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Short communication

Isolation and characterisation of two novel coliphages with high potential to control antibiotic-resistant pathogenic *Escherichia coli* (EHEC and EPEC)

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Abstract

Two newly isolated virulent coliphages (MVBS and MVSS) showed ability to lyse (form plaque on) a high percentage of pathogenic *Escherichia coli* strains of various serotypes and origins (292/310; 94.2%), whilst displaying low lytic (plaque-forming) capacity on non-pathogenic ECOR strains (MVBS and MVSS lysed 10/72 (13.9%) and 15/72 (20.8%) strains, respectively). In comparison, a higher percentage (196/310, 63.2%) of tested isolates exhibited resistance to a broad range of antibiotics. It was also observed that, in contrast to antibiotics, phage treatment did not induce Shiga toxin production. These findings suggest that the newly isolated bacteriophages have potential for biocontrol and as therapeutic agents for pathogenic *E. coli* (EHEC and EPEC) strains.

Keywords: Coliphages; Antibiotic-resistant; Multidrug-resistant; EHEC; EPEC

1. Introduction

Enterohaemorrhagic *Escherichia coli* (EHEC) is a causative agent for haemorrhagic colitis and haemolytic uremic syndrome [1] both in humans and animals, and appears to have evolved from enteropathogenic *E. coli* (EPEC). The gastrointestinal tract of domestic ruminants is considered as the major reservoir for EHEC [1], which may be transmitted to humans via contaminated food and water.

Whilst antibiotics are widely used to control bacterial pathogens, they have contributed to an increased incidence of antibiotic-resistant bacteria. In addition, the recommended antimicrobial agents such as fosfomycin and quinolones are known to be able to induce the production of Shiga toxin [2].

Use of a bacteriophage (phage) could be a natural, non-toxic, feasible alternative for controlling bacterial pathogens. A number of studies have described the efficacy of phages in treating experimentally infected animals [3]. Recently, delayed introduction of new antibiotics on the market as well as US Food and Drug Administration (FDA) approval of using *Listeria* phages in foods [4] has renewed interest in phage use as an antimicrobial agent.

In this study, two novel phages (MVBS and MVSS) against *E. coli* O157:H7 were isolated and characterised in order to evaluate their potential for control of pathogenic *E. coli* (EHEC and EPEC). Both phages exhibited lytic activity against 94.2% of tested pathogenic *E. coli* strains (310 isolates), whilst they lysed (formed plaques on) only a small portion (14–21%) of non-pathogenic ECOR strains (72 isolates). Furthermore, in contrast to antibiotic treatment, no induction of Shiga toxins was observed in phage-treated bacterial culture supernatants. These findings make both phages potential candidates for control of antibiotic-resistant pathogenic *E. coli*.

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2. Materials and methods

2.1. Bacterial strains and growth

Escherichia coli strains used in this study are summarised in Table 1. Bacteria were grown in either Luria–Bertani (LB) broth (Difco Laboratories, Detroit, MI) for *E. coli* or brain–heart infusion (Difco) for non-*E. coli* with constant shaking (240 rpm) at 37 °C, unless otherwise indicated.

2.2. Isolation of bacteriophages

Escherichia coli O157:H7 NCTC 12900 was used as an indicator strain for phage isolation. Bovine or water buffalo

Table 1
Comparison of bacterial resistance to bacteriophages (EOP < 10^{-7}) and antibiotics

Bacteria/origin	Serotype (no. tested)	No. of bacteriophage-resistant isolates (no plaque formation)		Number of antibiotics-resistant isolates[a]
		MVBS	MVSS	
EHEC[b] (VT+)[*]				
Human	O157 (50)	1	1	27
	O145 (4)	4	1	3
	O111 (3)	0	3	3
	O26 (2)	0	0	1
	O113 (1)	1	1	0
	O103 (2)	0	0	1
	O91 (1)	0	0	0
	O121 (1)	1	0	1
	O55 (1)	0	1	1
Bovine	O157 (144)	3	3	89
Water buffalo	O157 (36)	0	0	13
Swine	O157 (9)	3	3	7
Ovine	O157 (5)	2	2	2
Equine	O157 (1)	0	0	0
Deer	O157 (4)	0	0	2
Roe deer	O157 (1)	0	0	1
Total	265	15 (5.66%)	15 (5.66%)	151 (56.98%)
EPEC[c] (VT−)				
Rabbit	O103 (30)	0	0	30
	O149 (2)	0	0	2
	O141 (3)	1	1	3
	O132 (2)	0	0	2
	O8 (2)	0	0	2
	O153 (4)	0	0	4
	O15 (2)	2	2	2
Total	45	3 (6.67%)	3 (6.67%)	45 (100%)
Total	310	18 (5.81%)	18 (5.81%)	196 (63.23%)
ECOR[d]	72	62[e] (86.11%)	57[f] (79.17%)	NT

EOP, efficiency of plating; VT+, verotoxigenic; VT−, non-verotoxigenic; NT, not tested.

[a] Antimicrobial-containing disks (Oxoid) were carefully applied onto the lawn and incubated for 24 h at 37 °C, including ampicillin (10 μg), cefaclor (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), fosfomycin (50 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), oxolinic acid (2 μg), norfloxacin (10 μg), streptomycin (10 μg), sulfonamides (300 μg), tetracycline (30 μg) and co-trimoxazole (25 μg). The susceptibility of each isolate to the panel of antibiotics was revealed by the diameter size of the clear zones around the dish as directed by the manufacturer. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls. Any isolate resistant to three or more of different antimicrobial classes was defined as being multidrug-resistant.

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[e] Does not contain (resistant to plaque formation) ECOR strains 2, 3, 4, 9, 12, 13, 16, 22, 37 and 56. It contains strains 6 and 61, which formed a clear zone of lysis in the spot assay but were not able to form plaques (EOP < 10^{-7}). All of the pathogenic *E. coli* strains tested for EOP measurement allowed plaque formation but in various EOPs. Eighty percent showed an EOP ranging from 1 to 0.7, 15% from 0.7 to 0.1 and 5% from 0.1 to 0.01. The EOPs in 10 MVBS-positive (plaque-forming) clones (2, 3, 4, 9, 12, 13, 16, 22, 37 and 56) were 1 for clone 4, 0.84 for 13 and ca. 10^{-5} for the remaining clones.

[f] Does not contain ECOR strains 2, 3, 4, 9, 12, 13, 16, 22, 50, 56, 57, 63, 67, 69 and 70. It contains 61 and 62, which were lysed in the spot assay but were not able to form plaques (EOP < 10^{-7}). Among the 15 MVSS-positive ECOR strains (2, 3, 4, 9, 12, 13, 16, 22, 50, 56, 57, 63, 67, 69 and 70), the EOPs were various (2, 0.43, 2, 0.34, 0.27, 2, 0.05, 0.03, 0.007, 2, 0.001, 0.05, 0.12, 0.02 and 0.07, respectively).

Neither phage formed plaques in other Gram-negative bacteria (obtained from American Type Culture Collection (ATCC, Manassas, VA), including *Campylobacter jejuni* ATCC 33291, *Citrobacter freundii* ATCC 8090, *Yersinia enterocolitica* ATCC 9610, *Enterobacter aerogenes* ATCC 13048, *Salmonella typhimurium* ATCC 14028, *Vibrio parahaemolyticus* ATCC 17802, *Shigella sonnei* ATCC 25931, *Klebsiella pneumoniae* ATCC 27736 and *Pseudomonas aeruginosa* ATCC 27853), except in *S. sonnei* (EOP of 0.2 for MVBS and 0.1 for MVSS), *C. freundii* (EOP of 2×10^{-6} for both phages) and *C. jejuni* (4×10^{-4} for MVSS).

[*] Isolates ED91, ED228, ED229, ED234, ED235 and ED239 were VT–.