Bacteriophage therapy: coping with the growing antibiotic resistance problem

Nina Chanishvili

The Eliava Institute of Bacteriophage, Microbiology and Virology, Tbilisi 0160, Georgia
Email: nina.chanishvili@gmail.com

Rustam Aminov

School of Medicine and Dentistry
University of Aberdeen, Aberdeen
AB25 2ZD, United Kingdom
Email: rustam.aminov@abdn.ac.uk

The global problem of multidrug-resistant bacterial pathogens requires urgent actions, including the development of therapies supplementary or alternative to antibiotics. One of the infection control options could be phage therapy. This article gives a brief overview of phage therapy potentials as well as the challenges it faces in order to become a widely accepted form of infection treatment.

The history of antimicrobial drug discovery includes more than 15 classes of antimicrobials that became a cornerstone in microbial infection control and management and saved many lives[1]. Antimicrobial therapy indeed became one of the most successful forms of therapy in clinical medicine. However, the broad and often indiscriminate use of antimicrobials in human and veterinary medicine and in agriculture resulted in the widespread antimicrobial resistance in microbiota of many ecological compartments[2]. Especially worrisome is the rise of multidrug resistance among bacterial pathogens, which may severely limit our abilities to control infectious diseases. Very limited options, for example, exist to treat the so-called ESKAPE bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species)[3]. If no immediate actions are taken, the estimated death toll due to multidrug-resistant bacterial pathogens may reach 10 million by the year 2050[4].

The question is what went wrong with this initially very successful form of infectious disease treatment? Why resistance to antibiotics develops so rapidly? To understand this phenomenon, we have to take a closer look into the fundamental biological processes governing the ecology and evolution in microbial ecosystems. For a long time, the problem of antimicrobial resistance has been viewed in isolation, mainly from the clinical microbiology perspective, e.g. as associated exclusively with the use/overuse/misuse of antimicrobials in human medicine. Admittedly, this can be one of the factors contributing to the dissemination of antimicrobial resistance, but the problem has much wider implications and must be contemplated within a broader evolutionary and ecological context.

In natural ecosystems, antibiotics play an essential role in regulatory processes that are involved in many functions of microbial ecosystems[5]. While serving as signalling molecules at low concentrations in natural ecosystems[6], in human and animal infectious disease therapy they are mainly used for their bacteriolytic and bacteriostatic activities expressed at high concentrations. Also, they are widely used at subtherapeutic concentrations in food animal production for metaphylactic purposes. Extensive use of antibiotics in clinical medicine, veterinary, agriculture and other applications are the hot spots with persistent antibiotic influx into microbial ecosystems. These are the places, where naturally occurring antibiotic resistance genes are selected and amplified. At this stage, antibiotic resistance genes are integrated into the normal microbiota and the fitness cost associated with the antibiotic resistance gene carriage is reduced. Because of this, antibiotic resistance becomes very resilient against eradication, even in the absence of antibiotic selective pressure[7]. The pool of the antibiotic resistance genes, which is amplified at these hot spots is then released, together with the concomitant antibiotics, into other ecological compartments. These are further disseminated to even more distant ecological compartments, including pathogens, via extensive horizontal gene transfer (HGT) mechanisms[8].

The widespread antibiotic resistance in a variety of microbiota, including human and animal pathogens, is the consequence of extensive use of antibiotics in human and veterinary medicine and agriculture. What can be done to limit and contain it? It is clear that the massive antimicrobial usage, which inevitably results in selection of the corresponding resistance mechanisms, has to be lessened. Unfortunately, the trends in antibiotic production and consumption are quite opposite, suggesting a very substantial growth for both humans and animals[9,10].

All major classes of antimicrobials were discovered during the golden age of antibiotic discovery, which came to an end more than 50 years ago[1]. Since then, the main antimicrobial drug developments included extensive modifications of the existing natural compounds, which, however, cannot guarantee the rapid development of resistance even against the newer antimicrobial derivatives. There are many potential avenues for the development in addition to modification of the existing antimicrobials. Alternatives to antimicrobials are urgently needed, and one of the most promising approaches could be the phage employment.

The idea to treat infections with phage came out of the pioneering work of Félix d'Hérelle[11]. The discovery of antibiotics that offered more convenient means to control infectious diseases, however, overshadowed the phage therapy approach. It has been largely abandoned except in a several countries: Georgia, Poland, and Russia, where it has remained as a part of authorised therapy for treatment of certain bacterial infections. A renewed interest in phage therapy is dictated by the need of new approaches to control bacterial infections, especially multidrug-resistant, and by its advantages.

First, unlike the wide range of bacteria targeted by antibiotics, phages are very specific and do not affect other beneficial microbes. This prevents complications such as antibiotic-induced dysbiosis and secondary infections. Second, phages multiply at the sites where the targets are present thus amplifying the local antibacterial effects. Third, no side effects of phage therapy have been so far detected[12–14]. Fourth, phage-resistant bacteria remain sensitive to other phages and, according to post-soviet regulations and standards for production of commercial phage preparations, introduction of new phages is a much faster and cheaper process compared to the development of new antimicrobials. Fifth, phages may be a valuable source of enzymes, such as lysins, active against pathogens[15]. Sixth, bacteriophages could play a significant role in restricting the evolution and spread of antibiotic resistance[16,17]. Seventh, phages may be effectively used for diagnostic purposes[18]. Eighth, unlike antibiotics, phages are efficient against biofilm-forming pathogens. And finally, antibiotics can be complemented by phages: phage-antibiotic combined therapy of infections is more effective than either alone[19]. In practical terms, the recent approval of bacteriophages as food additives to control foodborne pathogens opened new opportunities for their use in 'biocontrol' processes[20].

Although phages have been applied for treatment of various diseases for a century, as mentioned above their use in practical medicine is still limited to several countries. Safety is one of the main concerns when considering the use of phages for therapy and prophylaxis since, unlike regular pharmacological products, they are living organisms. Besides, they may contribute to HGT in the form of transduction[21]. According to the technological requirements applicable in the countries currently manufacturing commercial phage preparations, before a new phage is considered for practical use, it must pass a number of tests confirming its lytic nature and ruling out a potential involvement in HGT. The advance of omics technologies helps to address this issue. Genome sequencing is an essential initial step for considering phage candidates since it identifies prophage genes such as integrases, repressors, excisases, recombinases, terminases and hence, allows predictions of potential prophage properties including virulence factors or prophage incompleteness[22].

Regulatory issues represent a major obstacle for the implementation of phage therapy: its efficacy has to be confirmed according to the current pharmacological standards. This requires that properly designed, randomised, placebo controlled, and double-blind clinical trials have to be performed. So far, 17 clinical trials have been registered between 1996 through 2018, but the majority of them could not recruit enough patients; the others were not well designed, therefore they fall far from providing statistically relevant conclusions about the efficacy of phage therapy[22]. For instance, the recently completed Phagoburn trial, which represented a public investment of 3.85 million euros, enrolled a total of 27 patients only among 11 centres[23]. This is much less than the pre-calculated 220 patients needed to provide statistically significant results for the study. Besides, the trial was designed for treatment of burn wound infections caused by *E. coli*, but the clinical results showed that *P. aeruginosa* was predominant. The inadequate trial outcome was significantly affected by the initial error in trial design as it is well known that burn wounds are mostly infected by *P. aeruginosa*, not *E. coli*. A randomised, placebo-controlled, double-blind clinical trial has been recently completed in Georgia. Patients with urinary tract infections caused by *E. coli*, *Enterococcus* spp., *Proteus* spp., *Streptococcus* spp., *S. aureus* and P. *aeruginosa* were enrolled in the study. The patients were treated with a commercial preparation

Pyo-bacteriophage, which is a mix of bacteriophages targeting all these pathogens. Preliminary results of the clinical trial have been published[24,25], which hopefully will result in a broader recognition of phage therapy for treatment of multidrug resistant infections. Once widely accepted, phage can be used as a valuable alternative option to lessen antibiotic usage and corresponding resistance.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This research did not receive any specific funding.

References

1. Aminov, R. (2017) History of antimicrobial drug discovery: major classes and health impact. *Biochem Pharmacol.* **133**, 4–19. doi:10.1016/j.bcp.2016.10.001
2. Aminov, R.I. (2010) A brief history of the antibiotic era: lessons learned and challenges for the future. *Front. Microbiol.* **1**, 134. doi:10.3389/fmicb.2010.00134
3. Boucher, H.W. *et al*. (2009) Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin. Infect. Dis.* **48**, 1–12. doi:10.1086/595011
4. O'Neill, J. (2014) The review on antimicrobial resistance. http://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf
5. Aminov, R.I. (2009) The role of antibiotics and antibiotic resistance in nature. *Environ. Microbiol.* **11**, 2970–2988. doi:10.1111/j.1462-2920.2009.01972.x
6. Davies, J. *et al*. (2006) The world of subinhibitory antibiotic concentrations. *Curr. Opin. Microbiol.* **9**, 445–453. doi:10.1016/j.mib.2006.08.006
7. Andersson, D.I. and Hughes, D. (2011) Persistence of antibiotic resistance in bacterial populations. *FEMS Microbiol. Rev.* **35**, 901–911. doi:10.1111/j.1574-6976.2011.00289.x
8. Aminov, R.I. (2011) Horizontal gene exchange in environmental microbiota. *Front. Microbiol.* **2**, 158. doi:10.3389/fmicb.2011.00158
9. Van Boeckel, T.P. *et al*. (2014) Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *Lancet Infect. Dis.* **14**, 742–750. doi:10.1016/S1473-3099(14)70780-7
10. Van Boeckel, T.P. *et al*. (2015) Global trends in antimicrobial use in food animals. *Proc. Natl. Acad. Sci. USA* **112**, 5649–5654. doi:10.1073/pnas.1503141112
11. d'Hérelle, F. (1917) Sur un microbe invisible antagoniste des bacilles dysentériques. *C. R. Acad. Sci. Ser. D* **165**, 373–375.
12. Borysowski, J. and Górski, A. (2008) Is phage therapy acceptable in the immunocompromised host? *Int. J. Infect. Dis.* **12**, 466–471. doi:10.1016/j.ijid.2008.01.006
13. Międzybrodzki, R. *et al*. (2012) Clinical aspects of phage therapy. *Adv. Virus Res.* **83**, 73–121. doi:10.1016/B978-0-12-394438-2.00003-7
14. McCallin, S. *et al*. (2013) Safety analysis of a Russian phage cocktail: from metagenomic analysis to oral application in healthy human subjects. *Virology* **443**, 187–196. doi:10.1016/j.virol.2013.05.022
15. Schmelcher, M. *et al*. (2012) Bacteriophage endolysins as novel antimicrobials. *Future Microbiol.* **7**, 1147–1171. doi:10.2217/fmb.12.97
16. Jalasvuori, M. *et al*. (2011) Bacteriophage selection against a plasmid-encoded sex apparatus leads to the loss of antibiotic-resistance plasmids. *Biol. Lett.* **7**, 902–905. doi:10.1098/rsbl.2011.0384
17. Zhang, Q.G. and Buckling, A. (2012) Phages limit the evolution of bacterial antibiotic resistance in experimental microcosms. *Evol. Appl.* **5**, 575–582. doi:10.1111/j.1752-4571.2011.00236.x
18. O'Sullivan, L. *et al*. (2016) Bacteriophage-based tools: recent advances and novel applications. *F1000Res.* **5**, 2782. doi:10.12688/f1000research.9705.1
19. Torres-Barceló, C. and Hochberg, M.E. (2016) Evolutionary rationale for phages as complements of antibiotics. *Trends Microbiol.* **24**, 249–256. doi:10.1016/j.tim.2015.12.011
20. Kazi, M. and Annapure, U.S. (2016) Bacteriophage biocontrol of foodborne pathogens. *J. Food Sci. Technol.* **53**, 1355–1362. doi:10.1007/s13197-015-1996-8
21. Lerminiaux, N.A. and Cameron, A.D.S. (2019) Horizontal transfer of antibiotic resistance genes in clinical environments. *Can. J. Microbiol.* **65**, 34–44. doi:10.1139/cjm-2018-0275
22. Sybesma, W. *et al*. (2018) Silk route to the acceptance and re-implementation of bacteriophage therapy—part II. *Antibiotics (Basel)* **7**, 1–23. doi:10.3390/antibiotics7020035
23. Jault, P. *et al*. (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. doi:10.1016/S1473-3099(18)30482-1
24. Leitner, L. *et al*. (2017) Bacteriophages for treating urinary tract infections in patients undergoing transurethral resection of the prostate: a randomized, placebo-controlled, double-blind clinical trial. *BMC Urol.* **17**, 90. doi:10.1186/s12894-017-0283-6
25. Ujmajuridze, A. *et al*. (2018) Adapted bacteriophages for treating urinary tract infections. *Front. Microbiol.* **9**, 1832. doi:10.3389/fmicb.2018.01832

Biographies

Prof Nina Chanishvili has vast experience working in the field of bacteriophage research and phage therapy. Her recent projects were dedicated to clinical studies of bacteriophage application for treatment of urinary tract infection; selection of bacteriophages against MDR Salmonella spp.; study of the potential role of *B. fragilis* bacteriophages for prophylaxis of colon cancer. She is full professor at the New Vision University (Tbilisi, Georgia) and an invited lecturer at the Iv. Javakhishvili Tbilisi State University. Prof Nina Chanishvili is a peer reviewer of the scientific journals: *Frontiers in Microbiology, Viruses, Pharmaceuticals*, *Antibiotics*, etc. She is a Vice President of the Georgian Association for General and Applied Microbiology (GAGAM) and a Board Member of the Eliava Foundation-Association for Development of Bacteriophages in Georgia. In 2000 she has been awarded by the American Society of Microbiology with the Morrison-Rogoza Award. She is an author of over 120 research articles, book chapters, abstracts and monographs.

Dr Rustam Aminov's main research interests are in antimicrobials, antimicrobial resistance and in gut microbiology. He has published more than 120 articles and book chapters and edited several research topics on these subjects. He serves as editor of *Frontiers in Microbiology* and *FEMS Microbiology Letters*. He is currently affiliated with the University of Aberdeen and Kazan Federal University, and is the recipient of several fellowships from JSPS.