Efficacy of bacteriophages in companion animals

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Introduction

In human and veterinary medicine, the distribution of multi-drug resistant (MDR) and often zoonotic bacteria is a major problem posing a global threat to public health. The use of bacteriophages as an alternative or additive to antibiotics has the potential to counteract this problem. The prerequisite for this is a differentiated and close-meshed diagnosis of the clinical course of the disease. The aim of this study was to determine the *in vitro* efficacy of bacteriophage cocktails, commercially available in Georgia for use in humans, against bacterial isolates from companion animals and to accompany initial case studies.

Material and Methods

Species identification focusing on bacterial field isolates of *Pseudomonas* spp., *Staphylococcus* spp., *Proteus mirabilis*, *Klebsiella* spp. and *Enterococcus* spp. from companion animals (dogs, cats, horses) 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 were tested by phagogram according to standard procedures established at Eliava Institute. Multiphage and monophage cocktails (Eliava BioPreparations) were tested as listed in **Tab. 1**. Lysis zones were scored as positive (S). Absent lysis zones were scored as resistant (R). In addition, the bacteriophage titer was determined by dilution series of the respective bacteriophage cocktails.

Results and Discussion

In vitro efficacy

Tab. 1: *In vitro* efficacy of six commercially available bacteriophage cocktails against selected bacterial isolates of companion animals. [1]Bacteriophage cocktails from Eliava BioPreparations, Georgia. Number of positive isolates/total number of tested isolates in brackets.

	Enco[1]	Fersisi[1]	Pyo[1]	Intesti[1]	Ses[1]	Staph[1]
P. aeruginosa			**81.9%** (68/83)	**85.5%** (71/83)		
Pr. mirabilis			**29.0%** (29/100)	**40.0%** (40/100)		
S. aureus	**70.0%** (7/10)	**80.0%** (8/10)	**70.0%** (7/10)	**70.0%** (7/10)	**80.0%** (8/10)	**60.0%** (6/10)
S. pseudintermedius	**7.1%** (1/14)	**7.1%** (1/14)	**14.3%** (2/14)	**7.1%** (1/14)	**7.1%** (1/14)	**7.1%** (1/14)
K. pneumoniae			**10.5%** (2/19)	**30.8%** (4/13)		
K. oxytoca			**0.0%** (0/19)	**30.8%** (4/13)		
E. faecalis		**51.4%** (18/35)	**31.4%** (11/35)	**71.4%** (25/35)	**54.3%** (19/35)	
E. faecium		**20.0%** (3/15)	**20.0%** (3/15)	**0.0%** (0/15)	**13.3%** (2/15)	

A lytic activity of commercially available bacteriophage cocktails for use in humans could be demonstrated against bacterial isolates of companion animals. Depending on the different bacteriophage cocktails, overall a good efficacy e.g. for *P. aeruginosa* of about 85.5% was observed **(Tab. 1)**. However, *Pr. mirabilis* showed a low efficacy of about 29.0% and for *S. aureus* the efficacy was amounted of 80.0%. The efficacy of the bacteriophage cocktails against *S. pseudintermedius* was about 7.1% adding that no bacteriophages against this strain was indicated in bacteriophage cocktails. The determination of an efficacy for *K. pneumonia* and for *K. oxytoca* was unexpected as *Klebsiella* phages are not indicated being part of the bacteriophage cocktails. However, probably due to a broad host range of the present *E. coli* phages also *Klebsiella* spp. strains were lysed. The same phenomenon was observed for *Enterococcus* spp. treated with Fersisi, Pyo and Ses, where probably the *Streptococcus* phages were interacting with different *Enterococcus* strains.

Case study

A dog named Buddy (cane corso, male, 2 years old) had a purulent, very painful, recurrent otitis externa due to an infection with *P. aeruginosa*. Multiple and prolonged antibiotic therapies (amoxicillin and clavulanic acid, marbofloxacin, polymyxin B) were unsuccessful. Accordingly, the therapy emergency was given as well as the veterinary indication to avoid unacceptable suffering of the animal (Article 112, EU reg. 2019/6). Treatment of otitis externa in dog Buddy caused by *P. aeruginosa* with 5 days of bacteriophage therapy (2 times a day, bacteriophage cocktail Pyo) followed by at least 5 days of local antibiotic therapy (gentamicin) and cold plasma therapy was successful. The treatment was carried out by a responsible veterinarian. Bacteriological monitoring and AST were performed before **(Fig. 1)** and during the whole period of therapy. Before the 5th bacteriophage treatment, a greatly reduced growth rate of freshly isolated *P. aeruginosa* was observed, indicating virulence reduction after bacteriophage treatment.

Till today, Buddy is doing very well, without any further clinical signs of otitis.

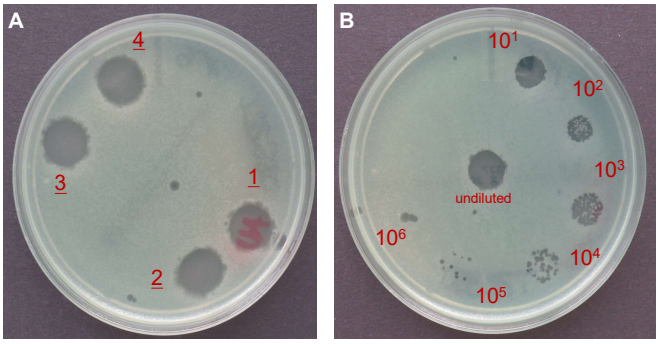

Fig. 1A.: Phagogramm of *P. aeruginosa* (isolated from otitis externa, dog), [1: Pyo (exp. date 12.2023), 2: Intesti (exp. date 12.20.23), 3: Pyo (exp. date 05.2024), 4: Intesti (exp. date 05.2024)]. **1B.: Dilution series of bacteriophage cocktail Pyo** (exp. date 05.2024).

Conclusion

This is the first preliminary study investigating the efficacy of commercially available bacteriophage cocktails from Eliava BioPreparations against bacterial isolates from companion animals. The five days treatment of dog “Buddy” 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. Further work is needed to clarify the interaction of phages and antibiotics, and to develop specific bacteriophage cocktails (e.g. *S. pseudintermedius*) with high efficacy against animal derived bacterial isolates.

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