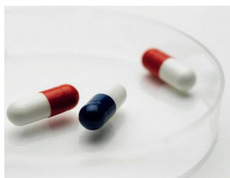
Susceptibility of ESBL-producing *Escherichia coli* to commercial bacteriophage coctails originated in Georgia

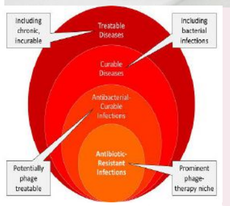
Aycan Gundogdu[1,2], Huseyin Kilic[1], Darajen Bolkvadze[3]

[1]Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Erciyes University, Kayseri, Turkey, [2] Genome and Stem Cell Center, Erciyes University, Kayseri, Turkey, [3] Laboratory of Molecular Biology, G. Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia

Escherichia coli is the leading bacterial pathogen responsible for intestinal as well as extraintestinal infections, including urinary tract infections and bacteremia. Extended spectrum beta-bactamases producing *E. coli* known to be resistant to ß-lactams, their combinations and non-ß-lactam antibiotics, might lead to infections with limited antibiotic therapy in clinical practice as well as unsuccessful treatment.



Due to global emergence of these types of multi drug resistant strains, alternative therapies for their infections are in seach worldwide. Bacteriophage therapy is among the alternatives, since bacteriophages have hight host specificity and actions unrelated of antibiotic targets. They also have self-propogating and self-limiting activities allowing low dosing and bacteriophage elimination following infection resolution.



Although phage therapy has been a successful part of standard healthcare practice for decades, in some eastern Europe countries , several camps have been sceptical to accept phages as alternative antinfectives.

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Objective of the study

To determine the *in vitro* activity of Georgian bacteriophage cocktails, that are used as a part of standard clinical practice in the Republic of Georgia on the extended spectrum beta-bactamases producing *E. coli* (ESBLEC) strains isolated from Turkish patients.

Methods:

- A total of 615 *E. coli* strains isolated from adult and pediatric patients (one isolate/patient) urine (n=433) or blood (n=182) cultures in Kayseri, Turkey, between 2014-2015 were included in this study.
- ESBL production of the isolates was screened according to the EUCAST criteria.
- All strains were typed using a Php-typing
- One representative from each PhP-types were included for in-vitro phage susceptibility assay.
- The *in vitro spot tests* were performed to determine the activities of four bacteriophage cocktails (Pyophage, Intestiphage, Enko and Ses) against each non-replicating ESBLEC.
- Observing confluent, semi-confluent, opaque lysis or individual plaques determined the isolate susceptibility. When the lysis was not possible, the corresponding isolates were determined to be resistant.

Phage cocktails	Active against
Pyophage	*Staphylococcus spp., Streptococcus spp, P. aeruginosa, P. mirabilis, P. vulgaris, E.coli*
Intestiphage	*Shigella spp, Salmonella spp., E.coli, Staphylococcus spp., Enterococcus spp., Proteus spp., P. aeruginosa*
Enko-phage	*S. typhimurium, S. enteritidis, S. heidelberg, S. newport, S. cholerae, S. oranienburg S. dublin, S. anatum, S. flexneri, S. sonnei,, S. aureus, S. epidermidis ,S. saprophyticus E.coli*
SESphage	*S. aureus, S. epidermidis, S. saprophyticus, S. pyogenes, S. sanguis, S. salivarius, S. agalactiae, E.coli*

Table 1. No and source of *E. coli*

	No of *E. coli*		
Urine	ESBL -ve	ESBL +ve	Total
Hosp-Adult	74	43	117
Out-Child	26	19	45
Hosp-Adult	92	26	118
Out-Child	131	22	153
Blood			
Children	12	13	25
Adult	105	52	157
Total	440	175	615

Results:

- 175 of *E. coli* isolates were found to be ESBL producer.
- Based on PhP, 142 unrelated ESBLEC were found.
- Majority (131/142, 92.3%) of ESBLEC strains were susceptible to at least one commercial phage cocktail. Phages were effective against all tested resistant types.
- Enko, Intestiphage and Pyophage preparations were active against more isolates than Ses (87.3%, 81.7%, 81.7% , 59.2%, respectively).
- 11 of the 142 ESBL-EC isolates were resistant to all phage cocktails.
- Of these, three isolates were susceptible to specifically prepared phages isolated from sewage water by an enrichment technique.

Table 2. Resistance rates for 142 ESBLEC. *intermadiate resistant

	SAM	CIP	GEN	SXT	AMC	TZP	AMK*	FEP	IPM	ETP
Urine (n=83)										
Hospi. (n=47)	82	73.3	42.3	74.5	51.2	30	5	100	2.2	2.2
Out (n=36)	79.2	60.5	24.4	60.2	47.6	20	0	100	0	0
Blood (n=59)	95	67.8	48	78	59.5	40.7	3.4	96.6	5.1	3.4

SAM; ampicillin sulbactam, CIP; ciprofloxasin; GEN; gentamycin, SXT;sulfamethoxazole, AMC; amoxicillin clavu., TZP; tazocin; AMK; Amikacin; FEP; cefepime, IPM; imipenem, ETP;etrapenem.

Conclusions:

- ✓ Commercial phage preparations were observed to have *in vitro* activity against ESBLEC isolated from children and adults in Turkey.
- ✓ The susceptibility rates are promising especially for infections that are difficult to treat caused by MDR pathogens.
- ✓ As this is a preliminary step to the potential clinical trials to be designed for the country, *in vitro* confirmation of their success on a ESBLEC collection should be accepted as an initial action, which is encouraging to consider clinical trials of phage therapy.
- ✓ Moreover, the effectiveness of generic phage cocktails which are not specifically produced for strains of concern is an implication that the spectrum of phages is large enough to allow their general use.