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vB_EcoM_kmuOR AND vB_EcoM_kmuGH: TWO BROAD HOST RANGE COLIPHAGES EFFECTIVE AGAINST *ESCHERICHIA COLI* O157:H7 AND *SHIGELLA FLEXNERI*

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ABSTRACT

E. coli O157:H7 is a foodborne pathogen mostly related to consumption of meat and vegetables. Although using antibiotics is not recommended in treatment of infections caused by this pathogen, antibiotic resistant isolates are reported. This has encouraged researchers to find alternative methods to treat and effective approaches to prevent the *E. coli* O157:H7 infections. Phage therapy is one of the techniques with therapeutic and biocontrol applications. The aim of this study was to find effective phages capable of lysing this pathogen. Twenty samples were searched for phages against *E. coli* O157:H7. The host range of the isolated phages was determined. The phages with broader host range were selected for structural study. The stability in different temperature and pH values were also performed. Phages kmuOR and kmuGH could lyse two strain of *E. coli* O157:H7, some clinical non-O157 *E. coli* strains, and a strain of *Shigella flexneri*, another enteric pathogen. Transmission electronic micrograph revealed that both phages belong to Myoviridae family. The two phages were resistant to pH 3 to 9.5 and temperatures 4-50°C and able to prevent the growth of *E. coli* O157:H7 in LB medium. Further study can prove the suitability of these phages as biocontrol or phage therapy agents.

Keywords: *Escherichia coli* O157:H7, *Shigella flexneri*, Lytic phages, Phage therapy, Antimicrobials

INTRODUCTION

Escherichia coli is a member of human microflora and mostly harmless, but some strains can cause different problems to human health. Enteric diseases, urinary tract infections, and sepsis arise from pathogenic *E. coli* strains **(Kim *et al.*, 2017)**. *E. coli* O157:H7, one of the predominant enterohaemorrhagic *E. coli* (EHEC) strains in human infections, can lead to hemorrhagic colitis and hemolytic uremic syndrome **(Mir and Kudva, 2019; Um *et al.*, 2018)**. However, it is harmless to its natural reservoirs (ruminants) and is spread via food **(Omisakin *et al.*, 2003)**. Generally, antibiotic therapy is not recommended in treatment of EHEC infections mainly due to the increased release or production of shiga toxins; however, some antibiotics such as fosfomycin and azithromycin may be useful in treatment of the infection and solve the problem related to bacterial shedding by carriers **(Agger *et al.*, 2015; Jost *et al.*, 2016; Kakoullis *et al.*, 2019)**. *Shigella* genus is also a foodborne pathogen causing shigellosis (bacillary dysentery) of which *Shigella flexneri* is the most prevalent strain in developing countries **(Shahin and Bouzari, 2018; Ye *et al.*, 2010)**.

Furthermore, antibiotic resistance is an important problem in healthcare system that needs urgent attention **(Mir and Kudva, 2019)**. This phenomenon is also present among *E. coli* O157 and *S. flexneri* isolates **(Cui *et al.*, 2015; Sabouri *et al.*, 2017; Soltan Dallal *et al.*, 2011)**. In a study conducted in Egypt, 57.4% of *E. coli* O157:H7 isolates were multidrug resistant **(Ahmed and Shimamoto, 2015)**. To be able to combat antibiotic resistant bacteria, scientists are searching for alternatives such as applying bacteriophages **(Chanishvili and Aminov, 2019; Mir and Kudva, 2019)**.

Bacteriophages (phages) are viruses that infect bacterial cells. These entities are categorized into lysogenic or lytic phages based on their replication cycle **(Cisek *et al.*, 2017)**. Lytic phages, which kill their host cells, can be used to treat bacterial infections (phage therapy) or to reduce the bacterial contamination of foods (biocontrol) **(Abedon *et al.*, 2017)**. Phage therapy is not a new concept. Actually, d'Herelle made the first attempts to treat infections using phages in 1919 **(Sulakvelidze *et al.*, 2001)**. This method was applied until the discovery of antibiotics **(Cisek *et al.*, 2017)**. Thereafter, it was faded in west and limited to Georgia, Russia, and Poland **(Chanishvili and Aminov, 2019)**. However, the vanished idea is back to the scene as a result of the emergence of antibiotic resistance among pathogens **(Lin *et al.*, 2017)**.

As it is valuable to find lytic phages against pathogens and characterize them for therapeutic and biocontrol application, the objective of our study was to isolate coliphages from sewage and water samples able to infect *E. coli* O157:H7 and study their Characteristics.

MATERIAL AND METHODS

Bacterial strains and phage isolation

E. coli O157:H7 UTMC01901 was used as the host strain. *E. coli* O157:H7 B-1 and *Shigella dysentriae* SD-T1 were characterized previously **(Shahrbabak *et al.*, 2013)** and the clinical isolates of *E. coli* were characterized based on biochemical reactions **(*Bergey's manual of systematic bacteriology*, 2005)**. All strains were cultured in LB (Luria–Bertani) medium to reach an OD_{600} of 0.3.

Twenty pond, lake, and raw urban and hospital wastewater samples were collected from different areas of Iran during summer. The samples were mixed by vortexing and left for 20 min. The particulate materials were isolated by centrifugation at 10000 g for 5 min and supernatants were kept at 4°C. Ten ml of each stored supernatant was added to 100 ml culture of *E. coli* O157:H7 UTMC01901 and incubated at 37°C overnight (shaking at 30 rpm). Then, chloroform (1/10) was added to lyse bacteria and the lysate settled for 1 h at 4°C. The upper part was collected in a new tube, centrifuged at 10000 g for 5 min, and left aside open-capped for 30 min in a laminar-flow cabinet **(Salem *et al.*, 2015)**. Spot assay method was used to detect the presence of phage particles and a single plaque for each positive sample was purified three times **(Shahrbabak *et al.*, 2013)**.

Determination of phage host range

Plates were seeded with bacterial strains and 5 µl drops of isolated phages were placed on top of the bacterial layer. In addition to the phage stock suspensions, serial dilutions were applied to rule out the lysis from without **(Delbruck, 1940)**. The plates were incubated overnight and the clear zones were observed the other day. Based on the results of this test, two more effective phages (kmuOR and kmuGH) were selected for further study.

Transmission Electron Microscopy (TEM)

High-titre (10^9 PFU.ml^{-1}) phage lysates of kmuOR and kmuGH were sedimented for 90 min at 20000 g at 4°C, washed twice in neutral ammonium acetate, deposited on carbon-coated Formvar films on copper grids, stained with 2% uranyl acetate, and examined in a zeiss EM900 transmission electron microscope.

Structural proteins

Purified phage particles (10^9 PFU.ml^{-1}) suspended in SDS-PAGE loading buffer (2% SDS, 10% glycerol, 2.5% b-mercaptoethanol, 0.0025% bromophenol blue, and 6.25 mM Tris/HCl, pH 6.8) were boiled for 10 min, then loaded onto 12% polyacrylamide gel and electrophoresed. The gel was stained with Coomassie brilliant blue R250 (Merck) and then destained with destaining solution (30% methanol and 10% acetic acid in distilled water) and investigated for protein bands.

Adsorption time

The host bacterium was grown to mid-log phase and each Phage (kmuOR and kmuGH) was added separately to the suspension at a multiplicity of infection (MOI) of 10^{-1}. Sampling was done at desired intervals. The samples were centrifuged to sediment bacterial cells and the adsorbed phage particles. To enumerate unadsorbed phage particles, soft agar overlay method was used. Briefly, the serially diluted supernatants were mixed with the host cells (grown to OD_{600} ~0.3), poured into 0.7% soft agar, plated on Nutrient Agar. The plates were incubated overnight and the formed plaques were counted the next day **(Adams, 1959)**.

Effect of temperature and pH on phages

Phage particles (10^7 PFU.ml^{-1}) were incubated at different temperatures (4, 25, 37, 42, 50, 60 and 70°C) at pH 7 or three pH values (2, 3, 7, 9.5 and 10.6) at 37°C for 30 minutes. One hundred µl of each sample was serially diluted and phage count was determined by the soft agar overlay method. Glycine-HCl (pH 2 and 3) and glycine NaOH (pH 9.5 and 10.6) were added to the culture medium to adjust the desired pH values **(Ruzin, 1999)**.

Determining the optimal MOI

Cultured host cells were diluted to obtain 1×10^5CFU.ml^{-1}. The dilution was mixed with phage particles at different MOIs (10^{-1}, 10^0, 10^1, and 10^2) and incubated at 37°C without shaking. Sampling was done at desired intervals (0, 2, and 4 h) and the absorbance at 600 nm was measured.

Statistical analysis

Experiments of adsorption time, effect of pH and temperature, and the optimal MOI were done 3 times. Data are expressed as means± standard deviation (SD). The results were analyzed by one way ANOVA (analysis of variance) in SPSS software version 18 (IBM Corp., Armonk, NY, USA). $p < 0.05$ was considered as the statistically significant level.

RESULTS AND DISCUSSION

Phage isolation and host range

Nine phages were isolated from 20 samples. All phages were tested to determine host range (Table 1). Two phages (named kmuOR and kmuGH), which could lyse two *E. coli* O157:H7 strains effectively, were chosen for further study. These two phages were also effective on *Shigella flexneri*. Since using broad host range phages in phage therapy is valuable **(Hyman, 2019)**, these phages can be considered as suitable choices for this purpose. There are reports of isolating anti-*E. coli* O157:H7 phages effective on other Enterobacteriaceae members. For example, HY01 is active on *Shigella flexneri*, SPF10 on *Salmonella enterica*, and AR1 on *Salmonella enterica* and *Shigella dysenteriae* **(Lee *et al.*, 2016; Liao *et al.*, 2011; Park *et al.*, 2012)**.

Table 1 Host range and sources of isolated phages

Sampling place	Sewage (Rasht, Ghaem hospital)	Urban sewage (Kerman)	Aradkouh Processing and disposal complex (Tehran)			Untreated sewage (Kerman)		Lake (Tehran, Eram park)	Zoo (Rasht, Mellat park)
Phage / Bacteria	GH	OR	P1	P2	P3	VK1	VK2	DS	BV
E. coli O157:H7 (UTMC01901)	+++	+++	++	++	+++	+++	+++	+++	++
E. coli O157:H7 (B-1)	+++	+++	+++	+	+	++	-	-	+++
E. coli O126:K71 (PTCC 1276)	++	+++	-	-	-	-	-	-	-
E. coli O55:K59 (PTCC 1269)	-	-	-	-	-	-	-	-	-
E. coli (PTCC 1330)	+++	+++	+++	+++	+++	+++	+++	+++	+++
E. coli (ATCC 25922)	+++	+++	+++	+++	+++	++	+++	+++	++
E. coli MA-1 (clinical isolate)	-	++	-	-	-	-	-	-	+
E. coli B1009 (clinical isolate)	-	+	-	-	-	-	-	-	-
E. coli B1010 (clinical isolate)	+	+++	-	-	-	-	-	-	+
E. coli B1047 (clinical isolate)	-	+	-	-	-	-	-	-	-
E. coli B1056 (clinical isolate)	-	++	-	-	-	-	-	-	-
E. coli B1076 (clinical isolate)	+	++	-	-	-	-	-	-	+
Shigella flexneri (PTCC 1234)	+++	+++	-	-	-	-	-	-	-
Shigella dysentriae SD-T1 (clinical isolate)	-	-	-	-	-	-	-	-	-
Salmonella typhi (PTCC 1609)	-	-	-	-	-	-	-	-	-

-: no lysis, Lysis at concentrations +: 10^6 and above, ++: 10^4 to 10^5, +++: 10^3 PFU.ml^{-1} and below

Table 2 Characteristics of phages kmuOR and kmuGH. All parameters are presented as nm.

Phage	Neck length	Head length	Head diameter	Non-contracted tail length and thickness	Contracted tail length and thickness
vB-EcoM-kmuOR	10	95	80	90× 15	35× 20
vB-EcoM-kmuGH	9.5	77	72	82.5× 11.5	38.5× 19

Transmission Electron Microscopy (TEM)

Both phages showed the characteristics of Myoviridae family of phages: an icosahedra head and a contractile tail (Figure 1). The result is not surprising as it is estimated that 96% of isolated phages are members of Caudovirales, which are tailed phages **(Brüssow and Hendrix, 2002)**. The head and tail sizes of phages kmuOR and kmuGH are presented in Table 2. It seems that both phages are large Myoviruses.

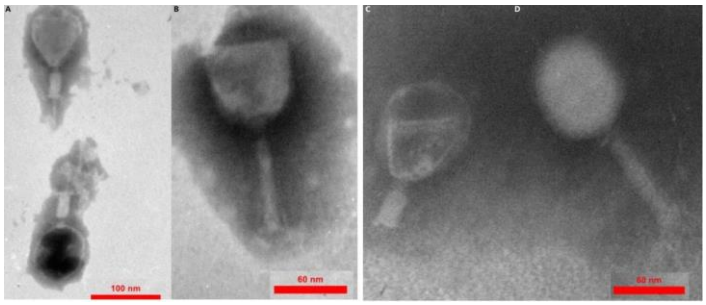

Figure 1 The electron micrographs of phages kmuOR with contracted (A) and non-contracted (B) tail and kmuGH with contracted (C) and non-contracted (D) tail stained with uranyl acetate

Structural proteins

Three sharp bands (~25, 35, 55 kDa) and some weaker bands were observed in Coomassie- stained SDS polyacrylamide gel of phages kmuOR and kmuGH (Figure 2). These sharp bands probably present the major proteins of capsid and tail. The pattern of the structural proteins of kmuOR and kmuGH are very similar that shows they are closely related (in addition to other findings).

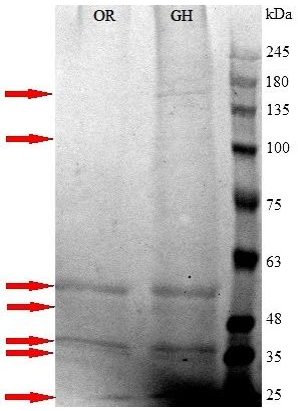

Figure 2 Structural proteins of Phages kmuOR and kmuGH separated by 12% SDS polyacrylamide gel and stained with Coomassie blue. A pre-stained protein size marker from Sinaclon was used. The sizes of protein bands are indicated on the right. The arrows show the protein bands of the phages.

Adsorption time of phages

For both phages, most particles are adsorbed within 4 minutes, a stage that depends on the specific phage receptors of the host cell **(Briggiler Marco *et al.*, 2015)**. The adsorption curve of kmuOR and kmuGH are the same with insignificant differences (Figure 3).

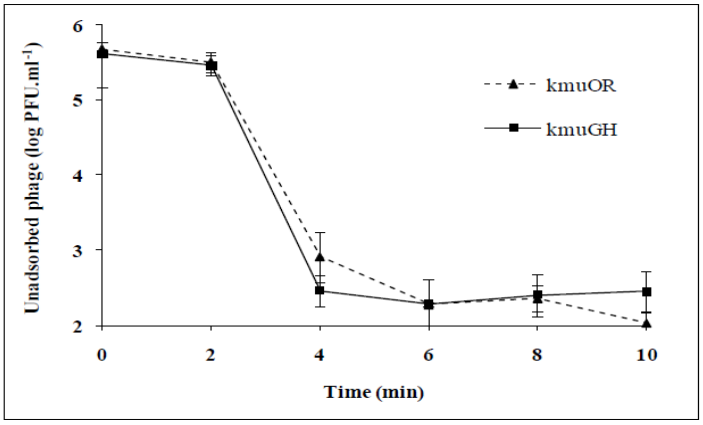

Figure 3 Adsorption times of phages kmuOR and kmuGH on *E. coli* O157:H7 UTMC01901. N=3

Effect of temperature and pH on phages

As seen in Figure 4A, phages kmuOR and kmuGH are stable at temperatures 4 to 60°C with no statistically significant differences (p-value>0.05). However, the active particles were significantly decreased at 70°C. Both phages were also stable at tested pH values at room temperature (Figure 4B) and there was no statistically significant difference between the four pH values (p-value>0.05) except for pH 2 in which there were no active phage particles at this pH value.It showes that phages kmuOR and kmuGH are atable at pH 3 to 10.6. Although the phage particles are slightly less at pH 10.6, the difference is not statistically significant. Stability at different pH values and temperatures is different for each phage **(Jonczyk *et al.*, 2011b)**. However, Ackermann *et al*. showed that lipid-containing phages are unstable while tailed phages are the more stable in long term preservation **(Ackermann *et al.*, 2004)**. pH and temperature affect the rate of adsorption of phages to their hosts **(Rakhuba *et al.*, 2010)**. In addition, temperature affects the phage's latent period and penetration. Resistance to low pH values is especially important for phage therapy of gastrointestinal diseases **(Nobrega *et al.*, 2016)**. Many phages are sensitive to low pH values. ISF001, a siphovirus against *S. flexneri*, was stable at pH 7 to 9 for 1 h **(Shahin and Bouzari, 2018)**. Phaegs e11/2 and e4/1c (against *E. coli* O157:H7) were stable at pH values from 3 to10 and 4 to 10, respectively **(Coffey *et al.*, 2011)**. T4 coliphage was also stable in pH range from 4 to 10 **(Taj *et al.*, 2014)**. Similarly, T7 coliphage was unstable at pH < 4 **(Jonczyk *et al.*, 2011a)**. It is notable that the aforementioned experiments have been performed in different buffers with different incubation periods.

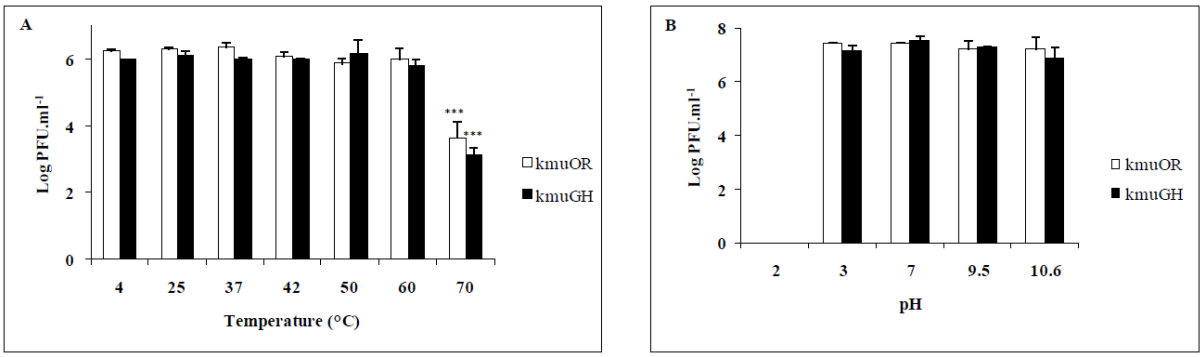

Figure 4 Effect of 30 min treatment of phages kmuOR and kmuGH at different temperatures (A) or pH values (B). N>3. There were no phage particles viable at pH 2.

Determining the optimal MOI

Both phages could effectively prevent the bacterial growth compared to the bacterial control at MOIs 0.1 to 100. While bacterial growth was observed at lower MOIs (0.01 and 0.001), even at these MOIs, the growth rate was lower than the control. The growth curves of the host infected with different MOIs of phages kmuOR and kmuGH are shown in Figure 5. One of the most problematic drawbacks of phage therapy is the emergence of phage-resistant cells especially when one phage strain is used. Therefore, using a phage cocktail can control or delay occurrence of the resistance. The resistance mostly occurs by deletion or alteration of phage receptor on the surface of the host cell, so using phages that utilize different receptors in the phage cocktail is important **(Sabouri *et al.*, 2017; Tanji *et al.*, 2004)**.

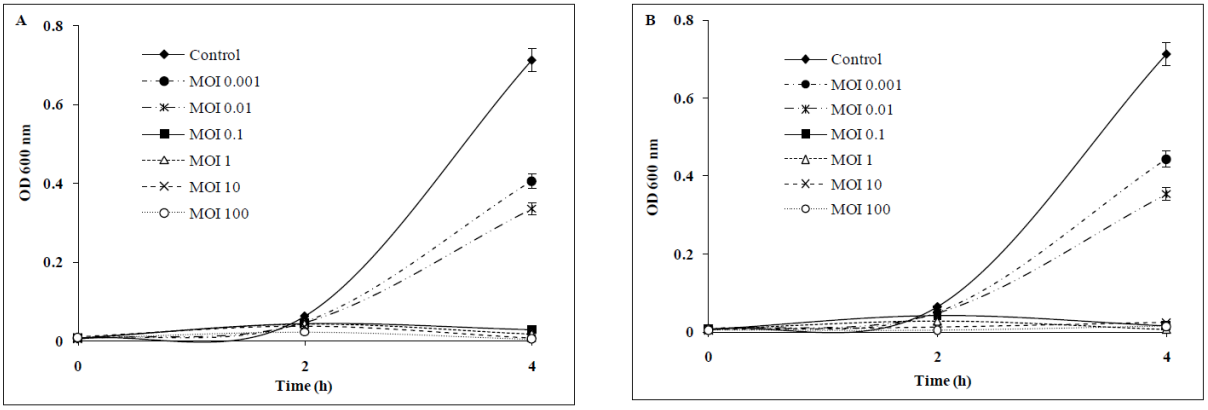

Figure 5 Effect of phages kmuOR (A) and kmuGH (B) on *E. coli* O157:H7 UTMC01901 in LB broth at different MOIs. No phage particles were added to the control. N>3.

CONCLUSION

Phages kmuOR and kmuGH behave similarly; they remain active at temperatures from 4 to 50°C and pH changes from 3 to 9.5, adsorb into the same host in the same conditions in a same period of time, share a common pattern of structural proteins, and belong to the same family. However, a notable difference is that kmuOR has a broader host range. As these two phages are effective in preventing the growth of *E. coli* O157:H7 in *in-vitro* conditions, they may be suitable candidates for phage therapy or bicontrol purposes.

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REFERENCES

Abedon, S. T., Garcia, P., Mullany, P., & Aminov, R. (2017). Editorial: Phage Therapy: Past, Present and Future. *Front Microbiol, 8*, 981. http://dx.doi.org/10.3389/fmicb.2017.00981

Ackermann, Hans-W, Tremblay, Denise, & Moineau, Sylvain. (2004). Long-term bacteriophage preservation. *WFCC Newsletter, 38*(1), 35-40.

Adams, MH. *Bacteriophages* (1959). Assay of phage by agar layer method. *New York: Interscience Publishers*, 450-451.

Agger, M., Scheutz, F., Villumsen, S., Molbak, K., & Petersen, A. M. (2015). Antibiotic treatment of verocytotoxin-producing *Escherichia coli* (VTEC) infection: a systematic review and a proposal. *J Antimicrob Chemother, 70*(9), 2440-2446. http://dx.doi.org/10.1093/jac/dkv162

Ahmed, A. M., & Shimamoto, T. (2015). Molecular analysis of multidrug resistance in Shiga toxin-producing *Escherichia coli* O157:H7 isolated from meat and dairy products. *Int J Food Microbiol, 193*, 68-73. http://dx.doi.org/10.1016/j.ijfoodmicro.2014.10.014

Bergey's manual of systematic bacteriology. (2005). (D. J. Brenner, N. R. Krieg & J. T. Staley Eds. 2 ed. Vol. 2): Springer.

Briggiler Marco, M., Reinheimer, J., & Quiberoni, A. (2015). Phage adsorption and lytic propagation in *Lactobacillus plantarum*: could host cell starvation affect them? *BMC Microbiol, 15*, 273. http://dx.doi.org/10.1186/s12866-015-0607-1

Brüssow, Harald, & Hendrix, Roger W. (2002). Phage genomics: small is beautiful. *Cell, 108*(1), 13-16.

Chanishvili, Nina, & Aminov, Rustam. (2019). Bacteriophage therapy: coping with the growing antibiotic resistance problem. *Microbiology Australia*.

Cisek, A. A., Dabrowska, I., Gregorczyk, K. P., & Wyzewski, Z. (2017). Phage Therapy in Bacterial Infections Treatment: One Hundred Years After the Discovery of Bacteriophages. *Curr Microbiol, 74*(2), 277-283. http://dx.doi.org/10.1007/s00284-016-1166-x

Coffey, B., Rivas, L., Duffy, G., Coffey, A., Ross, R. P., & McAuliffe, O. (2011). Assessment of *Escherichia coli* O157:H7-specific bacteriophages e11/2 and e4/1c in model broth and hide environments. *Int J Food Microbiol, 147*(3), 188-194. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.04.001

Cui, X., Wang, J., Yang, C., Liang, B., Ma, Q., Yi, S., . . . & Song, H. (2015). Prevalence and antimicrobial resistance of *Shigella flexneri* serotype 2 variant in China. *Front Microbiol, 6*, 435. http://dx.doi.org/10.3389/fmicb.2015.00435

Delbruck, M. (1940). The growth of bacteriophage and lysis of the host. *J Gen Physiol, 23*(5), 643-660.

Hyman, P. (2019). Phages for Phage Therapy: Isolation, Characterization, and Host Range Breadth. *Pharmaceuticals (Basel), 12*(1). http://dx.doi.org/10.3390/ph12010035

Jonczyk, E., Klak, M., Miedzybrodzki, R., & Gorski, A. (2011a). The influence of external factors on bacteriophages--review. *Folia Microbiol (Praha), 56*(3), 191-200. http://dx.doi.org/10.1007/s12223-011-0039-8

Jonczyk, E., Klak, M., Miedzybrodzki, R., & Gorski, A. (2011b). The influence of external factors on bacteriophages-review. *Folia Microbiol (Praha), 56*(3), 191-200. http://dx.doi.org/10.1007/s12223-011-0039-8

Jost, C., Bidet, P., Carrere, T., Mariani-Kurkdjian, P., & Bonacorsi, S. (2016). Susceptibility of enterohaemorrhagic *Escherichia coli* to azithromycin in France and analysis of resistance mechanisms. *J Antimicrob Chemother, 71*(5), 1183-1187. http://dx.doi.org/10.1093/jac/dkv477

Kakoullis, L., Papachristodoulou, E., Chra, P., & Panos, G. (2019). Shiga toxin-induced haemolytic uraemic syndrome and the role of antibiotics: a global overview. *J Infect, 79*(2), 75-94. http://dx.doi.org/10.1016/j.jinf.2019.05.018

Kim, N. H., Cho, T. J., & Rhee, M. S. (2017). Current interventions for controlling pathogenic *Escherichia coli*. *Adv Appl Microbiol, 100*, 1-47. http://dx.doi.org/10.1016/bs.aambs.2017.02.001

Lee, H., Ku, H. J., Lee, D. H., Kim, Y. T., Shin, H., Ryu, S., & Lee, J. H. (2016). Characterization and Genomic Study of the Novel Bacteriophage HY01 Infecting Both *Escherichia coli* O157:H7 and *Shigella flexneri*: Potential as a Biocontrol Agent in Food. *PLoS One, 11*(12), e0168985. http://dx.doi.org/10.1371/journal.pone.0168985

Liao, W. C., Ng, W. V., Lin, I. H., Syu, W. J., Liu, T. T., & Chang, C. H. (2011). T4-Like genome organization of the *Escherichia coli* O157:H7 lytic phage AR1. *J Virol, 85*(13), 6567-6578. http://dx.doi.org/10.1128/JVI.02378-10

Lin, D. M., Koskella, B., & Lin, H. C. (2017). Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World J Gastrointest Pharmacol Ther, 8*(3), 162-173. http://dx.doi.org/10.4292/wjgpt.v8.i3.162

Mir, Raies A, & Kudva, Indira T. (2019). Antibiotic-resistant Shiga toxin-producing *Escherichia coli*: An overview of prevalence and intervention strategies. *Zoonoses and public health*, *66*(1), 1-13.

Nobrega, F. L., Costa, A. R., Santos, J. F., Siliakus, M. F., van Lent, J. W., Kengen, S. W., Kluskens, L. D. (2016). Genetically manipulated phages with improved pH resistance for oral administration in veterinary medicine. *Sci Rep, 6*, 39235. http://dx.doi.org/10.1038/srep39235

Omisakin, F, MacRae, Marion, Ogden, Iain D, & Strachan, Norval James Colin. (2003). Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. *Appl. Environ. Microbiol., 69*(5), 2444-2447.

Park, M., Lee, J. H., Shin, H., Kim, M., Choi, J., Kang, D. H., . . . & Ryu, S. (2012). Characterization and comparative genomic analysis of a novel bacteriophage, SFP10, simultaneously inhibiting both *Salmonella enterica* and *Escherichia coli* O157:H7. *Appl Environ Microbiol, 78*(1), 58-69. http://dx.doi.org/10.1128/AEM.06231-11

Rakhuba, D. V., Kolomiets, E. I., Dey, E. S., & Novik, G. I. (2010). Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. *Pol J Microbiol, 59*(3), 145-155.

Ruzin, Steven E. (1999). *Plant microtechnique and microscopy* (Vol. 198): Oxford University Press, New York.

Sabouri, S., Sepehrizadeh, Z., Amirpour-Rostami, S., & Skurnik, M. (2017). A minireview on the *in vitro* and *in vivo* experiments with anti-*Escherichia coli* O157:H7 phages as potential biocontrol and phage therapy agents. *Int J Food Microbiol, 243*, 52-57. http://dx.doi.org/10.1016/j.ijfoodmicro.2016.12.004

Salem, M., Virtanen, S., Korkeala, H., & Skurnik, M. (2015). Isolation and characterization of *Yersinia*-specific bacteriophages from pig stools in Finland. *J Appl Microbiol, 118*(3), 599-608. http://dx.doi.org/10.1111/jam.12722

Shahin, K., & Bouzari, M. (2018). Bacteriophage application for biocontrolling *Shigella flexneri* in contaminated foods. *J Food Sci Technol, 55*(2), 550-559. http://dx.doi.org/10.1007/s13197-017-2964-2

Shahrbabak, S. S., Khodabandehlou, Z., Shahverdi, A. R., Skurnik, M., Ackermann, H. W., Varjosalo, M., . . . & Sepehrizadeh, Z. (2013). Isolation, characterization and complete genome sequence of PhaxI: a phage of *Escherichia coli* O157 : H7. *Microbiology, 159*(Pt 8), 1629-1638. http://dx.doi.org/10.1099/mic.0.063776-0

Soltan Dallal, Mohammad Mehdi, Ranjbar, Reza, & Pourshafie, Mohammad Reza. (2011). The study of antimicrobial resistance among *Shigella flexneri* strains isolated in Tehran, Iran. *Journal of Pediatric Infectious Diseases*, *6*(2), 125-129.

Sulakvelidze, A., Alavidze, Z., & Morris, J. G., Jr. (2001). Bacteriophage therapy. *Antimicrob Agents Chemother*, *45*(3), 649-659. http://dx.doi.org/10.1128/AAC.45.3.649-659.2001

Taj, MK, Ling, JX, Bing, LL, Qi, Z, Taj, I, Hassani, TM, . . . & Yunlin, W. (2014). Effect of dilution, temperature and pH on the lysis activity of T4 phage against *E. coli* bl21. *J. Anim. Plant Sci*, *24*(4), 1252-1255.

Tanji, Y., Shimada, T., Yoichi, M., Miyanaga, K., Hori, K., & Unno, H. (2004). Toward rational control of *Escherichia coli* O157:H7 by a phage cocktail. *Appl Microbiol Biotechnol*, *64*(2), 270-274. http://dx.doi.org/10.1007/s00253-003-1438-9

Um, Maryse Michèle, Brugere, Hubert, Kérourédan, Monique, Oswald, Eric, & Bibbal, Delphine. (2018). Antimicrobial resistance profiles of Enterohemorrhagic and Enteropathogenic *Escherichia coli* of serotypes O157: H7, O26: H11, O103: H2, O111: H8, O145: H28 compared to *Escherichia coli* isolated from the same adult cattle. *Microbial Drug Resistance*, *24*(6), 852-859.

Ye, C., Lan, R., Xia, S., Zhang, J., Sun, Q., Zhang, S., . . . & Xu, J. (2010). Emergence of a new multidrug-resistant serotype X variant in an epidemic clone of *Shigella flexneri*. *J Clin Microbiol*, *48*(2), 419-426. http://dx.doi.org/10.1128/JCM.00614-09