

In vitro efficacy and individual bacteriophage treatment of *P. aeruginosa* in dogs

Eva Maria Kalbhenn^{1,*}, Nele Pez¹, Wolfgang Beyer¹ and Elisabeth Müller^{1,2}

¹ Phage Center Bad Kissingen, Sieboldstraße 7, 97688 Bad Kissingen, Germany

² Laboklin GmbH & Co. KG, Steubenstraße 4, 97688 Bad Kissingen, Germany

* Corresponding author, contact: kalbhenn@laboklin.com

Introduction

Multi-drug resistant (MDR) and often zoonotic bacteria are a major problem in human and veterinary medicine posing a global threat to public health. The fight against antimicrobial resistance (AMR) is conducted in terms of the antimicrobial stewardship. Bacteriophages as an alternative or additive to antibiotics have the potential to counteract this problem. The aim of this study was to determine the *in vitro* efficacy of bacteriophage cocktails, commercially available in Georgia for use in humans, as well as a veterinary bacteriophage suspension against bacterial isolates from dogs and to accompany an initial case study of otitis externa caused by *Pseudomonas aeruginosa*.

Material and Methods

Species identification focusing on bacterial field isolates of *P. aeruginosa* from dogs was performed using MALDI-ToF MS (Bruker Cooperation, USA). Antimicrobial susceptibility tests (AST) were obtained using Micronaut Systems according to CLSI guidelines with resistance to at least three antimicrobial groups. The susceptibility of the bacterial isolates was tested by phagogram according to standard procedures established at Eliava Institute. Bacteriophage cocktails from Eliava BioPreparations and a bacteriophage suspension (VetPS) from Eliava Institute were tested. Lysis zones were scored as positive (S); absent lysis zones as resistant (R). In addition, the bacteriophage titer was determined by dilution series. The bacteriological monitoring during the case study included culture, identification by MALDI-ToF and AST and was performed by Laboklin GmbH & Co. KG.

Results and Discussion

In vitro efficacy of bacteriophage cocktail "Pyo", "Intesti" and a veterinary PS (VetPS)

A lytic activity of commercially available bacteriophage cocktails "Pyo" and "Intesti" for use in humans and of a VetPS could be demonstrated against bacterial isolates of dogs. Depending on the different batch of the bacteriophage cocktails (stated by batch numbers), overall a good efficacy against *P. aeruginosa* was observed (Fig. 1). For "Pyo" M1-1202 an *in vitro* efficacy of 80 % compared to "Pyo" M1-1301 of 76 % show slightly differences. In contrast to the bacteriophage cocktail "Intesti" M2-1202 with an *in vitro* efficacy of 83 % differs to "Intesti" M2-1301 with an efficacy of only 69 %. Differences between the batches have been observed due to the regular adaption of the bacteriophage cocktails to human isolates. However, the VetPS, which was adapted to animal derived isolates, shows an *in vitro* efficacy of 99 %.

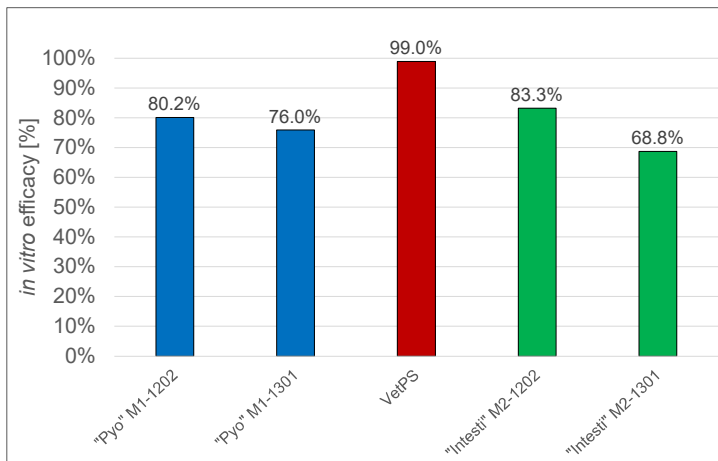


Fig. 1: *In vitro* efficacy of two commercially available bacteriophage cocktails with different batch numbers and one veterinary bacteriophage suspension (VetPS) against *P. aeruginosa* from dogs [n=96] were tested.

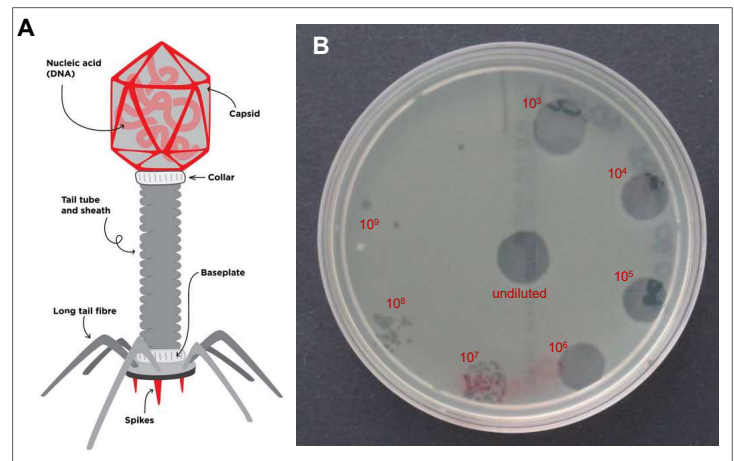


Fig. 2A: Schematic representation of bacteriophage (picture from Phagenzentrum). **2B:** Phagogramm (dilution series) of VetPS against *P. aeruginosa* (isolated from otitis externa, dog), Titer amounts 10^9 pfu/ml.

Case study of a dog with otitis externa caused by *P. aeruginosa*

A dog (Irish setter, 5 years old) suffering from purulent, painful, recurrent otitis externa caused by *P. aeruginosa* had limited treatment options due to intrinsic and acquired resistances of *P. aeruginosa*. Multiple and prolonged antibiotic therapies (amoxicillin and clavulanic acid, marbofloxacin, enrofloxacin, florfenicol) were unsuccessful. Thus, therapy emergency was given as well as the veterinary indication for not licensed treatment to avoid unacceptable suffering of the animal (Article 112, EU reg. 2019/6). A bacteriophage therapy was carried out by the treating veterinarian including thorough cleaning of the ears to remove secretions and cell detritus prior to application of the bacteriophages. Specific local bacteriophage treatment was carried out 5 consecutive days (5 ml, 2 times a day) (Fig. 2B), followed by 5 days of local antibiotic therapy (gentamicin). The treatment was successful with no recurrent otitis during time of clinical observation (9 months).

Conclusion

This is the first preliminary study investigating the *in vitro* efficacy of commercially available bacteriophage cocktails from Eliava BioPreparations and a veterinary bacteriophage suspension (VetPS) from Eliava Institute against bacterial isolates. The five days treatment of a dog with bacteriophages followed by five days treatment with antibiotic finally cured the infection. This case demonstrates that a meaningful combination of phage and antibiotic therapy is useful to combat infections with MDR bacteria also in the manner of One Health.

Acknowledgement

Mzia Kutateladze from Eliava Institute of Bacteriophages, Tbilisi, Georgia, and Vakhtang Pavlenishvili from Eliava BioPreparations, Tbilisi, Georgia