Escherichia coli
805 bacteriophage into eukaryotic neuroblastoma cells. Nature communications. 2017 Dec
806 4;8(1):1915.
807 78. Peng L, Chen BW, Luo YA, et al. Effect of mycobacteriophage to intracellular mycobacteria in
808 vitro. Chinese medical journal. 2006 Apr 20;119(8):692-5.
809 79. Lapenkova MB, Smirnova NS, Rutkevich PN, et al. Evaluation of the Efficiency of Lytic
810 Mycobacteriophage D29 on the Model of M. tuberculosis-Infected Macrophage RAW 264 Cell
811 Line. Bulletin of experimental biology and medicine. 2018 Jan;164(3):344-346.

812 80. Capparelli R, Parlato M, Borriello G, et al. Experimental phage therapy against Staphylococcus
813 aureus in mice. Antimicrobial agents and chemotherapy. 2007 Aug;51(8):2765-73.
814 81. Guang-Han O, Leang-Chung C, Vellasamy KM, et al. Experimental Phage Therapy for
815 Burkholderia pseudomallei Infection. PloS one. 2016;11(7):e0158213.
816 82. Carrigy NB, Larsen SE, Reese V, et al. Prophylaxis of Mycobacterium tuberculosis H37Rv
817 Infection in a Preclinical Mouse Model via Inhalation of Nebulized Bacteriophage D29.
818 Antimicrobial agents and chemotherapy. 2019;63(12):e00871-19.
819 83. Kolenda C, Josse J, Medina M, et al. Evaluation of the Activity of a Combination of Three
820 Bacteriophages Alone or in Association with Antibiotics on Staphylococcus aureus Embedded
821 in Biofilm or Internalized in Osteoblasts. Antimicrobial agents and chemotherapy. 2020 Feb
822 21;64(3):e02231-19.
823 84. Broxmeyer L, Sosnowska D, Miltner E, et al. Killing of Mycobacterium avium and
824 Mycobacterium tuberculosis by a mycobacteriophage delivered by a nonvirulent
825 mycobacterium: a model for phage therapy of intracellular bacterial pathogens. The Journal
826 of infectious diseases. 2002 Oct 15;186(8):1155-60.
827 85. Danelishvili L, Young LS, Bermudez LE. In vivo efficacy of phage therapy for Mycobacterium
828 avium infection as delivered by a nonvirulent mycobacterium. Microbial drug resistance. 2006
829 Spring;12(1):1-6.
830 86. Campoccia D, Montanaro L, Arciola CR. A review of the biomaterials technologies for infection-
831 resistant surfaces. Biomaterials. 2013 Nov;34(34):8533-54.
832 87. Fenton OS, Olafson KN, Pillai PS, et al. Advances in Biomaterials for Drug Delivery. Advanced
833 materials. 2018 May 7:e1705328.
834 88. Cinquerrui S, Mancuso F, Vladisavljevic GT, et al. Nanoencapsulation of Bacteriophages in
835 Liposomes Prepared Using Microfluidic Hydrodynamic Flow Focusing. Frontiers in
836 microbiology. 2018;9:2172.
837 89. Puapermpoonsiri U, Spencer J, van der Walle CF. A freeze-dried formulation of bacteriophage
838 encapsulated in biodegradable microspheres. European journal of pharmaceutics and
839 biopharmaceutics. 2009 May;72(1):26-33.
840 90. Singla S, Harjai K, Katare OP, et al. Encapsulation of Bacteriophage in Liposome Accentuates
841 Its Entry in to Macrophage and Shields It from Neutralizing Antibodies. PloS one.
842 2016;11(4):e0153777.
843 91. Chadha P, Katare OP, Chhibber S. Liposome loaded phage cocktail: Enhanced therapeutic
844 potential in resolving Klebsiella pneumoniae mediated burn wound infections. Burns : journal
845 of the International Society for Burn Injuries. 2017 Nov;43(7):1532-1543.
846 92. Ma Y, Pacan JC, Wang Q, et al. Microencapsulation of Bacteriophage Felix O1 into Chitosan-
847 Alginate Microspheres for Oral Delivery. Applied and environmental microbiology.
848 2008;74(15):4799.
849 93. Colom J, Cano-Sarabia M, Otero J, et al. Microencapsulation with alginate/CaCO(3): A strategy
850 for improved phage therapy. Scientific reports. 2017;7:41441-41441.
851 94. Loh B, Gondil VS, Manohar P, et al. Encapsulation and Delivery of Therapeutic Phages. Applied
852 and environmental microbiology. 2020:AEM.01979-20.
853 95. Fulgione A, Ianniello F, Papaianni M, et al. Biomimetic hydroxyapatite nanocrystals are an
854 active carrier for Salmonella bacteriophages. International journal of nanomedicine.
855 2019;14:2219-2232.
856 96. Dupuis M, Villion M, Magadán AH, et al. CRISPR-Cas and restriction-modification systems are
857 compatible and increase phage resistance. Nature communications. 2013;4:2087.
858 97. Enikeeva FN, Severinov KV, Gelfand MS. Restriction-modification systems and bacteriophage
859 invasion: who wins? Journal of theoretical biology. 2010 Oct 21;266(4):550-9.
860 98. Barrangou R, Fremaux C, Deveau H, et al. CRISPR provides acquired resistance against viruses
861 in prokaryotes. Science. 2007 Mar 23;315(5819):1709-12.

862 99. Watson BNJ, Vercoe RB, Salmond GPC, et al. Type I-F CRISPR-Cas resistance against virulent
863 phages results in abortive infection and provides population-level immunity. Nature
864 communications. 2019 Dec 4;10(1):5526.
865 100. Sousa JAM, Rocha EPC. Environmental structure drives resistance to phages and antibiotics
866 during phage therapy and to invading lysogens during colonisation. Scientific reports. 2019
867 2019/02/28;9(1):3149.
868 101. Nobrega FL, Costa AR, Santos JF, et al. Genetically manipulated phages with improved pH
869 resistance for oral administration in veterinary medicine. Scientific reports. 2016
870 2016/12/15;6(1):39235.
871 102. Trastoy R, Manso T, Fernandez-Garcia L, et al. Mechanisms of Bacterial Tolerance and
872 Persistence in the Gastrointestinal and Respiratory Environments. Clinical microbiology
873 reviews. 2018 Oct;31(4).
874 103. Fisher RA, Gollan B, Helaine S. Persistent bacterial infections and persister cells. Nature
875 reviews Microbiology. 2017 Aug;15(8):453-464.
876 104. Swift BM, Gerrard ZE, Huxley JN, et al. Factors affecting phage D29 infection: a tool to
877 investigate different growth states of mycobacteria. PLoS One. 2014;9(9):e106690.
878 105. Bryan D, El-Shibiny A, Hobbs Z, et al. Bacteriophage T4 Infection of Stationary Phase E. coli:
879 Life after Log from a Phage Perspective. Front Microbiol. 2016;7:1391.
880 106. Piuri M, Hatfull GF. A peptidoglycan hydrolase motif within the mycobacteriophage TM4 tape
881 measure protein promotes efficient infection of stationary phase cells. Molecular
882 microbiology. 2006 Dec;62(6):1569-85.
883 107. Kalapala YC, Sharma PR, Agarwal R. Antimycobacterial Potential of Mycobacteriophage Under
884 Disease-Mimicking Conditions. Frontiers in microbiology. 2020;11:583661.
885 108. Reyes-Robles T, Dillard RS, Cairns LS, et al. Vibrio cholerae Outer Membrane Vesicles Inhibit
886 Bacteriophage Infection. Journal of bacteriology. 2018 Aug 1;200(15):e00792-17.
887 109. Manning AJ, Kuehn MJ. Contribution of bacterial outer membrane vesicles to innate bacterial
888 defense. BMC microbiology. 2011 Dec 1;11:258.
889 110. Yang Y, Shen W, Zhong Q, et al. Development of a Bacteriophage Cocktail to Constrain the
890 Emergence of Phage-Resistant Pseudomonas aeruginosa. Frontiers in microbiology.
891 2020;11:327.
892 111. Takeuchi I, Osada K, Azam AH, et al. The Presence of Two Receptor-Binding Proteins
893 Contributes to the Wide Host Range of Staphylococcal Twort-Like Phages. Applied and
894 environmental microbiology. 2016 Oct 1;82(19):5763-74.
895 112. Gordillo Altamirano FL, Barr JJ. Unlocking the next generation of phage therapy: the key is in
896 the receptors. Current opinion in biotechnology. 2021 2021/04/01/;68:115-123.
897 113. Malik DJ, Sokolov IJ, Vinner GK, et al. Formulation, stabilisation and encapsulation of
898 bacteriophage for phage therapy. Advances in colloid and interface science. 2017
899 Nov;249:100-133.
900 114. Forti F, Roach DR, Cafora M, et al. Design of a Broad-Range Bacteriophage Cocktail That
901 Reduces Pseudomonas aeruginosa Biofilms and Treats Acute Infections in Two Animal Models.
902 Antimicrobial agents and chemotherapy. 2018 Jun;62(6):e02573-17.
903 115. Gu J, Liu X, Li Y, et al. A method for generation phage cocktail with great therapeutic potential.
904 PloS one. 2012;7(3):e31698.
905 116. Comeau AM, Tetart F, Trojet SN, et al. Phage-Antibiotic Synergy (PAS): beta-lactam and
906 quinolone antibiotics stimulate virulent phage growth. PloS one. 2007 Aug 29;2(8):e799.
907 117. Tagliaferri TL, Jansen M, Horz H-P. Fighting Pathogenic Bacteria on Two Fronts: Phages and
908 Antibiotics as Combined Strategy [Review]. Frontiers in cellular and infection microbiology.
909 2019 2019-February-18;9(22).
910 118. Krut O, Bekeredjian-Ding I. Contribution of the Immune Response to Phage Therapy. Journal
911 of immunology. 2018 May 1;200(9):3037-3044.

912 119. Hodyra-Stefaniak K, Miernikiewicz P, Drapala J, et al. Mammalian Host-Versus-Phage immune
913 response determines phage fate in vivo. Scientific reports. 2015 Oct 6;5:14802.
914 120. Kim K-P, Cha J-D, Jang E-H, et al. PEGylation of bacteriophages increases blood circulation time
915 and reduces T-helper type 1 immune response [https://doi.org/10.1111/j.1751-
916 7915.2008.00028.x]. Microbial Biotechnology. 2008 2008/05/01;1(3):247-257.
917 121. Agarwal R, Johnson CT, Imhoff BR, et al. Inhaled bacteriophage-loaded polymeric
918 microparticles ameliorate acute lung infections. Nature biomedical engineering. 2018
919 Nov;2(11):841-849.
920 122. Lu TK, Collins JJ. Dispersing biofilms with engineered enzymatic bacteriophage. Proceedings
921 of the National Academy of Sciences. 2007;104(27):11197.
922 123. Mahichi F, Synnott AJ, Yamamichi K, et al. Site-specific recombination of T2 phage using IP008
923 long tail fiber genes provides a targeted method for expanding host range while retaining lytic
924 activity. FEMS Microbiology Letters. 2009;295(2):211-217.
925 124. Wijagkanalan W, Kawakami S, Takenaga M, et al. Efficient targeting to alveolar macrophages
926 by intratracheal administration of mannosylated liposomes in rats. Journal of controlled
927 release. 2008 Jan 22;125(2):121-30.
928 125. Citorik RJ, Mimee M, Lu TK. Bacteriophage-based synthetic biology for the study of infectious
929 diseases. Current opinion in microbiology. 2014 Jun;19:59-69.
930 126. Nitta SK, Numata K. Biopolymer-based nanoparticles for drug/gene delivery and tissue
931 engineering. International journal of molecular sciences. 2013 Jan 14;14(1):1629-54.
932 127. Gestin M, Dowaidar M, Langel U. Uptake Mechanism of Cell-Penetrating Peptides. Advances
933 in experimental medicine and biology. 2017;1030:255-264.
934 128. Madani F, Lindberg S, Langel U, et al. Mechanisms of cellular uptake of cell-penetrating
935 peptides. Journal of biophysics. 2011;2011:414729.
936 129. Hussain S, Joo J, Kang J, et al. Antibiotic-loaded nanoparticles targeted to the site of infection
937 enhance antibacterial efficacy. Nature biomedical engineering. 2018 Feb;2(2):95-103.
938 130. Yang C, Krishnamurthy S, Liu J, et al. Broad-Spectrum Antimicrobial Star Polycarbonates
939 Functionalized with Mannose for Targeting Bacteria Residing inside Immune Cells. Advanced
940 healthcare materials. 2016 Jun;5(11):1272-81.
941 131. Nieth A, Verseux C, Barnert S, et al. A first step toward liposome-mediated intracellular
942 bacteriophage therapy. Expert opinion on drug delivery. 2015;12(9):1411-24.
943 132. Toti US, Guru BR, Hali M, et al. Targeted delivery of antibiotics to intracellular chlamydial
944 infections using PLGA nanoparticles. Biomaterials. 2011 Sep;32(27):6606-13.
945 133. Pei Y, Mohamed MF, Seleem MN, et al. Particle engineering for intracellular delivery of
946 vancomycin to methicillin-resistant Staphylococcus aureus (MRSA)-infected macrophages.
947 Journal of controlled release. 2017 Dec 10;267:133-143.
948 134. Fenaroli F, Robertson JD, Scarpa E, et al. Polymersomes Eradicating Intracellular Bacteria. ACS
949 nano. 2020 Jun 19;14(7):8287–8298.
950 135. Kamaly N, Yameen B, Wu J, et al. Degradable Controlled-Release Polymers and Polymeric
951 Nanoparticles: Mechanisms of Controlling Drug Release. Chemical reviews. 2016 Feb
952 24;116(4):2602-63.
953 136. Matsubara T, Emoto W, Kawashiro K. A simple two-transition model for loss of infectivity of
954 phages on exposure to organic solvent. Biomolecular engineering. 2007 Jun;24(2):269-71.
955 137. Yehl K, Lemire S, Yang AC, et al. Engineering Phage Host-Range and Suppressing Bacterial
956 Resistance through Phage Tail Fiber Mutagenesis. Cell. 2019 2019/10/03/;179(2):459-469.e9.
957 138. Moons P, Faster D, Aertsen A. Lysogenic conversion and phage resistance development in
958 phage exposed Escherichia coli biofilms. Viruses. 2013;5(1):150-161.
959 139. Zhang H, Fouts DE, DePew J, et al. Genetic modifications to temperate Enterococcus faecalis
960 phage Ef11 that abolish the establishment of lysogeny and sensitivity to repressor, and
961 increase host range and productivity of lytic infection. Microbiology. 2013 Jun;159(Pt 6):1023-
962 1035.

963 140. Haddad Kashani H, Schmelcher M, Sabzalipoor H, et al. Recombinant Endolysins as Potential
964 Therapeutics against Antibiotic-Resistant *Staphylococcus aureus*: Current Status of Research
965 and Novel Delivery Strategies. Clinical microbiology reviews. 2018;31(1):e00071-17.
966 141. Xu H, Bao X, Wang Y, et al. Engineering T7 bacteriophage as a potential DNA vaccine targeting
967 delivery vector. Virology Journal. 2018 2018/03/20;15(1):49.
968 142. Bhattarai SR, Yoo SY, Lee SW, et al. Engineered phage-based therapeutic materials inhibit
969 Chlamydia trachomatis intracellular infection. Biomaterials. 2012 Jul;33(20):5166-74.
970 143. Nobrega FL, Costa AR, Santos JF, et al. Genetically manipulated phages with improved pH
971 resistance for oral administration in veterinary medicine. Scientific reports. 2016 Dec
972 15;6:39235.
973 144. Vinner GK, Richards K, Leppanen M, et al. Microencapsulation of Enteric Bacteriophages in a
974 pH-Responsive Solid Oral Dosage Formulation Using a Scalable Membrane Emulsification
975 Process. Pharmaceutics. 2019 Sep 14;11(9):475.
976 145. Bus T, Traeger A, Schubert US. The great escape: how cationic polyplexes overcome the
977 endosomal barrier. Journal of materials chemistry B. 2018 Nov 21;6(43):6904-6918.
978 146. Freeman EC, Weiland LM, Meng WS. Modeling the proton sponge hypothesis: examining
979 proton sponge effectiveness for enhancing intracellular gene delivery through multiscale
980 modeling. Journal of biomaterials science Polymer edition. 2013;24(4):398-416.
981 147. Amarh ED, Dedrick RM, Garlena RA, et al. Genome Sequence of Mycobacterium abscessus
982 Phage phiT46-1. Microbiology Resource Announcements. 2021;10(2):e01421-20.
983 148. Ford ME, Sarkis GJ, Belanger AE, et al. Genome structure of mycobacteriophage D29:
984 implications for phage evolution. Journal of molecular biology. 1998 May 29;279(1):143-64.
985 149. Ford ME, Stenstrom C, Hendrix RW, et al. Mycobacteriophage TM4: genome structure and
986 gene expression. Tubercle and lung disease : the official journal of the International Union
987 against Tuberculosis and Lung Disease. 1998;79(2):63-73.
988 150. Mayer O, Jain P, Weisbrod TR, et al. Fluorescent Reporter DS6A Mycobacteriophages Reveal
989 Unique Variations in Infectibility and Phage Production in Mycobacteria. Journal of
990 bacteriology. 2016;198(23):3220-3232.
991 151. Ahamed ST, Roy B, Basu U, et al. Genomic and Proteomic Characterizations of Sfin-1, a Novel
992 Lytic Phage Infecting Multidrug-Resistant Shigella spp. and Escherichia coli C. Frontiers in
993 microbiology. 2019;10:1876-1876.
994 152. Doore SM, Schrad JR, Dean WF, et al. Phages Isolated during a Dysentery Outbreak Reveal
995 Uncommon Structures and Broad Species Diversity. Journal of virology. 2018;92(8):e02117-
996 17.
997 153. Faruque SM, Chowdhury N, Khan R, et al. Shigella dysenteriae type 1-specific bacteriophage
998 from environmental waters in Bangladesh. Applied and environmental microbiology.
999 2003;69(12):7028-7031.
1000 154. Lee S, Kim MG, Lee HS, et al. Isolation and Characterization of Listeria phages for Control of
1001 Growth of Listeria monocytogenes in Milk. Korean J Food Sci Anim Resour. 2017;37(2):320-
1002 328.
1003 155. Ahmadi H, Barbut S, Lim L-T, et al. Examination of the Use of Bacteriophage as an Additive and
1004 Determining Its Best Application Method to Control Listeria monocytogenes in a Cooked-Meat
1005 Model System [Original Research]. Frontiers in microbiology. 2020 2020-May-21;11(779).
1006 156. Guenther S, Huwyler D, Richard S, et al. Virulent Bacteriophage for Efficient Biocontrol of
1007 Listeria monocytogenes in Ready-To-Eat Foods. Applied and environmental microbiology.
1008 2009;75(1):93.
1009 157. Jung L-s, Ding T, Ahn J. Evaluation of lytic bacteriophages for control of multidrug-resistant
1010 Salmonella Typhimurium. Annals of clinical microbiology and antimicrobials. 2017
1011 2017/09/22;16(1):66.

158. Garcia E, Elliott JM, Ramanculov E, et al. The genome sequence of Yersinia pestis bacteriophage phiA1122 reveals an intimate history with the coliphage T3 and T7 genomes. Journal of bacteriology. 2003 Sep;185(17):5248-62.
159. Zhao X, Wu W, Qi Z, et al. The complete genome sequence and proteomics of Yersinia pestis phage Yep-phi. The Journal of general virology. 2011 Jan;92(Pt 1):216-21.
160. Xue Y, Zhai S, Wang Z, et al. The Yersinia Phage X1 Administered Orally Efficiently Protects a Murine Chronic Enteritis Model Against Yersinia enterocolitica Infection [Original Research]. Frontiers in microbiology. 2020 2020-March-06;11(351).
161. García P, Martínez B, Obeso JM, et al. Functional Genomic Analysis of Two Staphylococcus aureus Phages Isolated from the Dairy Environment. Applied and environmental microbiology. 2009;75(24):7663.
162. Śliwa-Dominiak J, Suszyńska E, Pawlikowska M, et al. Chlamydia bacteriophages. Archives of microbiology. 2013 Nov;195(10-11):765-71.
163. Mohamed A, Taha O, El-Sherif HM, et al. Bacteriophage ZCSE2 is a Potent Antimicrobial Against Salmonella enterica Serovars: Ultrastructure, Genomics and Efficacy. Viruses. 2020;12(4):424.
164. Scholl D, Merril C. The Genome of Bacteriophage K1F, a T7-Like Phage That Has Acquired the Ability To Replicate on K1 Strains of Escherichia coli. Journal of bacteriology. 2005;187(24):8499.
165. Hsia R, Ohayon H, Gounon P, et al. Phage infection of the obligate intracellular bacterium, Chlamydia psittaci strain guinea pig inclusion conjunctivitis. Microbes and infection. 2000 Jun;2(7):761-72.
166. Trigo G, Martins TG, Fraga AG, et al. Phage therapy is effective against infection by Mycobacterium ulcerans in a murine footpad model. PLoS neglected tropical diseases. 2013;7(4):e2183.
167. Kaur S, Harjai K, Chhibber S. Bacteriophage-aided intracellular killing of engulfed methicillin-resistant Staphylococcus aureus (MRSA) by murine macrophages. Applied microbiology and biotechnology. 2014 May;98(10):4653-61.
168. Porter J, Anderson J, Carter L, et al. In vitro evaluation of a novel bacteriophage cocktail as a preventative for bovine coliform mastitis. Journal of dairy science. 2016 Mar;99(3):2053-2062.
169. Jung LS, Ding T, Ahn J. Evaluation of lytic bacteriophages for control of multidrug-resistant Salmonella Typhimurium. Annals of clinical microbiology and antimicrobials. 2017 Sep 22;16(1):66.
170. Moller-Olsen C, Ross T, Leppard KN, et al. Bacteriophage K1F targets Escherichia coli K1 in cerebral endothelial cells and influences the barrier function. Scientific reports. 2020 Jun 1;10(1):8903.
171. Chhibber S, Kaur J, Kaur S. Liposome Entrapment of Bacteriophages Improves Wound Healing in a Diabetic Mouse MRSA Infection. Frontiers in microbiology. 2018;9:561.
172. United States of America clinical trial database. https://wwwclinicaltrialsgov/.
173. Merabishvili M, Pirnay JP, Verbeken G, et al. Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. PloS one. 2009;4(3):e4944.
174. Jault P, Leclerc T, Jennes S, et al. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by Pseudomonas aeruginosa (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. The Lancet Infectious diseases. 2019 Jan;19(1):35-45.
175. Leitner L, Sybesma W, Chanishvili N, et al. Bacteriophages for treating urinary tract infections in patients undergoing transurethral resection of the prostate: a randomized, placebo-controlled, double-blind clinical trial. BMC urology. 2017 Sep 26;17(1):90.
176. Ooi ML, Drilling AJ, Morales S, et al. Safety and Tolerability of Bacteriophage Therapy for Chronic Rhinosinusitis Due to Staphylococcus aureus. JAMA otolaryngology-- head & neck surgery. 2019 Jun 20;145(8):723-729.

1062 177. Sarker SA, Sultana S, Reuteler G, et al. Oral Phage Therapy of Acute Bacterial Diarrhea With
1063 Two Coliphage Preparations: A Randomized Trial in Children From Bangladesh. EBioMedicine.
1064 2016 Feb;4:124-37.

1065