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# The use of bacteriophages as One-Health approach to reduce multidrug-resistant bacteria

Sophie Kittler [a,*], Johannes Wittmann [b], Ruth Anna Lisa Perihan Mengden [a], Günter Klein [a], Christine Rohde [b], Hansjörg Lehnherr [c]

[a] Institute for Food Quality and Food Safety, University of Veterinary Medicine Hannover, Hannover, Germany
[b] Leibniz Institute, DSMZ, Braunschweig, Germany
[c] PTC Phage Technology Center GmbH, Kompetenzzentrum Bio-Security, Bönen, Germany

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ABSTRACT

Multidrug-resistant bacteria are increasingly present in humans, animals and the environment. They pose a serious health risk, especially for hospitalized patients. Policymakers, scientists and industry are called to intensify innovation and research for developing new vaccines, diagnostics and infection treatment options. However, very few new antibacterial drugs have been developed in the last decade and several experts already fear that the supply of new antimicrobial agents will one day dry up. Bacteriophages are viruses that specifically and exclusively attack bacteria. They can be isolated from all environments where their host bacteria exist and can be used for decontamination in all stages of food production, for clinical therapy and for prevention of animal disease in livestock. They can thus help us in terms of a One-Health strategy aiming at reducing the selective pressure for resistant bacteria due to reduced release of antibiotics into the environment. Bacteriophages can also help us to treat human and animal infections caused by multidrug-resistant bacteria.



## 1. Introduction

Multidrug-resistant bacteria are dramatically increasing in virtually all imaginable habitats, including the human and animal microbiomes and the environment. They pose a serious health risk, especially for hospitalized or immunocompromised patients (WHO, 2014). The World Health Organization (WHO) claims that the likelihood of fatality increases by 64% when patients are infected with multidrug-resistant staphylococci, compared to the non-resistant forms (WHO, 2015). In all regions of the world where data were collected by the WHO, very high rates of resistance have been observed in common bacterial infections (WHO, 2014). The immense antibiotic use and release into the environment for over half a century have provoked a constant selective pressure for resistant bacterial strains, their survival and transmission of resistance abilities in all habitats (WHO, 2014). Thus, antibiotic resistance is a serious and global One-Health problem concerning humans, animals and the environment. Corresponding to the One-Health approach (http://www.onehealthinitiative.com/) the mitigation of antibiotic resistance requires expanded interdisciplinary collaboration, acknowledging that human health is connected to the health of animals and the environment (Piffaretti, 2016).

Policymakers, scientists and industry are called to intensify innovation and research for developing new vaccines, diagnostics and infection treatment options (WHO, 2015). However, very few new antibacterial drugs have been developed in the last decade (WHO, 2011a, 2011b; Oliver, 2016).

Bacteriophages (phages) are the viruses of bacteria and represent the natural and specific predators of bacteria. They are principally present in all environments where their hosts exist (Gill and Hyman, 2010). For the last 90 years, in countries of Eastern Europe and especially in countries of the former Soviet Union, bacteriophages have been used in therapeutic applications for both humans and animals and as disinfecting agents (Monk et al., 2010). Bacteriophages can have a broad or narrow hosts range within a bacterial species, but rarely infect bacteria beyond the species border (Anand et al., 2015; Mapes et al., 2016; Yu et al., 2016). They can be used as monophage preparations, containing just one single type of phage or can be combined to cocktails to reduce the problem of occurring bacterial resistance development against single bacteriophages (Tanji et al., 2004). Thus far, bacteriophage treatments did not show any unwanted side effects in countless studies (Międzybrodzki et al., 2012; Pirnay et al., 2015). Bacteriophages can be used for decontamination in all stages of food production, for clinical therapy and for prevention of animal

* Corresponding author.
*E-mail address:* Sophie.Kittler@tiho-hannover.de (S. Kittler).

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disease in livestock (Knoll and Mylonakis, 2013). They can thus help us in terms of a One-Health strategy to reduce selective pressure for resistant bacteria due to reduced release of antibiotics into the environment, as well as helping us to treat infections caused by multiresistant bacteria. This review will try to summarize the need for improvements in application strategies aiming at a One-Health concept against resistant bacteria and for a reduction of the dramatic environmental antibiotic drug load by the use of bacteriophages.

### *1.1. The One-Health concept in fighting antimicrobial resistance: lessons learned*

Antibiotics are low molecular weight substances that can kill or inhibit the growth of susceptible bacteria. They can be naturally occurring, semi-synthetic or synthetic (Quinn et al., 2011). When antibiotics were first discovered in the early 20th century, and the large-scale production of penicillin became possible in the early 1940s, it seemed that mortality due to bacterial infections would soon be overcome. However, soon after introduction of these antimicrobial substances, the first drug-resistant microorganisms were described. While thousands of lives were saved by the use of antimicrobial drugs, resistant bacteria have become a global health problem and make bacterial disease a threat to human health again (Brandt et al., 2014). Development of resistant microorganisms is an inevitable consequence from the selective pressure that occurs wherever antimicrobials are used (Quinn et al., 2011). However, the use of antibiotics as growth promoters for fattening of livestock and other misuses accelerated the formation of resistant bacteria and prompted the EU to react with regulations for the use of antibiotics in livestock farming (EC, 2003). A susceptible bacterium can become resistant through a novel genetic mutation (chromosomal resistance) or through horizontal gene transfer - meaning the acquisition of mobile genetic elements from another bacterium that is already resistant (WHO, 2011a, 2011b). Under the selective pressure of an antimicrobial drug that kills susceptible bacteria, resistant bacteria can proliferate. These mechanisms can occur during antibiotic use in humans, animals and plants. The transmission of resistant bacteria can occur among humans, via food products of animal origin, via direct contact with animals and the animal environment or via the consumption of vegetables contaminated by animal waste or waste water (WHO, 2011a, 2011b). Negative impact on human health occurs, when resistant bacteria are pathogens that cause disease and cannot be treated with the commonly used therapeutics. Such situations may lead to longer duration of illness or, in the worst case, to the death of the patient (Bundesinstitut für Risikobewertung (BfR), 2014). About 700,000 deaths annually are estimated to ascribe to the lack of effective antibiotics (Debarbieux et al., 2016).

From the use of antibiotics we have learned a few lessons in how to use antimicrobial drugs and how to avoid bacterial resistance (Aminov, 2010):

1. The level of antibiotic-resistant infections strongly correlates with the level of antibiotic consumption (Goossens et al., 2005).
2. Knowledge and regulation of antibiotic use are helpful in order to prevent high prevalence of resistant bacteria (Grigoryan et al., 2007; Aminov, 2010).
3. Bacteria are not only exposed to selective pressure during antimicrobial therapy but also when antimicrobial agents are disseminated in the environment after therapeutic use (Hegreness et al., 2008; Chee-Sanford et al., 2009).
4. Many factors might influence the development of bacterial resistance and there is a huge lack of knowledge regarding these factors (Aminov, 2010).

In summary, antimicrobial resistance is not a black-and-white issue but a consequence of the use and misuse and an issue of (health) politics, economics, behavior and conflicting options (Dorman and Koeman, Nov. 13–15 2012). Since animal, human and environmental health is interrelated, solutions can only be found in a One-Health approach. This means that physicians, veterinarians and other scientific health and environmental professionals should think in larger dimensions and must find an agreement on how to use antibiotics and how to overcome bacterial resistance with the goal in mind: improving health on a world-wide level (Dorman and Koeman, Nov. 13–15 2012) in order to enable future generations to take advantage of the enormous progress of modern medicine and have life-saving methods available to treat infectious diseases.

New antimicrobial therapies are needed and among the suggested options phage application shall have its officially regulated role (Ormala and Jalasvuori, 2013).

Phages are viruses that can infect and kill bacteria by injecting their genetic material, taking over the bacterial metabolism, producing (viral) progeny and finally lysing the bacterium to set their progeny free (Thiel, 2004). Phages may have two modes of action dependent on the phage type. Lytic phages replicate immediately after infection and lyse the bacterial cell they have infected. In contrast, temperate phages may not directly induce bacterial cell lysis after injection but may integrate into the host genome and replicate their DNA along with the replication of bacterial cells. They remain in this state without damaging the host, until environmental conditions for bacterial growth decline. At this point the so-called prophage may enter a lytic lifestyle, produce virions and lyse the bacterial cell. For bacteriophage therapy, only lytic bacteriophages are suitable (Jassim and Limoges, 2014) as they will lead to the destruction of the bacterial cell. This is in contrast to many antibiotic drugs: they just inhibit bacterial growth.

Currently, in Western countries clinical phage therapy products are hardly available because the clinical trials that have been conducted in the former Soviet Union, do not meet Western standards. In Russia and Georgia, phages were used as therapeutics ever since their discovery, a century ago (Kutter et al., 2010; Parracho et al., 2012). For treatment of food products prior to market, some formulations are already available (Goodridge and Bisha, 2011). The U.S. Food and Drug Administration has approved several of these products and has recognized the phages in these applications as “GRAS” (generally recognized as safe) (FDA, 2006). Compared to antibiotics, phages are different in many aspects: They are self-replicating, have a narrow but specific host range, potentially co-evolve with and adapt to their host bacteria, they are abundant in the environment and novel, effective phages are easy to find. However, basic precautions in the implementation of phage application strategies must be followed in order to avoid mistakes that have been made in the use of antibiotics. Many authors have written recommendations for phage application and cocktail composition (Payne and Jansen, 2003; Weld et al., 2004; Bigwood et al., 2009; Gill and Hyman, 2010; Hagens and Loessner, 2010; Fischer et al., 2013; Kittler et al., 2013; Pirnay et al., 2015). Considering these ideas, the following improvements are necessary for phage application strategies aiming at a One-Health approach:

1. The phages should be selected based on a host range analysis across a biologically meaningful panel of bacterial isolates that means isolates with which they are likely to interact during application (Meaden and Koskella, 2013).
2. The phages should be tested for rapid adsorption kinetics and rapid killing of bacterial host cells under the conditions they will face during application e.g. in biofilms or non-media environments.

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3. Phages should always be applied in a sufficient concentration and with appropriate timing and quality standards (Gill and Hyman, 2010; Hagens and Loessner, 2010; Pirnay et al., 2015). If selected for application they should also be tested for their easy production on a non-pathogenic host and purification under industrial conditions as well as stability in vitro and in situ.
4. A better understanding and evaluation of the fitness costs that result from a resistance against a monophage preparation, compared to the costs of the resistance against a phage cocktail, as well as the knowledge about the synergies between phages and antibiotics would allow for an optimized application and could limit the spreading of resistance (Fischer et al., 2013; Kittler et al., 2014; Torres-Barcelo and Hochberg, 2016).
5. It is very unlikely that bacterial resistances to available phage products would impair treatment with phages in general because of their high abundance in all environments harboring their host bacteria (Ormala and Jalasvuori, 2013). However, the control of products for clinical use and rapidly responding regulations for the use of phages in agriculture should be carried out by national therapy centers that allow a reactive phage therapy program (e.g. correspondent to a flu vaccine) and carefully planned combination treatments of antibiotics and phages using synergistic effects (Verbeken et al., 2012; Meaden and Koskella, 2013; Pirnay et al., 2015). Positive examples for such optimized therapies (e.g. rational drug combinations in the therapy of tumor or HIV patients) rely on the understanding of pathogenesis and the knowledge about mechanisms of sensitivity and resistance (Torres-Barcelo and Hochberg, 2016).
6. Only lytic phages should be applied in order to avoid any potential spread of toxins and virulence factors by lysogenic conversion (Brüssow et al., 2004)
7. Phages have certain advantages over antibiotics regarding their impact on resistances in non-target microbiota. Due to their narrow host range they can eliminate a pathogenic strain without disrupting the normal microbiota or causing resistance (Carlton, 1999; Meaden and Koskella, 2013).

Considering the above said, phage application as natural tool is a realistic future approach to save human and animal life, to improve health and to reduce the environmental drug load, finally for the long term and future generations. Phages should be used instead of antimicrobial drugs wherever possible and in addition to antimicrobial drugs where appropriate. This sustainable One-Health approach may be verified stepwise but shall start at the junction between environmental and human microbiome: in livestock farming.

## Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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