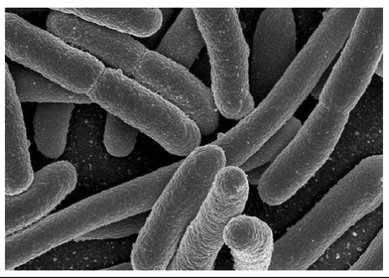
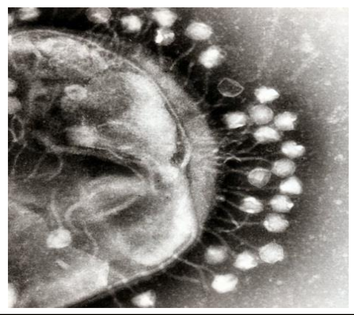


Characterisation of Bacteriophages with lytic activity against *E. coli* isolated from canine patients

Lucy Rhys-Davies[1], Arnoud H.M. van Vliet[1], Jennifer M Ritchie[2]

School of Veterinary Medicine[1] and School of Biosciences[2], Faculty of Health and Medical Sciences, University of Surrey, Guildford, GU2 7AL



Introduction

Phage therapy is the utilisation of Bacteriophages' natural ability to kill bacteria, and was initially explored as a possible treatment for infections in the early 1900s; however, research interests declined following the discovery of antibiotics. Today, as the availability of effective antibiotics is depleting, the potential of phage therapy as an alternative treatment for infections is being revisited.

Antibiotics are used routinely in companion animals to treat a range of infections, and as a result this sector of veterinary medicine is vulnerable to the threat of AMR. The utilisation of Phage therapy as an alternative antimicrobial could be tested and implemented in this area and could even pave the way to use in human medicine.

For this study, strains of *Escherichia coli* were obtained from canine patients with urinary tract infections (UTI) and surgical site infections (SSI). Whole genome sequencing revealed that these isolates were spread across 7 main phylogroups (A, B1, B2, C, D, E and F), which are the major phylogroups associated with the gut of warm-blooded animals, and most often implicated in extra-intestinal infections.

Methods

Phage extraction & isolation

- Samples of soil and sewage were suspended in LB broth
- *E. coli* NCTC 10538 was added to the samples as a phage host
- LB tubes were incubated overnight at 37°C, shaking at 200rpm
- Following incubation, samples were centrifuged for 10 mins at 13,000 rpm (x3)
- Supernatants were filtered through 0.22µm
- Plaque assays were carried out on filtered lysates, using *E. coli* NCTC 10538 as a host
- Plates were incubated overnight and observed for the presence of plaques
- Probable lytic phages were selected for further testing based on plaque morphology (Figure 2)

Figure 1: Phage extraction from environmental samples. Sample collection, incubation in broth, centrifugation and filtration.

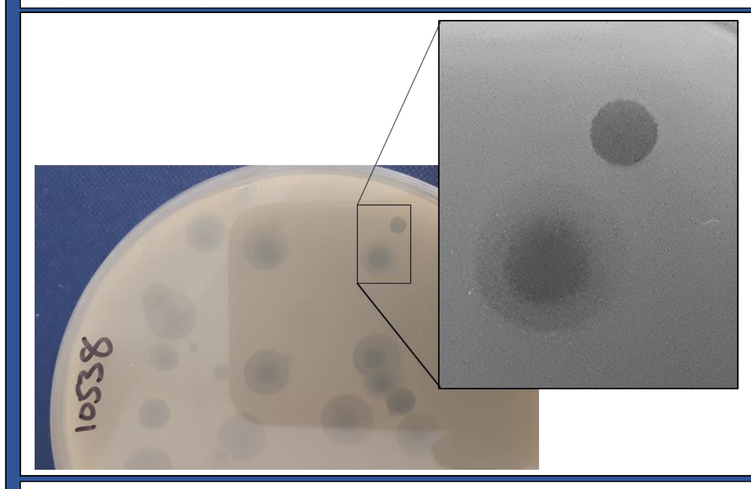


Figure 2: Plaque assay with *E. coli* strain 10538. Clear plaques are indicative of virulent lytic phages, "cloudy" plaques can indicate the presence of temperate (lysogenic) phages.

Phage characterisation

- Selected phages were tested for their ability to lyse host strain 10538, measured by a reduction of optical density over time (Figure 3)
- Five phages were selected for further testing against clinical isolates of *E. coli* obtained from canine patients
- Spot assays were carried out to give an indication of host range (Table 1)

Results & Future work

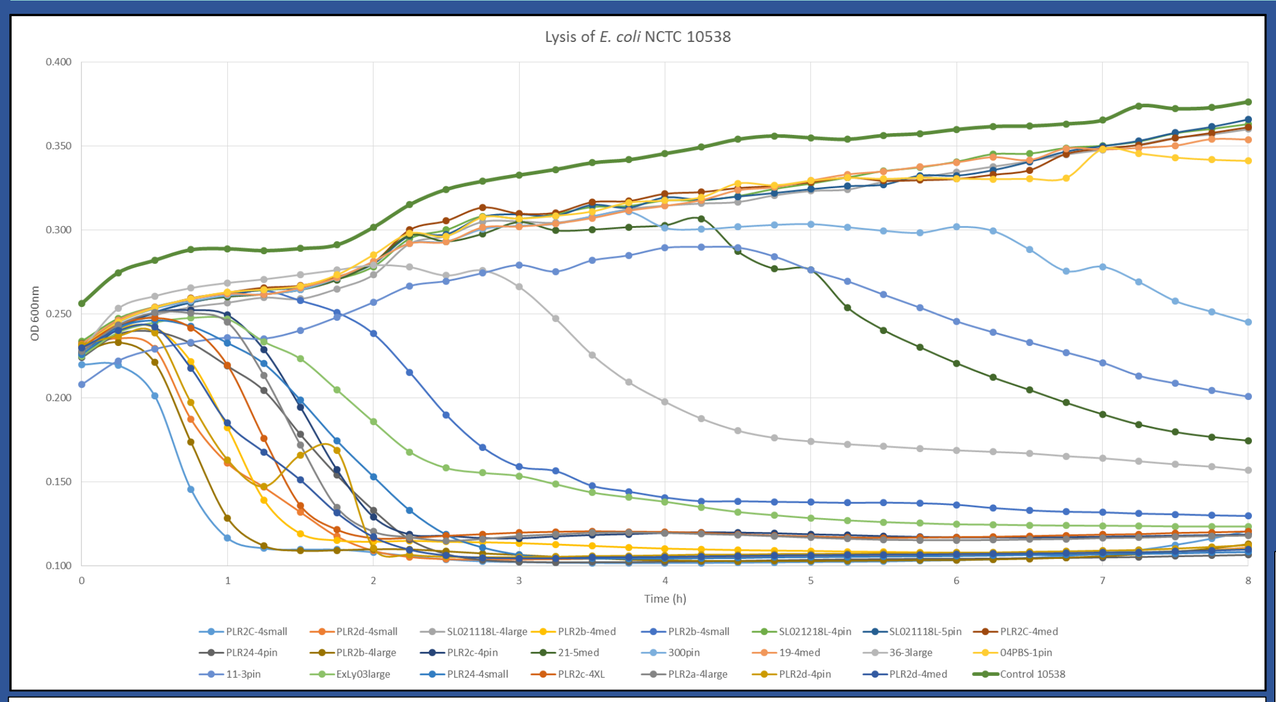


Figure 3: Lysis of E. coli NCTC 10538. Cultures of *E. coli* NCTC 10538 were infected with individual phage lysates, and their optical density was measured over 24 hours. A reduction in optical density was indicative of cell lysis by the infecting phage.

Phylogroup	Clinical source	Isolate	Phage01	Phage02	Phage03	Phage04	Phage05
D	SSI	913	✓	×	×	✓	✓
	SSI	917	✓	×	×	✓	✓
	SSI	1017	✓	✓	×	✓	×
B1	UTI	960	✓	✓	×	✓	×
	SSI	1048	×	×	✓	×	✓
B2	UTI	987	✓	×	×	✓	×
	SSI	1027	✓	✓	×	✓	✓
	UTI	1042	×	✓	×	×	✓
F	UTI	1024	✓	×	×	✓	×
C	UTI	1046	×	✓	✓	×	✓

Table 1: Clinical isolates & host range of selected phages. Clinical isolates are shown by phylogroup and clinical source. Five selected phages were tested against the clinical *E. coli* isolates using spot assays. Phage 04 and Phage 05 were selected for more characterisation to ensure coverage of all 10 clinical isolates.

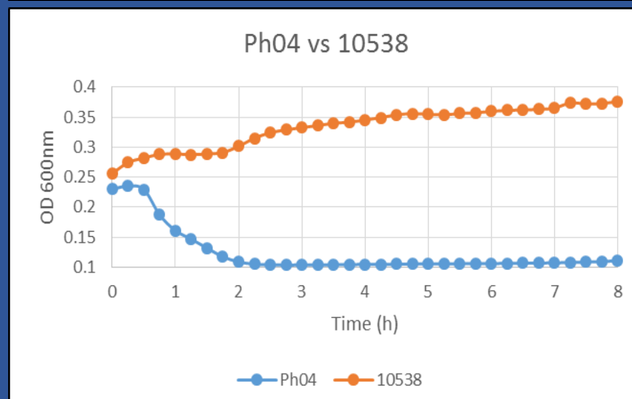


Figure 4: Lytic activity of Phage 04 against *E. coli* NCTC 10538 indicated by a reduction in optical density over time.

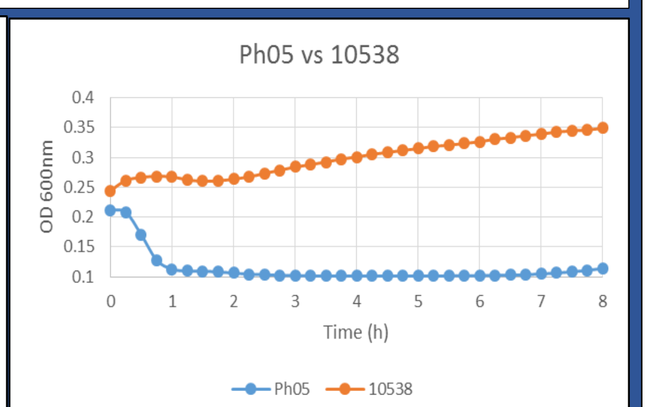


Figure 4: Lytic activity of Phage 05 against *E. coli* NCTC 10538 indicated by a reduction in optical density over time.

- The five selected phages were measured individually for lytic activity against *E. coli* NCTC 10538 and the 10 clinical *E. coli* strains
- Further testing will include:
  - Adsorption rates
  - Burst size
  - Stability (pH and temperature)
  - Time-kill
  - Structure and morphology (visualisation of phage using Transmission Electron Microscopy)
  - Genome sequencing