*Review*

# Phage Therapy: Past History and Future Prospects

Richard M. Carlton

Exponential Biotherapies, Inc., 150 Main Street, Port Washington, NY 11050, USA

**Abstract**. Bacterial viruses (bacteriophages, also called "phages") can be robust antibacterial agents *in vitro*. However, their use as therapeutic agents, during a number of trials from the1920s to the 1950s, was greatly handicapped by a number of factors. In part, there were certain limitations inherent in phage physiology (e. g. narrow host range, and rapid clearance from the body); in part there were technological limitations in the era (e.g. lysogeny not yet discovered); but the greatest limitation was the highly inadequate scientific methodologies used by practitioners at the time (e.g., their failure to conduct placebo-controlled studies, to remove endotoxins from the preparations, and to re-confirm phage viability after adding sterilizing agents to the preparations). In recent years, well-controlled animal models have demonstrated that phages can rescue animals from a variety of fatal infections, while non-controlled clinical reports published in Eastern Europe have shown that phages can be effective in treating drug-resistant infections in humans. This encouraging data, combined with the fact that drug-resistant bacteria have become a global crisis, have created a window of opportunity for phage therapy to be tested anew, this time using modern technologies and placebo-controlled designs. If successful, it can be used as a stand-alone therapy when bacteria are fully resistant to antibiotics, and as a valuable adjunct to antibiotics when the bacteria are still susceptible.

**Key words**: bacteriophage; phage; bacterial viruses; bacterial infections; multidrug resistance.

Phages are a kingdom of viruses that infect bacteria, and are distinct from the animal and plant viruses. Phages can have either a "lytic" or a "lysogenic" life cycle. The lytic phages are the most suitable candidates for phage therapy, because they quickly reproduce within and lyse the bacteria in their host range, growing exponentially in number in the process. Depending on the species and conditions, each "parent" phage can produce on average approximately 200 "daughters" per lytic cycle. If each daughter infects and kills a host bacterium there will be 40 000 progeny at the end of the 2nd cycle; 8 million at the end of the 3rd cycle; 1.6 billion at the end of the 4th cycle; and so on.

Some practitioners used phages as therapeutic agents in the West, from the 1920s to the early 1950s (referred to hereinafter as the "historic era"). This re-view will describe: 1) some of the key reasons this form of therapy failed to take root in the West; 2) its previous and current use in some enclaves of Eastern Europe; 3) recent animal models which suggest that phage therapy might be useful for humans; 4) the fact that the emergence of antibiotic-resistant infections has opened a second window of opportunity for phage therapy; and 5) the advantages that might be gained by administering phages along with antibiotics, as a combination therapy.

## Past History

*General information*

A number of reviews provide details on phage therapy's ascent and decline in the historical era[1–3, 13, 14].

**Table 1**. Attributes of phages that tend to favor a therapeutic response

| The issue | Limitations of antibiotics | Advantages of phages |
| --- | --- | --- |
| Fate of the "drug" molecule | Metabolic destruction of the molecule, as it works | Exponential growth in numbers, so that the "drug" makes more of itself at the site of infection, where it is needed |
| Concentration of the "drug" required to kill a given bacterium within the spectrum | Numerous molecules of the antibiotic are needed to kill a given bacterium. During initiation of therapy (and between doses), the sub-lethal dose that bacteria "see" affords them the opportunity to express resistance genes | "All or nothing" effect: one phage particle is sufficient to kill a given bacterium |
| Ability to overcome bacterial resistance | Antibiotics are fixed, immutable chemicals that cannot adapt to a bacterial mutation and therefore become obsolete. Bacteria that have resisted them can pass along the resistance trait within and between species | Phages are "living" organisms that undergo mutations, some of which can overcome bacterial mutations. E. g., mutated phage tail fibers can allow binding to a mutant bacterial receptor, or mutated phage DNA can escape cleavage by mutant bacterial endonucleases |
| Spread of bacterial resistance | The antibiotics in use tend to be broad spectrum, thereby provoking resistance in several species and genera of bacteria (in addition to the one targeted) | Although there are some exceptions, phages tend not to cross species boundaries. Thus even though the targeted bacterial species may become resistant to the phage, it is unlikely that other species will |

We will summarize some of the more salient features of this history.

Phages were discovered in 1915 by British microbiologist Felix Twort, and, independently in 1917, by French-Canadian microbiologist Felix d'Hérelle. Twort did not pursue his discovery, whereas d'Hérelle systematically investigated the nature of bacteriophages and explored their ability to function as therapeutic agents[5, 6].

D'Hérelle received a fair measure of fame for his discovery. He was appointed Professor of Protobiology at Yale University Medical Center, and was also on the staff of the Pasteur Institute. In 1931 he gave a series of monthly lectures on phage therapy to the New York Academy of Medicine. He established phage therapy centers in several countries, including the U. S., France, and Soviet Georgia. A fictionalized account of his work was depicted in Arrowsmith, the Pulitzer-prize winning novel by Sinclair Lewis.

There are many attributes of phages (see Table 1) that would tend to favor a positive outcome in therapy.

Despite these attributes of phages, there were so many problems with the way phage therapy was practiced in the historical era that, by the time antibiotics were introduced in mid-century, it was already in sharp decline in the West. The investigators who developed antibiotics did not make the kinds of mistakes exhibited by the early phage investigators.

*Key problems with phage therapy, and how the problems can be overcome*

Problem 1. Host range

*The issue*. Phages tend to have a relatively narrow host range, posing certain disadvantages. A disadvantage is that one should administer only those phage strains shown to be strongly lytic for the bacterial strain infecting the given patient. If the patient's condition is too critical to take the time required for this matching, then one should use a grouping (a panel) of phages, where each of the phages therein has a broad-enough host range that most strains of the bacterial target are likely to be targeted. In his lectures to the New York Academy of Medicine in 1931, d'Hérelle cited the reports of other colleagues whose initial trials used phages "off the shelf" (without being shown to be virulent for the bacteria infecting the patient) and had negative outcomes, but who did match the phage to the bacteria in subsequent trials and obtained positive outcomes.

*The solution*. 1) Screen the bacteria infecting a given patient against a panel of phages, to ensure that one of the phage strains will be lytic (analogous to the "culture and sensitivity test" that physicians should perform; and 2) develop "multivalent" phages that lyse all or most of the bacterial strains within a given species of pathogen.

Problem 2. Bacterial debris present in the phage preparations

*The issue*. Injection of even minute amounts of endotoxin and other bacterial debris can be fatal to patients. Unfortunately, many of the phage preparations used by practitioners in the historical era were crude lysates. When these preparations were injected i.v., i.p., and in some cases even intrathecally, any beneficial effect of the phages would likely have been counteracted by illness and deaths resulting from the endotoxin.

*The solution*. Modern technology allows density centrifugation, banding, and other methods of purification.

Problem 3. Attempts to remove host bacteria from therapeutic preparations

*The issue:* In order to ensure that phage preparations would not contain live bacteria, some early investigators added mercurials and/or oxidizing agents, while others heated them. It is now known that such agents and procedures will denature or otherwise inactivate the phage coat proteins. These investigators did not check for continued viability of the phages. The false-negative results of such studies were the unintended (but inevitable) consequence of such practices.

*The solution*: Sterile filtration. If chemical agents must be used, retitrate the preparation over time to ensure that the phage remain viable.

Problem 4. Rapid clearance of phages

*The issue*. In fairness to phage investigators in the historical era, at the time it was not an accepted practice, in any discipline, to conduct pharmacokinetic studies. However, had the early phage investigators conducted such studies, they would have discovered that bacteriophages (being foreign proteins) tend to be rapidly cleared from the circulation. This clearance problem was first documented by Merril and his colleagues in 1973 who injected high titers of phage lambda into non-immune germ-free mice. They discovered that the phages were rapidly cleared by the spleen, liver and other filtering organs of the reticulo-endothelial system (RES)[7]. This was a seminal observation, given Gunther Stent's widely-accepted statement that one of the principal reasons phages had failed as a therapeutic was their supposed inactivation by pre-existing antibodies to them. However, any clearance of the phages from the bloodstream of the germ-free animals used by Merril and his group (ref.[7]) would not be due to antibodies, since those animals had never previously been exposed to bacteria or bacteriophages (and so would not have antibodies). Moreover, the phages in Merril's experiment remained viable in the spleens of these animals over a period of several days, indicating that they were neither neutralized by antibody nor engulfed by macrophages. Rather, they appeared to have been passively entrapped in (sequestered by) these filtering organs. Such trapped phages would be unavailable to reach bacteria.

*The solution*. The author of this review collaborated with investigators at the U.S. National Institutes of Health (MERRIL et al.[11]) in the development of a method to isolate and amplify phage strains that are cleared at a slower rate. We reasoned that in all species of phage, minor variations in coat proteins might be present that would enable some variants to be less easily recognized by the RES organs and to thereby remain in the circulation for longer periods of time than the "average" wild-type phage. In this "serial passage" method, the wild-type preparation is injected into an animal, and then blood samples are taken at progressively longer time points. Any phages found in the blood sample are grown to high titer and reinjected. Through iterative rounds of passage, one can amplify the long-circulating strains being isolated. U.S. and PCT patents have been granted on this method.

For coliphage lambda as well as for salmonella phage P22, phage variants were isolated in this manner that were much longer-circulating than the wild-type. For example, for every 100 000 particles of the wild-type lambda used at baseline, only one particle remained in circulation at 18 h; whereas for the long-circulating phage mutant isolated at the 8th round of serial passage, for every 100 000 injected, at 18 h 62 500 particles remained in circulation. For each moment of time, far more of these long-circulating phages are propagating exponentially, as compared to the situation for the wild-type phages.

As predicted, these long-circulating phages were far superior to the wild-types from which they were derived, in terms of rescuing animals from an otherwise-fatal fulminant bacteremia: 1) with no treatment, all animals were dead within 48 h; 2) treatment with the wild-type phages prevented death, but the animals became critically ill (a human with such degrees of illness would be in the intensive care unit); and 3) in contrast, with administration of the long-circulating phage strain, the only sign of illness seen was mild lethargy. These results were published in the Proceedings of the National Academy of Sciences (ref. [11]), and were accompanied by a Commentary by Nobel laureate Dr. JOSHUA LEDERBERG[8].

We have elucidated the molecular basis of the mutation in lambda that reduced its rate of clearance: a single point mutation, an A to G transition, had occurred in the gene encoding the major head protein E. This mutation substituted a basic amino acid (lysine) for an acidic one (glutamic acid), causing a double charge shift readily seen on 2D gel electrophoresis. Computer modeling predicted that the mutation occurred in a loop of the E protein that sticks out into space and that therefore may interact with the external environment. A double charge shift in this region of a protein that is highly represented on the surface of the virion could conceivably alter the phage's interaction with the microcirculation of the spleen, in such a way that the mutant phage is less easily entrapped than the wild-type.

Problem 5. Lysogeny

*The issue.* It was not until the late 1950s that Lwoff demonstrated the ability of some phage genomes to integrate into the bacterial chromosome as "prophages". After a period of time (up to days or weeks, or longer), such prophages can enter the lytic cycle, and will thus appear as plaques on a bacterial lawn. It is likely that some phage therapy trials in the historic era had a negative outcome due to the inadvertent use of phage strains that, being lysogens, could not provide the rapid lysis and exponential growth in numbers that are needed for full efficacy.

*The solution.* Use only phages that are lytic; sequence phages that are strong candidates for clinical trials, looking for (among other things) homologies to known genes of lysogeny.

Problem 6. Anti-phage antibodies

*The issue.* There are reports in the literature[20] that neutralizing antibodies appear a few weeks after administering phages to humans or animals. Given the time lag, antibodies would not seem likely to interfere with an acute treatment lasting a week or so. However, in chronic treatment, or in treatment of a recurrence of the same bacterial infection, the neutralizing antibodies might prevent some proportion of the administered dose of phages from being able to adhere to the bacterial target.

*The solution.* In treating chronic or recurrent infections it may be possible to administer a higher dose of phage, to compensate for those that are rendered non-viable by interaction with neutralizing antibodies. In any case, the types and titers of antibodies that develop should be systematically studied in humans.

Problem 7. Failure to establish scientific proof of efficacy

In scholarly reviews of comparative styles of research, Dutch historian Ton van Helvoort[24] has discussed d'Hérelle's systematic failure to conduct double-blind studies. As van Helvoort pointed out, while it is true that ethical problems are faced by anyone who has to administer placebo to some patients (in order to prove efficacy), nevertheless the investigators who later tested antibiotics did conduct double-blind, placebo-controlled trials. Van Helvoort points out that, even when using phages to treat an epidemic of diarrhea in poultry on a French farm, d'Hérelle failed to use a placebo on half the flock (a situation where ethical considerations would not have been an issue). As a consequence, all reports of phage therapy's successes in the historical era were anecdotal. No systematic proof was available to demonstrate that the results were reliable and repeatable.

Problem 8. The scientific style of phage investigators in the historical era

D'Hérelle's failure to conduct placebo-controlled studies, even on chickens, is an important example of his style. This story is a notable example of the negative impact an investigator's personality can have on the outcome of a discovery, and d'Hérelle's style contrasts sharply to the strongly positive influence that other scientists (such as Pasteur) have had on the outcomes of their discoveries. Whereas Pasteur excelled at conceiving of definitive experiments, and was persuasive in style, d'Hérelle failed to conduct definitive experiments, and was antagonistic rather than persuasive.

For example, d'Hérelle maintained to the end that phages are the sole mechanism of defense against bacterial infection. While he may have been correct in his view that epidemics can sometimes be checked by the spontaneous appearance of a lytic strain of phage, nevertheless he was incorrect in categorically dismissing the discoveries of Nobel laureates Metchnikoff and Ehrlich, who had shown that cellular elements (white blood cells) and humoral elements (antibodies and complement) constitute the innate host defenses against infection. D'Hérelle was afforded many opportunities to integrate his discovery with those of Metchnikoff and Ehrlich, but refused to the end (see below).

In addition to the damage he was doing to himself and his cause with this adamance, d'Hérelle was attacked by Nobel laureate Jules Bordet (for whom *Bordetella pertussis* was named), who had an intense dislike not just for d'Hérelle's science but also for the man himself. Bordet used his considerable influence to discredit D'Hérelle[5].

D'Hérelle retreated from attacks by Bordet and others, and moved to Soviet Georgia in the 1930s (see ref. [13]). An ardent communist, he dedicated the last of his published treatises to Josef Stalin. He was in Paris at the outbreak of World War II, refused to offer his skills with phage therapy to the Germans*, and spent the occupation years in prison. By the time of the liberation his health had been broken. He was invited to a post-War international scientific symposium, where colleagues made a last effort to see if they could help him bridge the gulf. He persisted in his belief that phages were the body's sole mechanism of defense against bacteria ("Ce n'est *que* la phage…"), and he died in isolation in 1949.

Surely the prospects of phage therapy in the historical era would have been better served if d'Hérelle had possessed some of the personality traits and scientific style of Pasteur.

## Animal Models of Phage Therapy

From the 1950s to the 1980s there was little published on the subject of phage therapy. Then papers began to appear demonstrating the utility of phage therapy in animal models. For example, phages were shown to be effective in rescuing rats from fatal systemic infections (induced with *E. coli*)[14] in rescuing calves and lambs from fatal diarrhea (induced with *E. coli*)[15, 16], in rescuing chicks from fatal diarrhea (induced with *S. typhimurium*)[4], and in preventing destruction of skin grafts in burned rabbits by *Pseudomonas aeruginosa*[18]. As mentioned above MERRIL et al.[11] demonstrated in 1996 that mice with fulminant *E. coli* bacteremia could be rescued by phages, and that long-circulating phage variants were superior to the wild-types (see below).

In one of those studies cited, Smith and Huggins (ref. [6]) demonstrated that, in rats inoculated with a lethal intramuscular dose of *E. coli*, a single injection of a phage preparation was more effective than multiple injections of antibiotics (chloramphenicol, tetracycline, etc.). This work was replicated in 1997 by LEVIN and BULL[9], who used mathematical modeling in a population dynamics approach to study the titers of phages and bacteria in the animals. The investigators concluded that the reason a single injection of phage was superior to multiple injections of antibiotics was that

the phages grew exponentially in number, overwhelming the bacteria present.

## Current Status of Human Phage Therapy Efforts

*Poland.* Phage therapy is practiced in Poland, albeit on a small scale. In the mid-1980s a series of papers was published by a group led by the late Prof. S. Ślopek and his colleagues, including Dr. M. Mulczyk and Dr. B. Weber-Dąbrowska, working at the L. Hirszfeld Institute of Immunology and Experimental Therapy (a branch of the Polish Academy of Sciences). These papers[20–23] reported on 550 cases of suppurative bacterial infections (empyemas, peritonitis, osteomyelitis, etc.) in humans. Most of the cases were chronic; most were resistant to all available antibiotics; and most had not been referred for this form of therapy until all else had failed, meaning that it was often quite late in the disease progression.

The bacterial pathogens targeted included *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *E. coli*. The phages used by these investigators are reported to have cured approximately 90% of the cases. The criteria of cure were cessation of suppuration and, where applicable, complete closure of wounds/fistulae (many of which had been draining for months).

These investigators administer phages orally, because they are aware of the hazards of administering them parenterally (not all of the bacterial debris has been removed). They pre-treat the patients with antacids and gelatin in order to protect the phages from destruction by gastric acidity. These same investigators have published evidence that phages administered orally to humans in this manner do in fact reach the bloodstream[26].

The Polish investigators have been rigorous in matching the phages to the bacterial strain infecting the given patients. Their practice, as stated in the published reports, is to culture the bacteria during the course of treatment, so that the occurrence of a mutant resisting the phage can be countered by switching to a different phage strain. The group also has panels of multivalent phages available, for use in fulminant infections (such as septicemia with acute respiratory distress syndrome) where time is insufficient to classify the offending bacteria or to match phages to bacteria.

The group now has statistics on the treatment of approximately 1 300 cases. The overall cure rate across the spectrum of pathogens and sites of infection is approximately 86% (personal communication from Dr. B. Weber-Dąbrowska).

A criticism of the work by Ślopek's group is that

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* The push of the German army into the region of Georgia was intended not only to capture the region's oil wells, but also to obtain the collection of phages manufactured at the Eliava-d'Hérelle Institute in Tblisi. That institute was providing phages to the Russian army, to control dysentery, *Staphylococcus aureus* infections of wounds, and other bacterial problems associated with war.

the absence of placebo controls means the power of suggestion cannot be definitively ruled-out. It is clear that the difficulties of that nation's economy over recent decades has denied the investigators the financial resources needed to enroll matched cohorts in a placebo arm of a clinical trial. While the criticism is valid, and absolute proof of principle can be obtained only through placebo-controlled trials, nevertheless the usefulness of the data is improved by the detailed statistical accounting of the percentages of complete, partial and nil response. One of the factors that enables this author to find the data from Poland more believable (even in the absence of double-blind proof) is that in conditions such as emphysema where phage efficacy might be somewhat impeded, the group's statistics show that the success rate is considerably lower than for other conditions where such impediments do not obtain*.

*The Republic of Georgia.* The work started in Tblisi in the 1930s by d'Hérelle and his Georgian colleague, Eliava, continues to this day. In the 1970s, under the direction of Dr. Teimuraz Chanishvili, the Eliava-d'Hérelle Institute had a large staff manufacturing considerable quantities of phage preparations per year, primarily for the control of dysentery in the troops of the Soviet Army. This group has anecdotal evidence of the efficacy of phage therapy. They report, for example, that in certain adult and pediatric hospitals it is routine for their phage preparations to be administered topically on surgical incisions. Given the lack of statistical analysis, there is little to be said other than the anecdotal reports are encouraging that phage therapy can be useful.

## Multidrug-Resistant (MDR) Bacteria Have Created a Need for Phage Therapy

Several species of bacteria have become resistant to most antibiotics, with some strains being resistant to all antibiotics. One example is vancomycin-resistant *Enterococcus faecium* (VRE), a low-virulence pathogen that now frequently causes fatal bacteremias due to complete resistance[2]. Another example is vancomycin intermediate-resistant *Staphylococcus aureus* (VISA), strains of which have recently emerged in three nations (Japan, U.S. and Scotland), and are known to have killed 4 patients to date. Such strains spread throughout Japanese hospitals within a year of their first appear-

ance. Unfortunately, it has been demonstrated that some hospital strains of methicillin-resistant *S. aureus* (MRSA) that are widespread have become vancomycin resistant upon exposure of the patients to vancomycin[1, 2]. Experts predict that *S. aureus* will progress to become completely resistant to vancomycin (the antibiotic of last resort for most strains of this pathogen), and that when this occurs, millions of people will die each year from infections that had until recently been fairly easy to control. Based on such developments and impending developments with pathogens such as MRSA and VRE, opinion leaders have been warning that we are entering the "Post-Antibiotic Era".

While pharmaceutical companies are developing new antibiotics to counter the trend, it has been shown that half a century of global antibiotic abuse has equipped the surviving bacteria with "supergenes" that enable them to quickly resist new classes of antibiotics, even those to which they have never been exposed[1]. Examples of the "supergenes" are mutations that 1) enable bacteria to pump out several classes of antibiotics (through an efficient efflux pump), or that 2) alter the antibiotic binding sites on ribosomal subunits, so that several different classes of antibiotics can no longer inhibit those subunits. As a consequence, in recent years, by the time newer antibiotics have gone through clinical trials and have reached the market, 20% or more of clinical isolates in the hospitals are already resistant to them at the time of regulatory approval, and within a few more years the majority of strains are resistant.

## Future Prospects for Phage Therapy

Infectious disease experts have warned that there is now a compelling need to develop totally new classes of antibacterial agents, ones that cannot be resisted by the same genes that render bacteria resistant to antibiotics.

Phage therapy represents such a "new" class. We believe that the impediments cited above (bacterial debris in the preparations, rapid clearance in the body, etc.) can be overcome, freeing up the phages so that their attributes (such as exponential growth, and the ability to mutate against resistant bacteria) can be used to great advantage.

There are 3 additional attributes of phages that should be noted:

*Host specificity.* While the host specificity is somewhat of a drawback (requiring a matchup of phage to bacterial target, and/or the development of highly multivalent phages), it also offers the great advantage that the phages will not kill other species of bacteria.

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* Conditions where phage efficacy is predicted to be reduced would include 1) hypoxic sites, where bacterial replication is slower and therefore phage replication is reduced; and 2) chronic obstructive pulmonary disease, where high acidity and proteases would be expected to inactivate some percentage of the phages.

Thus, e.g., phage therapy is not likely to kill off the healthy flora of the intestines, lungs or urogenital tract, and it is therefore unlikely to provoke the illnesses and deaths seen when antibiotics cause overgrowth of pathogens (such as *Clostridia difficile* and *Candida albicans*).

*Genetic engineering*. It is possible to genetically engineer phages to express new traits of potential value. In so doing, scientists will have to deal with the legitimate concerns of regulatory agencies concerning recombinant organisms. The regulatory obstacles may be well worth the price, given the powerful engineering tools that are currently available.

*Ideal candidates for co-therapy with antibiotics*. If a given bacterium acquires resistance to a phage (e.g. by a mutation in the receptor site or in the endonuclease enzymes), that mutation is not likely to "teach" the bacterium to resist the antibiotics (which do not target those structures). Similarly, if a given bacterium acquires resistance to an antibiotic (e. g. by a mutation in the reflux pump or in the ribosomal subunits), that mutation is not likely to "teach" the bacterium to resist the phage (which does not target those structures). Thus, if the bacterium is exposed to both agents, the odds are remote that any resistance genes it starts to express (or acquires anew) will enable it to survive. There are reports that bacteria tend to mutate against antibiotics once in every $10^6$ divisions, while they tend to mutate against phages once in every $10^7$ divisions. Therefore the odds of a given bacterium mutating against a phage and an antibiotic at the same time would be the product of $10^6 \times 10^7$, meaning it would likely take $10^{13}$ bacterial divisions for such a double mutation to occur. Given that low probability, the co-administration of phages and antibiotics may help prevent the emergence of bacterial resistance to antibiotics, thereby greatly prolonging their clinical usefulness (and *vice versa*). Just as multiple classes of anti-HIV medications are administered to AIDS patients, to prevent the emergence of resistant strains of that virus, so it is that co-therapy with phages and antibiotics may also prove to be of great clinical value.

## Conclusion

Multidrug-resistant bacteria have opened a second window for phage therapy. Modern innovations, combined with careful scientific methodology, can enhance mankind's ability to make it work this time around. Phage therapy can then serve as a stand-alone therapy for infections that are fully resistant. It will also then be able to serve as a co-therapeutic agent for infections

that are still susceptible to antibiotics, by helping to prevent the emergence of bacterial mutants against either agent.

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