Susceptibility of extended-spectrum- β-lactamase-producing Escherichia coli to commercially available and laboratory-isolated bacteriophages.

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The susceptibility of extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* to commercial and laboratory bacteriophages

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Running title: Susceptibility of ESBL *E. coli* to bacteriophages

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Sir,

Extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E), particularly *Escherichia coli* and *Klebsiella pneumoniae*, are resistant to β-lactam antibiotics, β-lactam combinations and often, non-β-lactam antibiotics. ESBL-E infections are associated with longer hospital stays and often poorer outcomes. Alternative or complementary therapies for ESBL-E infections are required. In response to the global emergence of antibiotic resistance, there is renewed interest in bacteriophage treatment of bacterial infections. Bacteriophages have high specificity (owing to narrow host ranges), modes of actions unrelated to antibiotic targets and self-propagating and self-limiting activities, facilitating low dosing and bacteriophage elimination following infection resolution [1]. We determined the *in vitro* susceptibility of 100 previously characterised ESBL-producing *E. coli* (ESBL-EC) [2] to four bacteriophage cocktails, used as part of standard clinical practice in the Republic of Georgia.

ESBL-production of ESBL-EC was confirmed according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) criteria [3] in Beaumont Hospital, Dublin, Ireland and were mainly isolated from urine, blood and respiratory specimens. As found for other ESBL-EC collections the majority belonged to phylogenetic groups B2 and D (80/100. 80%), but groups A and B1 were also represented[2]. The activities of four bacteriophage cocktails (Pyo-Phage, Intesti-Phage, Enko, Ses) were determined against each isolate using *in vitro* spot tests. Isolates were susceptible if confluent, semi confluent, opaque lysis or individual plaques ($n \geq 1$) were observed (single plaques may be propagated to generate bacteriophage with improved lytic spectra) and resistant if lysis was not visible. The

bacteriophage cocktails originated in Georgia and are sterile filtrates of phage lysates of bacterial species including *E. coli* serovar O25b [4].

Widespread susceptibility to bacteriophage preparations was found among ESBL-EC with the majority (89/100, 89 %) susceptible to at least two commercial phage cocktails. Ses and Enko phage preparations were active against more isolates than Pyo or Intesti (36%, 53%, 87%, 89%, isolates susceptible to Pyo, Intesti, Ses, Enko, respectively). Ses bacteriophage cocktail contains phage lysates against staphylococci, streptococci and enteropathogenic *E. coli* (011, 055, 026, 0125, 0119, 018, 044, 025, 020 serovars). Enko contains phage lysates for various serovars of salmonella, shigella, *E. coli* and staphylococci. These preparations are used for treatment of purulent-septic infections of skin or visceral organs, and intestinal disorders. The bacteriophage susceptibility of isolates, according to their phylogenetic group is shown in Table 1. All phylogenetic group B2 isolates, which included all members of the O25B-ST131 clone, were susceptible to at least two commercial bacteriophage preparations (Table 1). The 11 isolates (11 %) poorly susceptible to commercial phage preparations, were sporadically-occurring strains of phylogenetic groups A (5/100, 5%), B1 (3/100, 3 %), D (2/100, 2 %) or were unassignable to a phylogenetic group (1/100, 1%).

The susceptibility to other bacteriophage preparations or to strain-specific bacteriophages was demonstrated for 11 ESBL-EC isolates, resistant to the commercial bacteriophage cocktails. Three Eliava laboratory bacteriophages previously isolated against O-type *E. coli* strains were active against 3/11 (27%) ESBL-EC; five bacteriophages prepared for individual patients (autophages) as part of their treatment for chronic urinary tract infection were active against 6/11 (55%) ESBL-EC. Nine of 11 ESBL-EC isolates (82%) were

susceptible to specifically-prepared bacteriophages isolated from sewage water by an enrichment technique using the ESBL-EC as host [5].

The global dissemination of NDM1-mediated carbapenem resistance among ESBL-E will make treatment of ESBL-E infections increasingly challenging. Bacteriophage preparations, used to treat human infection in the Republic of Georgia, have *in vitro* activity against ESBL-EC types that are prevalent and problematic in our hospital and across the globe [2, 6]. Furthermore, isolates resistant to commercial bacteriophages, were susceptible to specifically-isolated bacteriophages. Bacteriophage therapy is part of standard healthcare in Georgia and Russia, but there remains no acceptance of bacteriophages as alternative anti-infectives outside these countries[1]. Early scientific studies using bacteriophages do not meet the standards required for modern clinical trials and the case for using these agents is reliant on anecdotal evidence of their success. A small number of early-phase clinical trials involving bacteriophages are reported in the English literature[7-10], one involving safety testing of an *E. coli* T4 oral phage preparation[7]. However, to date there have been no *in vitro* studies of ESBL-E isolates or clinical trials involving ESBL-E infections. Clinical trials that comply with the regulatory standards of Europe and the United States of America are necessary to test the safety and efficacy of bacteriophages for human therapeutic applications. However, the confirmation of *in vitro* bacteriophage susceptibility of a well characterised isolate collection, as described in this study, is an initial and encouraging development.

Transparency declaration

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Table 1. Susceptibility of ESBL-EC belonging to different phylogenetic groups to commercial bacteriophages.

Phylogenetic group (n)	Bacteriophage susceptibility(n)	Type of lysis observed[a]
B2 (62)	Susceptible to 4 bacteriophage preparations (24)	CL, SCL, OL or IPO_n/IPC_n with Enko, Ses, Intesti, Pyo
	Susceptible to 3 bacteriophage preparations (12)	CL, SCL, OL or IPO_n/IPC_n with Intesti, Enko, Ses; no lysis with Pyo
	Susceptible to 3 bacteriophage preparations (1)	IPC_n with Intesti, Enko, Pyo; no lysis with Ses
	Susceptible to 2 bacteriophage preparations (25)	SCL, OL with Enko and Ses; no lysis with Intesti and Pyo
D (18)	Susceptible to 4 bacteriophage preparations(8)	CL, SCL, OL or IPO_n with Enko, Ses, Intesti, Pyo
	Susceptible to 3 bacteriophage preparations (4)	OL or IPO_n with Intesti, Enko, Ses; no lysis with Pyo
	Susceptible to 2 bacteriophage preparations (4)	SCL or OL with Enko and Ses; no lysis with Intesti and Pyo
	Resistant to all commercial bacteriophage preparations(2)	No lysis with any commercial bacteriophage preparation
A (10)	Susceptible to 3 bacteriophage preparations (2)	SCL or OL with Enko, Ses, Intesti, no lysis with Pyo
	Susceptible to 2 bacteriophage preparations (2)	OL with Enko and Ses; no lysis with Intesti and Pyo
	Susceptible to 1 bacteriophage preparation (1)	IPC_n with Enko, no lysis with Ses, Intesti, Pyo
	Resistant to all commercial bacteriophage preparations (5)	No lysis with any commercial bacteriophage preparation
B1 (7)	Susceptible to 4 bacteriophage preparations (2)	SCL, OL or IPC_n with Enko, Ses, Intesti, Pyo
	Susceptible to 2 bacteriophage preparations (2)	OL with Enko and Ses; no lysis with Intesti and Pyo
	Resistant to all commercial bacteriophage preparations (3)	No lysis with any commercial bacteriophage preparation
U[b] (3)	Susceptible to 4 bacteriophage preparations (1)	OL with Intesti, Enko, Ses, Pyo
	Susceptible to 2 bacteriophage preparations (1)	SCL/OL with Enko/ Ses; no lysis with Intesti and Pyo
	Resistant to all commercial bacteriophage preparations (1)	No lysis with any commercial bacteriophage preparation

[a]CL-Confluent lysis, SCL-Semi-confluent lysis, OL-opaque lysis, IPO_n- individual turbid plaques (where n=number of plaques; 3-30), IPC_n- individual clear plaques (where n=number of plaques; 3-30). [b]U-unassigned phylogenetic group,

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