

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

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

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

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14 **group**

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21 Sir,

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

34 ESBL-production of ESBL-EC was confirmed according to the European Committee
35 for Antimicrobial Susceptibility Testing (EUCAST) criteria ³ in Beaumont Hospital, Dublin,
36 Ireland and were mainly isolated from urine, blood and respiratory specimens. As found for
37 other ESBL-EC collections the majority belonged to phylogenetic groups B2 and D (80/100.
38 80%), but groups A and B1 were also represented². The activities of four bacteriophage
39 cocktails (Pyo-Phage, Intesti-Phage, Enko, Ses) were determined against each isolate using *in*
40 *vitro* spot tests. Isolates were susceptible if confluent, semi confluent, opaque lysis or
41 individual plaques ($n \geq 1$) were observed (single plaques may be propagated to generate
42 bacteriophage with improved lytic spectra) and resistant if lysis was not visible. The

43 bacteriophage cocktails originated in Georgia and are sterile filtrates of phage lysates of
44 bacterial species including *E. coli* serovar O25b⁴.

45 Widespread susceptibility to bacteriophage preparations was found among ESBL-EC
46 with the majority (89/100, 89 %) susceptible to at least two commercial phage cocktails. Ses
47 and Enko phage preparations were active against more isolates than Pyo or Intesti (36%,
48 53%, 87%, 89%, isolates susceptible to Pyo, Intesti, Ses, Enko, respectively). Ses
49 bacteriophage cocktail contains phage lysates against staphylococci, streptococci and
50 enteropathogenic *E. coli* (011, 055, 026, 0125, 0119, 018, 044, 025, 020 serovars). Enko
51 contains phage lysates for various serovars of salmonella, shigella, *E. coli* and staphylococci.
52 These preparations are used for treatment of purulent-septic infections of skin or visceral
53 organs, and intestinal disorders. The bacteriophage susceptibility of isolates, according to
54 their phylogenetic group is shown in Table 1. All phylogenetic group B2 isolates, which
55 included all members of the O25B-ST131 clone, were susceptible to at least two commercial
56 bacteriophage preparations (Table 1). The 11 isolates (11 %) poorly susceptible to
57 commercial phage preparations, were sporadically-occurring strains of phylogenetic groups
58 A (5/100, 5%), B1 (3/100, 3 %), D (2/100, 2 %) or were unassignable to a phylogenetic group
59 (1/100, 1%).

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

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

68 The global dissemination of NDM1-mediated carbapenem resistance among ESBL-E
69 will make treatment of ESBL-E infections increasingly challenging. Bacteriophage
70 preparations, used to treat human infection in the Republic of Georgia, have *in vitro* activity
71 against ESBL-EC types that are prevalent and problematic in our hospital and across the
72 globe ^{2,6}. Furthermore, isolates resistant to commercial bacteriophages, were susceptible to
73 specifically-isolated bacteriophages. Bacteriophage therapy is part of standard healthcare in
74 Georgia and Russia, but there remains no acceptance of bacteriophages as alternative anti-
75 infectives outside these countries¹. Early scientific studies using bacteriophages do not meet
76 the standards required for modern clinical trials and the case for using these agents is
77 reliant on anecdotal evidence of their success. A small number of early-phase clinical trials
78 involving bacteriophages are reported in the English literature⁷⁻¹⁰, one involving safety
79 testing of an *E. coli* T4 oral phage preparation⁷. However, to date there have been no *in*
80 *vitro* studies of ESBL-E isolates or clinical trials involving ESBL-E infections. Clinical trials that
81 comply with the regulatory standards of Europe and the United States of America are
82 necessary to test the safety and efficacy of bacteriophages for human therapeutic
83 applications. However, the confirmation of *in vitro* bacteriophage susceptibility of a well
84 characterised isolate collection, as described in this study, is an initial and encouraging
85 development.

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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	
			96
			97
B2 (62)	Susceptible to 4 bacteriophage preparations (24)	CL, SCL, OL or IPO _n /IPC _n with Enko, Ses, Intesti, Pyo	98
	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	99
	Susceptible to 2 bacteriophage preparations (25)	SCL, OL with Enko and Ses; no lysis with Intesti and Pyo	100
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	101
	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	102
A (10)	Susceptible to 3 bacteriophage preparations (2)	SCL or OL with Enko, Ses, Intesti, no lysis with Pyo	103
	Susceptible to 2 bacteriophage preparations (2)	OL with Enko and Ses; no lysis with Intesti and Pyo	104
	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	105
B1 (7)	Susceptible to 4 bacteriophage preparations (2)	SCL, OL or IPC _n with Enko, Ses, Intesti, Pyo	106
	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	107
U ^b (3)	Susceptible to 4 bacteriophage preparations (1)	OL with Intesti, Enko, Ses, Pyo	108
	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	109

^aCL-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). ^bU-unassigned phylogenetic group,

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