






The Future of Bacteriophage Therapy Will Promote Antimicrobial Susceptibility

 Olivia Williams Barber,^a Iria Mañas Miramontes,^a Manu Jain,^b  Egon A. Ozer,^c  Erica M. Hartmann^a

^aDepartment of Civil and Environmental Engineering, Northwestern University, Evanston, Illinois, USA

^bDivision of Pulmonary and Critical Care Medicine, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

^cDepartment of Medicine, Division of Infectious Diseases, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

ABSTRACT Rising antimicrobial resistance severely limits efforts to treat infections and is a cause for critical concern. Renewed interest in bacteriophage therapy has advanced understanding of the breadth of species capable of targeting bacterial antimicrobial resistance mechanisms, but many questions concerning ideal application remain unanswered. The following minireview examines bacterial resistance mechanisms, the current state of bacteriophage therapy, and how bacteriophage therapy can augment strategies to combat resistance with a focus on the clinically relevant bacterium *Pseudomonas aeruginosa*, as well as the role of efflux pumps in antimicrobial resistance. Methods to prevent antimicrobial efflux using efflux pump inhibitors and phage steering, a type of bacteriophage therapy, are also covered. The evolutionary context underlying antimicrobial resistance and the need to include theory in the ongoing development of bacteriophage therapy are also discussed.

KEYWORDS *Pseudomonas aeruginosa*, antibiotic resistance, bacteriophage therapy, evolutionary rescue

Antimicrobial resistance (AMR) is a major threat to modern medical advancements. Although antimicrobial drugs create a hostile environment for bacteria, antimicrobials cannot change their mechanism of action, while bacteria can evolve defenses and experience evolutionary rescue. Evolutionary rescue causes even new antimicrobials to often become ultimately ineffective against the most resistant bacteria (1). The rise of resistance requires us to develop novel solutions to combat infection. Bacteriophage, or phage, therapy uses viruses that infect bacteria to treat human infections, often in conjunction with antimicrobial treatment (2–4). Phage not only provide an alternative method to kill pathogens and an additional selective pressure, but also coevolve with their bacterial hosts.

While phage therapy was used widely prior to the discovery of antibiotics (5), the initial availability of antibiotics exclusively to the Allies in World War II, confusion about the science of phages, and suspicion of Soviet scientific results after the war led to the lack of adoption outside the former Soviet Union (3). In Tbilisi, Georgia, the George Eliava Institute of Bacteriophage, Microbiology and Virology has been conducting phage therapy research and treatment for almost a century (6). Although Georgia, Russia, and a few other Eastern European countries widely use phage-based treatments (7), there are no Food and Drug Administration (FDA)-approved phage therapies, and use in the United States is reserved for compassionate treatment (8).


One type of phage therapy that considers evolutionary pathways of resistance is phage steering. Phage steering intentionally combats resistant infections by forcing bacteria to resist the selection pressure of either phage or antimicrobials (9). Currently, most phage steering applications use natural phages that are prescreened for efficacy against a specific bacterial target. Using phage steering to target AMR mechanisms can

Citation Barber OW, Miramontes IM, Jain M, Ozer EA, Hartmann EM. 2021. The future of bacteriophage therapy will promote antimicrobial susceptibility. *mSystems* 6: e00218-21. <https://doi.org/10.1128/mSystems.00218-21>.

Editor Mark J. Mandel, University of Wisconsin-Madison

Copyright © 2021 Barber et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Erica M. Hartmann, erica.hartmann@northwestern.edu.

 Pathogens evolve, but antibiotics don't. We look at the use of phage as drugs to keep up with the evolution of resistance. Researchers should consider the evolutionary context of pathogens when designing new drugs for immediate and long-term efficacy.

Published 20 July 2021

potentially extend the life span of current antimicrobials by anticipating that bacteria will develop resistance (10). AMR in *Pseudomonas aeruginosa* is particularly concerning because it is a common cause of acute and chronic infections in humans and is predisposed to the development of resistance (11, 12).

The following review focuses on countering resistance in *P. aeruginosa* and how bacteriophage therapy can improve existing approaches. A brief overview of bacterial resistance methods, including efflux pumps, and the evolutionary theory behind antimicrobial resistance is provided. How these mechanisms influence bacteriophage therapy, and the potential repercussions, will be considered along with improvements to phage treatment. The use of phage steering and how it enhances current strategies targeting the bacterial efflux system, as well as its use with other phage and antimicrobial treatments, will be highlighted.

BACTERIA USE BOTH BIOCHEMICAL AND PHYSICAL STRATEGIES TO RESIST ANTIMICROBIALS

Resistance to toxic substances is an intrinsic protective strategy that has long predated clinical use of antimicrobials (13). Increased clinical, industrial, and agricultural use of antimicrobials such as β -lactams, aminoglycosides, and quinolones has promoted multidrug resistance in many clinically relevant bacterial organisms, including *P. aeruginosa* (14).

Bacteria employ many resistance strategies such as production of degradative enzymes, formation of biofilm structures regulation or alteration of outer membrane proteins, and antimicrobial secretion through efflux pumps. Bacteria can inactivate antimicrobials using enzymes like adenylyltransferase ANT(II)-Ia, which results in resistance to three antimicrobials in the aminoglycoside family (gentamicin, tobramycin, and kanamycin) (15). However, the mechanisms and regulation of enzyme-induced AMR, such as AmpC β -lactamase overproduction (16) or the influence of protein CATB7 in chloramphenicol resistance (17), are not always well understood.

Biofilm growth is often associated with increased AMR compared to that of planktonic growth of bacteria, in part because not all members of the biofilm need to express drug-inactivating enzymes to protect the community as a whole. Commonly, susceptible bacteria will reside alongside drug-resistant bacteria within the structure, creating an internal diversification of bacterial activities, which can be an impediment to successful antimicrobial therapy (18). While biofilms are considered generally recalcitrant to antimicrobials (19, 20), not all studies have found significant differences in resistance (21).

Both biofilm growth and biofilm resistance have been linked to efflux systems embedded in the membrane (22–25). Regulation of membrane proteins is a common resistance strategy. Analysis of the *P. aeruginosa* membrane proteome found a strong association between the abundance of certain outer membrane efflux transporters and the degree of resistance to ampicillin, kanamycin, and tetracycline (18). These results suggest that among the outer membrane protein markers found, some appear to be upregulated across antimicrobial classes while other markers show more regulation specificity, which is dependent on the specific antimicrobial substrate, hence making a case for the future investigation of shared homologous targets.

Efflux pumps are a focus of resistance treatment due to their substrate promiscuity.

Efflux systems located in the cell membrane have both generalized and specific responses to antimicrobials. *P. aeruginosa* possesses multiple efflux pump systems that have been extensively characterized, including MexAB-OprM, MexCD-OprJ, and MexXY-OprM, which often promote resistance to multiple antimicrobials (26–28). Both pharmaceutical and biological methods for efflux inhibition have been investigated. This includes efflux pump inhibitors (EPIs), which prevent removal of antimicrobials through methods such as downregulation of efflux pump expression, efflux competition, or blocking the outer membrane channel (Fig. 1A to D). Efflux competition between the EPI Phe-Arg- β -naphthylamide (PA β N) and antimicrobials leads to PA β N being preferentially pumped out and allows antimicrobials to reach lethal

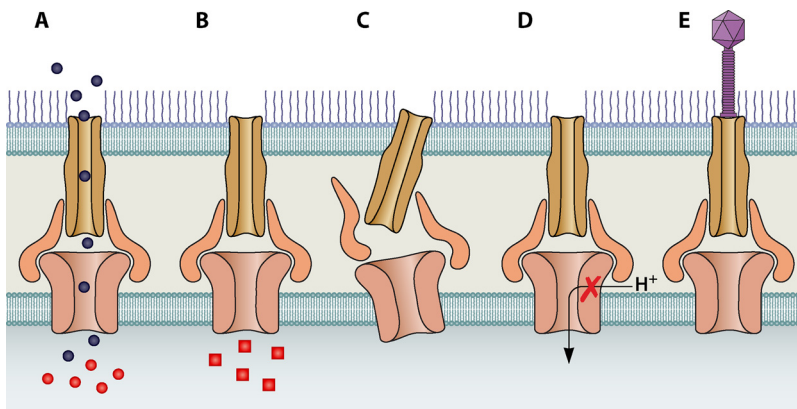


FIG 1 Efflux pump inhibitor (EPI) mechanisms (A to D) compared to phage steering (E). The figure illustrates (A) substrate competition causing efflux of EPIs (black circles) while retaining antimicrobials (red circles), (B) altered antimicrobial structure by EPIs (red squares) prevents recognition by the efflux system, (C) disruption of pump assembly, (D) disruption of the proton motive force required for efflux, and (E) phage steering using the outer efflux protein as a receptor to block efflux in addition to actively destroying pathogens.

concentrations (29). Other EPIs such as spermine are thought to physically block the membrane channel of OprD in *P. aeruginosa* (30).

EPIs can have knock-on effects that further reduce pathogenicity. For example, efflux inhibition in *P. aeruginosa* and other pathogens affected quorum sensing-dependent virulence factors (31), invasiveness (32), and biofilm growth (25, 33), which could further increase antimicrobial efficacy. While EPIs are a potential method to combat resistance, some research outcomes highlight the question of whether efflux pumps are always a main driver of *P. aeruginosa* resistance (34, 35). Additionally, both EPIs and antimicrobials require continued development in response to the evolution of bacterial resistance.

The evolutionary context underlying AMR is important to develop treatments.

AMR can be considered a form of evolutionary rescue (36). Evolutionary rescue occurs when adaptation allows a declining population to recover and avoid extinction (37). Treatments that minimize the probability of evolutionary rescue are expected to be the most effective (38).

The probability of rescue can increase due to existing mutations within a population, periods of environmental quality restoration, and gradual decay of environmental conditions (36). Additionally, extreme environmental changes, such as an application of concentrated antimicrobials, can be beneficial for the survival of mutants within the population (39). The role of migration, or horizontal gene transfer in antimicrobial resistance, can also favor evolutionary rescue, as seen by the transfer of a plasmid containing a β -lactamase gene from one *Escherichia coli* strain to a β -lactam-susceptible strain, allowing the latter to survive high levels of ampicillin (40).

Greater population size and initial genetic diversity have also been correlated with a higher likelihood of antimicrobial resistance evolution (41). Maintenance of genetic diversity through multiple adaptations moving as a “soft sweep” through a population may also increase the likelihood of evolutionary rescue (42). Diversity could be one predictor of drug efficacy, with diverse populations being more likely to survive (42).

Survival through evolution is a trait of not only bacteria but also their phage predators. Infection of “suboptimal” bacterial hosts by cyanophages led to diverse evolutionary outcomes in phage, which suggested that host availability affected bacteriophage evolution (43). The coevolution of phage and bacteria is a central concept behind the development of bacteriophage therapy.

THE PRINCIPLE OF BACTERIOPHAGE THERAPY AND ITS PLACE IN MODERN MEDICINE

Phage and bacteria coevolve, with host efflux pumps often being used for recognition. Bacteriophages are DNA or RNA viruses that infect specific bacterial hosts through recognition of one or multiple receptor binding proteins (RBPs) on the cell

surface, including efflux systems (44). Efflux pump proteins are the RBPs for multiple phages. Examples include *P. aeruginosa* phage OMK01, which uses the M protein (45), *Vibrio cholerae* phage VP3, and *E. coli* phages U136B and TLS, which use the TolC protein as a coreceptor (46–48). TolC is also a receptor for the ST27, ST29, and ST35 bacteriophages, which are active against several *Salmonella* serovars, many with high TolC sequence similarity (49). However, despite TolC sequence similarity in some *Enterobacteriaceae*, the ST27, ST29, and ST35 are inactive against many *Enterobacteriaceae* species, demonstrating the host specificity of phages (49).

In response to phage predation, bacterial hosts have evolved both broad and specialized strategies to resist infection through inhibition of phage adsorption, injection, or replication. Adsorption can be prevented through biofilm production, as observed in biofilm protection of embedded *Staphylococcus epidermidis* from phage Sepunavirus philBB-SEP1 (50). Additionally, adsorption can be blocked by RBP modification, even while maintaining antimicrobial efflux dependent on the same protein (47). RBP mutations not only block phage attachment, but can also confer cross-resistance to multiple phage species targeting the same binding site (51). Furthermore, several phage resistance mechanisms can coexist, as observed in *E. coli* O157:H7 where exposure to phage PP01 changed in both the outer membrane lipopolysaccharides and outer membrane protein C channel expression (52).

While bacteria evolve to resist attack, phage coevolve to overcome defense mechanisms. Parallel evolution of four cloned bacteriophage PP01 populations converged to have the same point mutations, which improved binding to the *E. coli* O157:H7 outer membrane protein C receptor (53). Phages can even influence host quorum sensing to increase the ease of infection, as demonstrated by phage DMS3 lysogeny in *P. aeruginosa* that inhibited quorum sensing-controlled biofilm production, swarming behavior, and even anti-phage defenses (54, 55). The ability of phage to evolve in response to bacterial resistance is one reason bacteriophage treatment is appealing compared to fixed antimicrobial formulas.

Resistance to bacteriophage and antimicrobials can be synergistic or antagonistic.

An appealing aspect of phage therapy is that it uses the host specificity that phages naturally possess to target pathogens of interest. However, in the context of divergent evolution of pathogens, this specificity is a double-edged sword. A substantial barrier to effective phage therapy is understanding how pathogens evolve resistance, both to phage and antimicrobials.

Biofilm formation is one resistance mechanism that could provide protection against both phage and antimicrobials. *P. aeruginosa* PAO1 was resistant to phage 14/1 in sublethal concentrations of gentamicin (56). The outcome was hypothesized to be a result of the generalized resistance promoted by biofilms (56), which commonly develop in sublethal antimicrobial conditions (57, 58).

Although biofilm formation can inhibit both antimicrobials and phage activity, some studies have identified certain phage enzymes as potential biofilm treatment agents. *P. aeruginosa* biofilms experienced a 99% reduction in bacterial exopolysaccharide viscosity through exposure to phages producing polysaccharide depolymerase (59). An enzyme produced by phage PT-6 hydrolyzed biofilms of *P. aeruginosa* strains isolated from patients with cystic fibrosis, a finding which could ultimately improve patient outcomes by disrupting biofilm growth (60). Additionally, biofilm degradation using *P. aeruginosa* phage LKA1 lysate did not affect ciprofloxacin or gentamicin activity (61), which is an important factor to consider when combining phage and antimicrobial treatment. Thus, phage enzymes directed toward biofilm degradation could become important auxiliary aids in bacteriophage therapeutics. However, due to the complexity of bacterial biofilm structures, phage enzymes require additional research to investigate their potential clinical applicability.

THE DUAL ROLE OF EFFLUX PUMPS IN ANTIMICROBIAL RESISTANCE AND PHAGE SUSCEPTIBILITY

Bacteriophage steering deliberately uses the coevolution of phage and their hosts to treat bacterial infections (62). There have been multiple studies focused on phage

steering of OMKO1 in *P. aeruginosa* (9, 45, 63). In one therapeutic investigation, phage OMKO1 directed evolutionary changes in multidrug-resistant *P. aeruginosa* to make it susceptible either to phage or to one of four antimicrobials of different classes (63). Through downregulation of the efflux system protein OprM, which OMKO1 uses as a receptor, the bacteria developed resistance to OMKO1, but also became susceptible to antimicrobials (Fig. 1E) (63). In computational modeling of *in vitro* phage-antimicrobial synergy, combination therapy of antimicrobials with phage OMKO1 against *P. aeruginosa* resistant to either phage or antimicrobials resulted in a treatment efficacy higher than that of either therapy individually (64). Successful therapeutic application of OMKO1 to treat a chronic *P. aeruginosa* infection in a patient demonstrated that prior knowledge of the evolutionary resistance pathways against antimicrobials and phage is critical for positive outcomes (45). Outcomes can be improved using screening methods, such as intentional overexpression of outer membrane proteins, to identify phage that target the ideal host receptor to introduce antimicrobial susceptibility (49).

Other phage steering mechanisms have been identified, such as the use of *Acinetobacter baumannii* phages Φ FG02 and Φ CO01 (65). Phage resistance resulted in bacterial capsule loss and subsequent susceptibility to certain antimicrobials and other phages (65). An earlier study found that *Acinetobacter baumannii* with multidrug resistance to more than three antimicrobials, either within or across classes, were significantly more likely to be phage susceptible, with phage infection rates over 80% compared to around 50% for cells with no AMR (66). However, due to the multitude of resistance strategies, including enzyme production, and the possibility that some anti-phage adaptations could still maintain AMR (46), application of a single phage in conjunction with a single antimicrobial is unlikely to be the most effective treatment. Treatment combining ϕ PA01 and ϕ PA02 in a phage cocktail suppressed *P. aeruginosa* growth for 20 h compared to between 8 and 9 h for either phage applied individually (67). Furthermore, the use of the phage cocktail combined with either ciprofloxacin or meropenem was even more effective than the cocktail alone and suppressed growth for 96 h (67). Designing phage cocktails to contain multiple phages that steer evolution toward antimicrobial susceptibility would improve phage therapy outcomes. The evolutionary design is in contrast to selection of phages based only on host specificity or growth inhibition, which are some of the most common methods currently used to choose cocktail candidates (68, 69).

Bacteriophage as a therapeutic agent faces production and delivery challenges.

Current limitations to bacteriophage therapy include the lack of purity and stability in solution as well as the reduction in concentration between the point of administration and the site of bacterial infection (70). Phage purification has been achieved using naturally occurring phage aggregation and microfiltration (71, 72). Microencapsulation of precipitated phages has been suggested to protect phages from stomach acid after oral administration (73). As the number and availability of phage products increase, production standards and regulatory frameworks must be created to provide the same safe outcomes and ease of access expected of antimicrobial therapeutics (74–76).

Successful clinical outcomes using phage treatment have been reported (45, 75, 77, 78), but to date there has been a lack of robust, double-blind phase III trials (76, 78). A double-blind phase I/II clinical trial for phage treatment of *P. aeruginosa* ear infections demonstrated reduced infection levels (79). Conversely, another double-blind phase I/II clinical trial that treated burn wounds using a *P. aeruginosa* phage cocktail cream resulted in slower recovery compared to that of conventional treatment (80). Ideal treatment schedules also remain relatively unexplored, although one study found that the application of streptomycin 12 h after phage led to the greatest reduction in *P. aeruginosa* POA1 density *in vitro* (81). Mixed outcomes in studies are caused by wide variations in experimental design, including phage species used, concentrations of phage or antimicrobial applied, application methods, and timing of therapy. To adopt phage therapy, it is imperative that more high-quality clinical trials are undertaken and barriers to implementation are resolved.

DISCUSSION

Evolution continues to play a central role in the rise of antimicrobial resistance. If resistance is a form of evolutionary rescue, phage steering can decrease the probability of rescue. Rather than simply identifying phages that target bacteria of interest, *in silico* experiments and screening to invoke evolutionary pathways as well as direct treatment to specifically inhibit the evolution of resistance will bring a more directed approach to phage therapy (82, 83). Phage steering with antimicrobials has been investigated in only a few host-phage combinations (46, 63, 65). Expanding the host strains and types of phage included in studies will lead to a greater understanding of phage steering applications as well as phage-antimicrobial synergies (84, 85). Phages are advantageous due to their abundance, natural antibacterial activity, bacterial specificity, and ability to evolve. Ultimately, a better understanding of phage ecology and evolution will establish phage steering as a major tool for clinicians and researchers to not only combat AMR, but further explore the mechanisms of resistance development.

REFERENCES

- Ventola CL. 2015. The antibiotic resistance crisis: part 1: causes and threats. *Pharmacol Ther* 40:277–283.
- Harada LK, Silva EC, Campos WF, Del Fiol FS, Vila M, Dąbrowska K, Krylov VN, Balcão VM. 2018. Biotechnological applications of bacteriophages: state of the art. *Microbiol Res* 212–213:38–58. <https://doi.org/10.1016/j.micres.2018.04.007>.
- Summers WC. 2001. Bacteriophage therapy. *Annu Rev Microbiol* 55:437–451. <https://doi.org/10.1146/annurev.micro.55.1.437>.
- Kortright KE, Chan BK, Koff JL, Turner PE. 2019. Phage therapy: a renewed approach to combat antibiotic-resistant bacteria. *Cell Host Microbe* 25:219–232. <https://doi.org/10.1016/j.chom.2019.01.014>.
- Chanishvili N. 2012. Chapter 1 - phage therapy—history from twort and d'herelle through soviet experience to current approaches, p 3–40. In Łobocka M, Szybalski W (ed), *Advances in Virus Research*, vol 83. Academic Press.
- Myelnikov D. 2018. An alternative cure: the adoption and survival of bacteriophage therapy in the USSR, 1922–1955. *J Hist Med Allied Sci* 73:385–411. <https://doi.org/10.1093/jhmas/jry024>.
- Brüssow H. 2012. What is needed for phage therapy to become a reality in Western medicine? *Virology* 434:138–142. <https://doi.org/10.1016/j.virol.2012.09.015>.
- McCallin S, Sacher JC, Zheng J, Chan BK. 2019. Current state of compassionate phage therapy. *Viruses* 11 <https://doi.org/10.3390/v11040343>.
- Gurney J, Pradier L, Griffin JS, Gougat-Barbera C, Chan BK, Turner PE, Kaltz O, Hochberg ME. 2020. Phage steering of antibiotic-resistance evolution in the bacterial pathogen *Pseudomonas aeruginosa*. *Evol Med Public Health* 2020:148–157. <https://doi.org/10.1093/emph/eoaa026>.
- Saha DA-O, Mukherjee RA-O. 2019. Ameliorating the antimicrobial resistance crisis: phage therapy. *IUBMB Life* 71:781–790. <https://doi.org/10.1002/iub.2010>.
- Harper DR, Enright MC. 2011. Bacteriophages for the treatment of *Pseudomonas aeruginosa* infections. *J Appl Microbiol* 111:1–7. <https://doi.org/10.1111/j.1365-2672.2011.05003.x>.
- Santajit S, Indrawattana N. 2016. Mechanisms of antimicrobial resistance in ESKAPE pathogens. *Biomed Res Int* 2016:2475067. <https://doi.org/10.1155/2016/2475067>.
- Munita JM, Arias CA. 2016. Mechanisms of antibiotic resistance. *Microbiol Spectr* 4 <https://doi.org/10.1128/microbiolspec.VMBF-0016-2015>.
- Henrichfreise B, Wiegand I, Pfister W, Wiedemann B. 2007. Resistance mechanisms of multiresistant *Pseudomonas aeruginosa* strains from Germany and correlation with hypermutation. *Antimicrob Agents Chemother* 51:4062–4070. <https://doi.org/10.1128/AAC.00148-07>.
- Cox G, Stogios PJ, Savchenko A, Wright GD. 2015. Structural and molecular basis for resistance to aminoglycoside antibiotics by the adenyllyltransferase ANT(2^{''})-Ia. *mBio* 6 <https://doi.org/10.1128/mBio.02180-14>.
- Schmidtke AJ, Hanson ND. 2006. Model system to evaluate the effect of ampD mutations on AmpC-mediated β -lactam resistance. *Antimicrob Agents Chemother* 50:2030–2037. <https://doi.org/10.1128/AAC.01458-05>.
- White PA, Stokes HW, Bunny KL, Hall RM. 1999. Characterisation of a chloramphenicol acetyltransferase determinant found in the chromosome of *Pseudomonas aeruginosa*. *FEMS Microbiol Lett* 175:27–35. <https://doi.org/10.1111/j.1574-6968.1999.tb13598.x>.
- Singh R, Ray P, Das A, Sharma M. 2009. Role of persisters and small-colony variants in antibiotic resistance of planktonic and biofilm-associated *Staphylococcus aureus*: an in vitro study. *J Med Microbiol* 58:1067–1073. <https://doi.org/10.1099/jmm.0.009720-0>.
- Machado I, Lopes SP, Sousa AM, Pereira MO. 2012. Adaptive response of single and binary *Pseudomonas aeruginosa* and *Escherichia coli* biofilms to benzalkonium chloride. *J Basic Microbiol* 52:43–52. <https://doi.org/10.1002/jobm.201100137>.
- De Oliveira A, Cataneli Pereira V, Pinheiro L, Moraes Riboli DF, Benini Martins K, Ribeiro de Souza da Cunha MD. 2016. Antimicrobial resistance profile of planktonic and biofilm cells of *Staphylococcus aureus* and coagulase-negative staphylococci. *Int J Mol Sci* 17:1423. <https://doi.org/10.3390/ijms17091423>.
- Spoering AL, Lewis K. 2001. Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials. *J Bacteriol* 183:6746–6751. <https://doi.org/10.1128/JB.183.23.6746-6751.2001>.
- Zhang L, Mah TF. 2008. Involvement of a novel efflux system in biofilm-specific resistance to antibiotics. *J Bacteriol* 190:4447–4452. <https://doi.org/10.1128/JB.01655-07>.
- Soto SM. 2013. Role of efflux pumps in the antibiotic resistance of bacteria embedded in a biofilm. *Virulence* 4:223–229. <https://doi.org/10.4161/viru.23724>.
- Bay DC, Stremick CA, Slipski CJ, Turner RJ. 2017. Secondary multidrug efflux pump mutants alter *Escherichia coli* biofilm growth in the presence of cationic antimicrobial compounds. *Res Microbiol* 168:208–221. <https://doi.org/10.1016/j.resmic.2016.11.003>.
- Kvist M, Hancock V, Klemm P. 2008. Inactivation of efflux pumps abolishes bacterial biofilm formation. *Appl Environ Microbiol* 74:7376–7382. <https://doi.org/10.1128/AEM.01310-08>.
- Wolloscheck D, Krishnamoorthy G, Nguyen J, Zgurskaya HI. 2018. Kinetic control of quorum sensing in *Pseudomonas aeruginosa* by multidrug efflux pumps. *ACS Infect Dis* 4:185–195. <https://doi.org/10.1021/acscinfecdis.7b00160>.
- Masuda N, Sakagawa E, Ohya S, Gotoh N, Tsujimoto H, Nishino T. 2000. Substrate specificities of MexAB-OprM, MexCD-OprJ, and MexXY-oprM efflux pumps in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 44:3322–3327. <https://doi.org/10.1128/AAC.44.12.3322-3327.2000>.
- Mima T, Joshi S, Gomez-Escalada M, Schweizer HP. 2007. Identification and characterization of TriABC-OprM, a triclosan efflux pump of *Pseudomonas aeruginosa* requiring two membrane fusion proteins. *J Bacteriol* 189:7600–7609. <https://doi.org/10.1128/JB.00850-07>.
- Lamers RP, Cavallari JF, Burrows LL. 2013. The efflux inhibitor phenylalanine-arginine beta-naphthylamide (PA β N) permeabilizes the outer membrane of Gram-negative bacteria. *PLoS One* 8:e60666. <https://doi.org/10.1371/journal.pone.0060666>.
- Kwon DH, Lu CD. 2007. Polyamine effects on antibiotic susceptibility in bacteria. *Antimicrob Agents Chemother* 51:2070–2077. <https://doi.org/10.1128/AAC.01472-06>.
- Rampioni G, Pillai CR, Longo F, Bondi R, Baldelli V, Messina M, Imperi F, Visca P, Leoni L. 2017. Effect of efflux pump inhibition on *Pseudomonas aeruginosa* transcriptome and virulence. *Sci Rep* 7:11392. <https://doi.org/10.1038/s41598-017-11892-9>.

32. Hirakata Y, Kondo A, Hoshino K, Yano H, Arai K, Hirotani A, Kunishima H, Yamamoto N, Hatta M, Kitagawa M, Kohno S, Kaku M. 2009. Efflux pump inhibitors reduce the invasiveness of *Pseudomonas aeruginosa*. *Int J Antimicrob Agents* 34:343–346. <https://doi.org/10.1016/j.ijantimicag.2009.06.007>.
33. Fleeman RM, Debevec G, Antonen K, Adams JL, Santos RG, Welmaker GS, Houghten RA, Giulianotti MA, Shaw LN. 2018. Identification of a novel polyamine scaffold with potent efflux pump inhibition activity toward multi-drug resistant bacterial pathogens. *Front Microbiol* 9:1301. <https://doi.org/10.3389/fmicb.2018.01301>.
34. McFarland AG, Bertucci HK, Littman E, Shen J, Huttenhower C, Hartmann EM. 2021. Triclosan tolerance is driven by a conserved mechanism in diverse *Pseudomonas* species. *Appl Environ Microbiol* 87. <https://doi.org/10.1128/AEM.02924-20>.
35. Santi I, Manfredi P, Maffei E, Egli A, Jenal U. 2021. Evolution of antibiotic tolerance shapes resistance development in chronic *Pseudomonas aeruginosa*. *Infections mBio* 12:e03482-20. <https://doi.org/10.1128/mBio.03482-20>.
36. Wu Y, Saddler CA, Valckenborgh F, Tanaka MM. 2014. Dynamics of evolutionary rescue in changing environments and the emergence of antibiotic resistance. *J Theor Biol* 340:222–231. <https://doi.org/10.1016/j.jtbi.2013.09.026>.
37. Carlson SM, Cunningham CJ, Westley PA. 2014. Evolutionary rescue in a changing world. *Trends Ecol Evol* 29:521–530. <https://doi.org/10.1016/j.tree.2014.06.005>.
38. Orr HA, Unckless RL. 2014. The population genetics of evolutionary rescue. *PLoS Genet* 10:e1004551. <https://doi.org/10.1371/journal.pgen.1004551>.
39. Uecker H, Otto SP, Hermisson J. 2014. Evolutionary rescue in structured populations. *Am Nat* 183:E17–35. <https://doi.org/10.1086/673914>.
40. Ojala V, Mattila S, Hoikkala V, Bamford JK, Jalasvuori M. 2014. Evolutionary rescue of bacteria via horizontal gene transfer under a lethal beta-lactam concentration. *J Glob Antimicrob Resist* 2:198–200. <https://doi.org/10.1016/j.jgar.2014.02.005>.
41. Ramsayer J, Kaltz O, Hochberg ME. 2013. Evolutionary rescue in populations of *Pseudomonas fluorescens* across an antibiotic gradient. *Evol Appl* 6:608–616. <https://doi.org/10.1111/eva.12046>.
42. Wilson BA, Pennings PS, Petrov DA. 2017. Soft selective sweeps in evolutionary rescue. *Genetics* 205:1573–1586. <https://doi.org/10.1534/genetics.116.191478>.
43. Enav H, Kirzner S, Lindell D, Mandel-Gutfreund Y, Beja O. 2018. Adaptation to sub-optimal hosts is a driver of viral diversification in the ocean. *Nat Commun* 9:4698. <https://doi.org/10.1038/s41467-018-07164-3>.
44. Dowah ASA, Clokie MRJ. 2018. Review of the nature, diversity and structure of bacteriophage receptor binding proteins that target Gram-positive bacteria. *Biophys Rev* 10:535–542. <https://doi.org/10.1007/s12551-017-0382-3>.
45. Chan BK, Turner PE, Kim S, Mojibian HR, Eleftheriades JA, Narayan D. 2018. Phage treatment of an aortic graft infected with *Pseudomonas aeruginosa*. *Evol Med Public Health* 2018:60–66. <https://doi.org/10.1093/emph/eoy005>.
46. Burmeister AR, Fortier A, Roush C, Lessing AJ, Bender RG, Barahman R, Grant R, Chan BK, Turner PE. 2020. Pleiotropy complicates a trade-off between phage resistance and antibiotic resistance. *Proc Natl Acad Sci U S A* 117:11207–11216. <https://doi.org/10.1073/pnas.1919888117>.
47. German GJ, Misra R. 2001. The TolC protein of *Escherichia coli* serves as a cell-surface receptor for the newly characterized TLS bacteriophage. *J Mol Biol* 308:579–585. <https://doi.org/10.1006/jmbi.2001.4578>.
48. Fan F, Li X, Pang B, Zhang C, Li Z, Zhang L, Li J, Zhang J, Yan M, Liang W, Kan B. 2018. The outer-membrane protein TolC of *Vibrio cholerae* serves as a second cell-surface receptor for the VP3 phage. *J Biol Chem* 293:4000–4013. <https://doi.org/10.1074/jbc.M117.805689>.
49. Ricci V, Piddock LJ. 2010. Exploiting the role of TolC in pathogenicity: identification of a bacteriophage for eradication of *Salmonella* serovars from poultry. *Appl Environ Microbiol* 76:1704–1706. <https://doi.org/10.1128/AEM.02681-09>.
50. Melo LDR, Pinto G, Oliveira F, Vilas-Boas D, Almeida C, Sillankorva S, Cerca N, Azeredo J. 2020. The protective effect of *Staphylococcus epidermidis* biofilm matrix against phage predation. *Viruses* 12:1076. <https://doi.org/10.3390/v12101076>.
51. Wright RCT, Friman VP, Smith MCM, Brockhurst MA. 2019. Resistance evolution against phage combinations depends on the timing and order of exposure. *mBio* 10. <https://doi.org/10.1128/mBio.01652-19>.
52. Mizoguchi K, Morita M, Fischer CR, Yoichi M, Tanji Y, Unno H. 2003. Coevolution of bacteriophage PP01 and *Escherichia coli* O157:H7 in continuous culture. *Appl Environ Microbiol* 69:170–176. <https://doi.org/10.1128/AEM.69.1.170-176.2003>.
53. Akusobi C, Chan BK, Williams E, Wertz JE, Turner PE. 2018. Parallel evolution of host-attachment proteins in phage PP01 populations adapting to *Escherichia coli* O157:H7. *Pharmaceuticals* 11:60. <https://doi.org/10.3390/ph11020060>.
54. Zegans ME, Wagner JC, Cady KC, Murphy DM, Hammond JH, O'Toole GA. 2009. Interaction between bacteriophage DMS3 and host CRISPR region inhibits group behaviors of *Pseudomonas aeruginosa*. *J Bacteriol* 191:210–219. <https://doi.org/10.1128/JB.00797-08>.
55. Shah M, Taylor VL, Bona D, Tsao Y, Stanley SY, Pimentel-Elardo SM, McCallum M, Bondy-Denomy J, Howell PL, Nodwell JR, Davidson AR, Moraes TF, Maxwell KL. 2021. A phage-encoded anti-activator inhibits quorum sensing in *Pseudomonas aeruginosa*. *Mol Cell* 81:571–583.e6. <https://doi.org/10.1016/j.molcel.2020.12.011>.
56. Moulton-Brown CE, Friman VP. 2018. Rapid evolution of generalized resistance mechanisms can constrain the efficacy of phage-antibiotic treatments. *Evol Appl* 11:1630–1641. <https://doi.org/10.1111/eva.12653>.
57. Aka ST, Haji SH. 2015. Sub-MIC of antibiotics induced biofilm formation of *Pseudomonas aeruginosa* in the presence of chlorhexidine. *Braz J Microbiol* 46:149–154. <https://doi.org/10.1590/S1517-838246120140218>.
58. Mangalappalli-Illathu AK, Korber DR. 2006. Adaptive resistance and differential protein expression of *Salmonella enterica* serovar Enteritidis biofilms exposed to benzalkonium chloride. *Antimicrob Agents Chemother* 50:3588–3596. <https://doi.org/10.1128/AAC.00573-06>.
59. Hanlon GW, Denyer SP, Olliff CJ, Ibrahim LJ. 2001. Reduction in exopolysaccharide viscosity as an aid to bacteriophage penetration through *Pseudomonas aeruginosa* biofilms. *Appl Environ Microbiol* 67:2746–2753. <https://doi.org/10.1128/AEM.67.6.2746-2753.2001>.
60. Glonti T, Chanishvili N, Taylor PW. 2010. Bacteriophage-derived enzyme that depolymerizes the alginate acid capsule associated with cystic fibrosis isolates of *Pseudomonas aeruginosa*. *J Appl Microbiol* 108:695–702. <https://doi.org/10.1111/j.1365-2672.2009.04469.x>.
61. Olszak T, Shneider MM, Latka A, Maciejewska B, Browning C, Sycheva LV, Cornelissen A, Danis-Wlodarczyk K, Senchenkova SN, Shashkov AS, Gula G, Arabski M, Wasik S, Miroshnikov KA, Lavigne R, Leiman PG, Knirel YA, Drulis-Kawa Z. 2017. The O-specific polysaccharide lyase from the phage LKA1 tailspike reduces *Pseudomonas* virulence. *Sci Rep* 7:16302. <https://doi.org/10.1038/s41598-017-16411-4>.
62. Gurney J, Brown SP, Kaltz O, Hochberg ME. 2020. Steering phages to combat bacterial pathogens. *Trends Microbiol* 28:85–94. <https://doi.org/10.1016/j.tim.2019.10.007>.
63. Chan BK, Siström M, Wertz JE, Kortright KE, Narayan D, Turner PE. 2016. Phage selection restores antibiotic sensitivity in MDR *Pseudomonas aeruginosa*. *Sci Rep* 6:26717. <https://doi.org/10.1038/srep26717>.
64. Rodriguez-Gonzalez RA, Leung CY, Chan BK, Turner PE, Weitz JS. 2020. Quantitative models of phage-antibiotic combination therapy. *mSystems* 5. <https://doi.org/10.1128/mSystems.00756-19>.
65. Gordillo Altamirano F, Forsyth JH, Patwa R, Kostoulas X, Trim M, Subedi D, Archer SK, Morris FC, Oliveira C, Kieley L, Korneev D, O'Bryan MK, Lithgow TJ, Peleg AY, Barr JJ. 2021. Bacteriophage-resistant *Acinetobacter baumannii* are resensitized to antimicrobials. *Nat Microbiol* 6:157–161. <https://doi.org/10.1038/s41564-020-00830-7>.
66. Chen L-K, Kuo S-C, Chang K-C, Cheng C-C, Yu P-Y, Chang C-H, Chen T-Y, Tseng C-C. 2017. Clinical antibiotic-resistant *Acinetobacter baumannii* strains with higher susceptibility to environmental phages than antibiotic-sensitive strains. *Sci Rep* 7:6319. <https://doi.org/10.1038/s41598-017-06688-w>.
67. Ong SP, Azam AH, Sasahara T, Miyanaaga K, Tanji Y. 2020. Characterization of *Pseudomonas* lytic phages and their application as a cocktail with antibiotics in controlling *Pseudomonas aeruginosa*. *J Biosci Bioeng* 129:693–699. <https://doi.org/10.1016/j.jbiosc.2020.02.001>.
68. Gu J, Liu X, Li Y, Han W, Lei L, Yang Y, Zhao H, Gao Y, Song J, Lu R, Sun C, Feng X. 2012. A method for generation phage cocktail with great therapeutic potential. *PLoS One* 7:e31698. <https://doi.org/10.1371/journal.pone.0031698>.
69. Tanji Y, Shimada T, Fukudomi H, Miyanaaga K, Nakai Y, Unno H. 2005. Therapeutic use of phage cocktail for controlling *Escherichia coli* O157:H7 in gastrointestinal tract of mice. *J Biosci Bioeng* 100:280–287. <https://doi.org/10.1263/jbb.100.280>.
70. Malik DJ, Sokolov IJ, Vinner GK, Mancuso F, Cinquerrui S, Vladislavjevic GT, Clokie MRJ, Garton NJ, Stapley AGF, Kirpichnikova A. 2017. Formulation, stabilisation and encapsulation of bacteriophage for phage therapy. *Adv Colloid Interface Sci* 249:100–133. <https://doi.org/10.1016/j.cis.2017.05.014>.

71. Drab M. 2018. Phage aggregation-dispersion by ions: striving beyond antibacterial therapy. *Trends Biotechnol* 36:875–881. <https://doi.org/10.1016/j.tibtech.2018.03.002>.
72. Szermer-Olearnik B, Drab M, Mąkosa M, Zembala M, Barbasz J, Dąbrowska K, Boratyński J. 2017. Aggregation/dispersion transitions of T4 phage triggered by environmental ion availability. *J Nanobiotechnology* 15:32. <https://doi.org/10.1186/s12951-017-0266-5>.
73. Bicalho MLS, Machado VS, Nydam DV, Santos TMA, Bicalho RC. 2012. Evaluation of oral administration of bacteriophages to neonatal calves: phage survival and impact on fecal *Escherichia coli*. *Livest Sci* 144:294–299. <https://doi.org/10.1016/j.livsci.2011.12.007>.
74. Merabishvili M, Pirnay JP, Verbeken G, Chanishvili N, Tediashvili M, Lashkhi N, Glonti T, Krylov V, Mast J, Van Parys L, Lavigne R, Volckaert G, Mattheus W, Verween G, De Corte P, Rose T, Jennes S, Zizi M, De Vos D, Vaneechoutte M. 2009. Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. *PLoS One* 4:e4944. <https://doi.org/10.1371/journal.pone.0004944>.
75. Aslam S, Lampley E, Wooten D, Karris M, Benson C, Strathdee S, Schooley RT. 2020. Lessons learned from the first 10 consecutive cases of intravenous bacteriophage therapy to treat multidrug-resistant bacterial infections at a single center in the United States. *Open Forum Infect Dis* 7. <https://doi.org/10.1093/ofid/ofaa389>.
76. Chan BK, Abedon ST, Loc-Carrillo C. 2013. Phage cocktails and the future of phage therapy. *Future Microbiol* 8:769–783. <https://doi.org/10.2217/fmb.13.47>.
77. Rubalskii E, Ruemke S, Salmoukas C, Boyle EC, Warnecke G, Tudorache I, Shrestha M, Schmitto JD, Martens A, Rojas SV, Ziesing S, Bochkareva S, Kuehn C, Haverich A. 2020. Bacteriophage therapy for critical infections related to cardiothoracic surgery. *Antibiotics* 9:232. <https://doi.org/10.3390/antibiotics9050232>.
78. Vandenheuveld D, Lavigne R, Brussow H. 2015. Bacteriophage therapy: advances in formulation strategies and human clinical trials. *Annu Rev Virol* 2:599–618. <https://doi.org/10.1146/annurev-virology-100114-054915>.
79. Wright A, Hawkins CH, Anggard EE, Harper DR. 2009. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. *Clin Otolaryngol* 34:349–357. <https://doi.org/10.1111/j.1749-4486.2009.01973.x>.
80. Jault P, Leclerc T, Jennes S, Pirnay JP, Que YA, Resch G, Rousseau AF, Ravat F, Carsin H, Le Floch R, Schaal JV, Soler C, Fevre C, Arnaud I, Bretaudeau L, Gabard J. 2019. 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. *Lancet Infect Dis* 19:35–45. [https://doi.org/10.1016/S1473-3099\(18\)30482-1](https://doi.org/10.1016/S1473-3099(18)30482-1).
81. Torres-Barcelo C, Arias-Sanchez FI, Vasse M, Ramsayer J, Kaltz O, Hochberg ME. 2014. A window of opportunity to control the bacterial pathogen *Pseudomonas aeruginosa* combining antibiotics and phages. *PLoS One* 9:e106628. <https://doi.org/10.1371/journal.pone.0106628>.
82. Valero-Rello A. 2019. Diversity, specificity and molecular evolution of the lytic arsenal of *Pseudomonas* phages: in silico perspective. *Environ Microbiol* 21:4136–4150. <https://doi.org/10.1111/1462-2920.14767>.
83. Schooley RT, Strathdee S. 2020. Treat phage like living antibiotics. *Nat Microbiol* 5:391–392. <https://doi.org/10.1038/s41564-019-0666-4>.
84. Gu Liu C, Green SI, Min L, Clark JR, Salazar KC, Terwilliger AL, Kaplan HB, Trautner BW, Ramig RF, Maresso AW. 2020. Phage-antibiotic synergy is driven by a unique combination of antibacterial mechanism of action and stoichiometry. *mBio* 11:e01462-20. <https://doi.org/10.1128/mBio.01462-20>.
85. Uchiyama J, Shigehisa R, Nasukawa T, Mizukami K, Takemura-Uchiyama I, Ujihara T, Murakami H, Imanishi I, Nishifuji K, Sakaguchi M, Matsuzaki S. 2018. Piperacillin and ceftazidime produce the strongest synergistic phage-antibiotic effect in *Pseudomonas aeruginosa*. *Arch Virol* 163:1941–1948. <https://doi.org/10.1007/s00705-018-3811-0>.

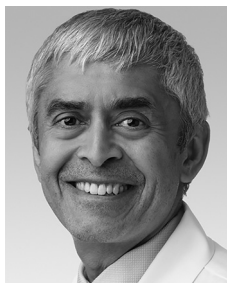
Olivia Barber is an environmental engineer interested in the human impact on antimicrobial resistance and how it impacts health outcomes. She received her bachelor's degree in Civil and Environmental Engineering from University of Maryland, College Park where her research focused on optimizing developments to minimize stormwater flooding. She then moved to England to study in the Environmental Engineering master's degree program at Imperial College London. Her dissertation focused on barriers to the implementation of novel microbial wastewater treatment in Serbia. After completing her master's, she spent a year working in industry as a consultant engineer for drinking water projects before returning to academia. Her research as a Ph.D. candidate at Northwestern currently focuses on how disinfectant use impacts antimicrobial resistance and strategies to combat the resistance trend.



Iria Mañas began her training at the University of Edinburgh Honors where she received a Biotechnology degree with honors. She then completed her Master's in Biotechnology at Northwestern University with a "La Caixa" Foundation fellowship. She also has a postgraduate certificate in Drug Discovery and Protein Biotechnology from the University of Edinburgh. Her current research interests include the characterization of antibiotic resistance development using different bioinformatics tools and researching new ways to implement bacteriophage diversity in clinical settings for different therapeutic applications.



Manu Jain is a Professor of Medicine and Pediatrics at Northwestern University's Feinberg School of Medicine. He then completed his M.D. at the University of Chicago as well as his internal medicine residency and pulmonary critical care fellowship. He joined the faculty at Northwestern in 1996 and obtained a master's degree in clinical investigation. He has risen to the ranks at Northwestern and is now a Full Professor. He has received funding from the NIH, Veteran's Administration, and CF foundation for his investigator-initiated research. He has also been the director of the adult CF program at Northwestern since 1997 as well as the site co-PI for the Therapeutic Development Network (TDN). He has published more than 35 peer-reviewed articles, is on the editorial board for the AJRCCM, and is a regular reviewer for multiple journals. He has also served as an ad-hoc reviewer for the NIH, CFF, and CF Canada.



Egon A. Ozer received his Ph.D. in Molecular Biology and his M.D. through the Medical Scientist Training Program at the University of Iowa Carver College of Medicine. He joined the Physician Scientist Training Program at the Northwestern University Feinberg School of Medicine in 2006 to perform his residency in Internal Medicine and fellowship in Infectious Disease before joining the faculty in 2012. His research focuses on genomic explorations of microbial pathogens and includes uncovering genetic determinants of virulence and antimicrobial resistance and using genomic features to investigate the transmission and evolution of bacterial, fungal, and viral pathogens. Over the last year, Dr. Ozer has adapted his expertise to perform whole-genome sequencing of SARS-CoV-2 to track the population structure of the virus in Illinois and study genomic correlations with clinical features in COVID-19 patients.



Erica Marie Hartmann is an environmental microbiologist interested in the interaction between anthropogenic chemicals and microorganisms, as well as bio-inspired mechanisms for controlling microbial communities. Her career began at the Johns Hopkins Bloomberg School of Public Health, where she worked on mass spectrometry-based methods for detecting microbial enzymes necessary for bioremediation. She then moved to Arizona State University where she was the first graduate of the interdisciplinary Biological Design Ph.D. program. She then moved to France on a Fulbright, studying microbes that degrade carcinogenic pollutants at the Commission for Atomic Energy. She began leading studies on antimicrobial chemicals and microbes found in indoor dust at the Biology and the Built Environment Center at the University of Oregon. She is currently continuing that work, as well as developing novel nonchemical antimicrobials, as an assistant professor at Northwestern University.

