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**Research Article** **Open Access**

# Identification and Characterization of a Bacteriophage with Lytic Activity against Multidrug Resistant *E. coli*

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## ABSTRACT

*Escherichia coli* strains are increasingly becoming resistant to antibiotics and emerging globally. Bacteriophage is re-explored for the development of an alternative and safe agent to control a bacterial infection, especially with multi-drug resistant (MDR) bacteria. Here, we reported the identification of AAA1 phage from a sewage site near Baghdad's Medical City. Morphological analysis using Transmission Electron Microscopy (TEM) suggested that the AAA1 phage had an icosahedral head of width ~ 70 nm and a non-contractile tail of ~ 400 nm belonging to the *Siphoviridae* family from the *Caudovirales* order. The plaque observation showed that plaques size and shape differ after prolonged incubation. The optimal multiplicity of infection was 0.1. The one-step growth curve was assessed and the result showed that AAA1 latent period was 10 minutes; burst size was 93PFU/cell and the burst period of 30 minutes. Based on characteristics, AAA1 phage is a potential candidate to control MDR *E. coli* infection.

**KEYWORDS:** Pathogenic *E. coli*; bacteriophages; phage therapy; sewage.

**الخلاصة**

إن بكتريا الاشيريكيا القولونية هي واحدة من أكثر مسببات الامراض شيوعا وتشكل معظم السلالات مقاومة لأكثر من عائلة من المضادات وهي ذات إنتشار عالمي مما يؤدي الى تقييد الخيارات العلاجية، تعد العاثيات التي تم اعادة الاهتمام بها لتطوير علاج امن ضد الاصابات البكتيرية وخصوصا المقاومة لاكثر من نوع من المضادات. في هذه الدراسة تم عزل عاثي من مياه التصريف الصحي بالقرب من مدينة الطب في بغداد. اظهرت نتائج فحص المجهر الاكتروني الى ان العاثي AAA1 ذو قطر رأس يساوي تقريبا 70 nm وله ذيل طويل غير قابل للتقلص يساوي طوله تقريبا 400 nm إذ أنه ينتمي للعائلة *Siphoviridae* والتي تنتمي للصنف *Caudovirales*. تم ملاحظة التغير بحجم وشكل plaque بعد الحضن لفترة زمنية. تم تحديد MOI المثلى للعاثي وكانت 0.1. تم اجراء تجربة منحنى النمو ذو الخطوة الواحدة واوضحت النتائج بان فترة سكون العاثي هي 10 دقيقة وان حجم الانفجار كان 93 PFU/cell للعاثي AAA1 وان فترة الانفجار هي 30 دقيقة.اعتمادا على النتائج فان العاثي AAA1 يعد مرشح واعد لعلاج بكتريا الاشيريكيا القولونية المقاومة للمضادات.

## INTRODUCTION

*Escherichia coli* has long been known as one of the opportunistic nosocomial pathogens causing the major worldwide threat. *E. coli* colonizes the gut of many animals, including humans' gut as commensals [1]. With the uncontrolled use of antibiotics, the emergence of multidrug-resistant strains increased dramatically [2,3]. Several local studies in Iraq indicated a high prevalence of MDR *E. coli* isolates among Iraqi patients. In one study, 87.5% of *E. coli* was MDR while 75% of *K. pneumonia* was MDR among Iraqi patients [4]. Another study showed high resistant rates to β-lactams by *E. coli* ST131 isolates among Iraqi patients with acute urinary tract infection (UTI) in Waist Province [5]. In Babylon province, results of one study confirmed dissemination of MDR *E. coli* (88.09%) and XDR (11.90%) in patients with UTIs [6]. *E. coli* is considered one of the main reservoirs of antibiotic resistance genes that may contribute to treatment failure and has the ability to pass these genes horizontally to different bacterial species [7]. When bacteria become resistant to three and more antibiotic groups, they become MDR and treatment options available then become limited [8]. The rise in infections severity and treatment failure requires safe and efficient alternatives. Bacteriophages have been characterized in recent years for their antibacterial and anti-biofilm activities [9-12]. In addition, bacteriophages are also used in different fields, such as DNA delivery [13] and bio-control [14]. Bacteriophages are specific in their activity,

unlike antibiotics, not being infective to humans or their normal flora and self-limiting [15]. Following their infection of the bacterial cell, they control the metabolic machinery to synthesize new phage particles [16]. Phage therapy is suggested as an alternative to the eradication or prevention of many bacterial infections including *E. coli, Staphylococcus aureus* and *Klebsiella pneumonia* [17,18]. In a previous study, the virulent phage (AAA1) that infects MDR *E. coli* was isolated from the Tigris River in the center of Baghdad, Iraq, near the sewage lines of the Medical City hospitals. Multiple tests were performed on the AAA1 phage including pH, thermal stability, host range, phage cocktail activity and protein profile [19]. Here we studied the phage morphology and plaque morphology, the optimum multiplicity of infection (MOI), latency period of 10 min and burst size of 93 PFU/host cell. Our findings provide support data and confirmed that AAA1 phage is a promising candidate to perform against pathogenic *E. coli*.

## MATERIALS AND METHODS

### Isolation and identification of host bacterium

The isolation and identification of *E. coli* was performed based on previous study [19]. Briefly, 1 meter away from the edge and 50 cm in depth, a one water sample (50 ml) was collected from Medical City hospitals sewage, Baghdad, IRAQ in 2021 in sterile cup and preserved in ice (4°C). The sample was centrifuged 5000rpm/15 min, 100 µl from the supernatant were culture on MacConkey agar and Eosin methylene blue (EMB) agar and incubated at 37°C for 24 hrs for host isolation. The colonies with distinct morphologies were picked, subjected to biochemical test and preserved [20].

### Isolation and identification of bacteriophage

The centrifuged water sample used above was filtered first thorough 0.45µm and then through 0.22µm membrane filter (Millipore, USA) for phage isolation. Different *E. coli* isolates were tested for host identification and phage isolation. The agar overlay method was used to culture phage with each *E. coli* isolate. Briefly, 100µl of the filter was mixed with 100µl of the host bacterium at log phage (0.5 McFarland) for 10 min at room temperature, then 4 ml from soft agar was added and the mixture was poured on fresh nutrient agar and incubated for 24 hrs at 37°C. The next day, an individual plaque was picked by micropipette tip and the agar overlay method was repeated several times for phage purification. For the host, the VITEK-2 test was applied for phenotypic identification and antibiotics profile and 16S rRNA gene also was applied for genotypic identification [20].

### Phage plaque morphology

The plate that contained uniform plaques obtained from the purification process was used to observe plaque morphology. Briefly, a plaque was picked by micropipette tip and re-suspended in 3 ml Phosphate buffer saline. The titre was determined and the purified phage lysate infected the host bacterium and was incubated for 18, 24, and 48 hrs and the produced plaques shape and size were observed.

### Transmission Electron Microscopy (TEM)

To determine bacteriophage morphology, 10 ml of phosphate buffer saline was added to one plate, left for 2 hrs, collected in a sterile tube and centrifuged for 30 min at 15000 rpm and passed then through 0.22 µm Millipore filter. The supernatant titre was measured by agar overlay and a high titre sample was transferred in a sterile tube and sent in an icebox for Transmission Electron Microscopy (TEM) examination at Basra University- Pharmacy College. The purified phage lysate was negatively stained and examined by TEM. Briefly, sample preparation included a drop of ~$10^{10}$ PFU/ml to the surface of a copper grid (200mesh coated with Formvar film thickness 50nano) negatively stained with 2% uranyl acetate and lead citrate drops, allowed to dry by placing the grid on a filter paper and then examined with ZEISS FESEM Supra 55vp (Germany).

### Determination of optimal multiplicity of infection

The optimal multiplicity of infection (MOI) is expressed as the optimal ratio of the phage particles to the number of host cells required to reach the maximum phage titre [21]. For that, the host bacterium was cultured in nutrient broth and CFU/ml was determined according to CFU/ml = (Number of colonies*dilution factor) / volume plated. The PFU/ml for phages were also calculated according to PFU/ml = (Number of Plaques*dilution factor) / volume plated. The MOI was determined by dividing the number of infectious particles (phage) by the target cell (bacterial cell). The AAA1 phage and host

bacterium were mixed at different MOI: 0.001, 0.01, 0.1, 1 and 10 then agar overlay was used on nutrient agar and incubated for 24 hrs. at 37 ◦C. Later each MOI was calculated as PFU/ml. This experiment was repeated in three independent experiments [22].

### One-step growth curve

The host *E. coli* was grown on nutrient broth at 37°C and when it reached exponential-growth-phase $10^8$ CFU/ml, they were mixed with the AAA1 phage ($10^7$) at optimal MOI of 0.1 for AAA1 phage. The host-phage mixture was incubated for 10 min at 37°C to allow phage adsorption. The mixture was then centrifuged at 14,000 *gx* for 2 min to remove non-adsorbed phages. The precipitate was washed twice with fresh nutrient broth and re-suspended finally in 10 ml, left for 60 min at 37°C. Every 10 min, 100 µl of the host-phage mixture was taken over a period of 60 min in a sterile tube and kept at 4◦C and the phage titre was determined using the double layer agar plate method. The burst size was calculated as the ratio of the final titre of liberated phages to the initial count of infected bacterial cells. All assays were repeated in three independent experiments. The numbers of phages produced at the plateau stage/the original numbers of infected cells was the burst size [22].

## RESULTS AND DISCUSSION

### Host bacterium identification

Water sample obtained from Baghdad Medical city sewage showed different gram-negative bacteria on MacConkey agar and EMB agar as shown in Figure 1. Colonies with green metallic sheen and pink colonies on EMB and MacConky agar were picked for further species identification. The isolates identified as E. coli were mixed with the water filter and the isolate that was lysed by phage were chosen as the host.

The chosen host were subjected for antibiotics profile using VITEK-2 test and the result showed that the host was resistant to Ampicillin, Cefazolin, Cefuroxime Axetil, Cefuroxime, Ceftazidime, Ceftriaxone, Cefepime, Sulfamethoxazole antibiotics according to VITEK-2 report. For the genetic identification, a specific primer for polymerase chain reaction (PCR) amplification according to the presence of the 16S rRNA gene was used. The result of agarose gel electrophoresis showed a band with correct sizes of the PCR products (382bp) Figure 2.

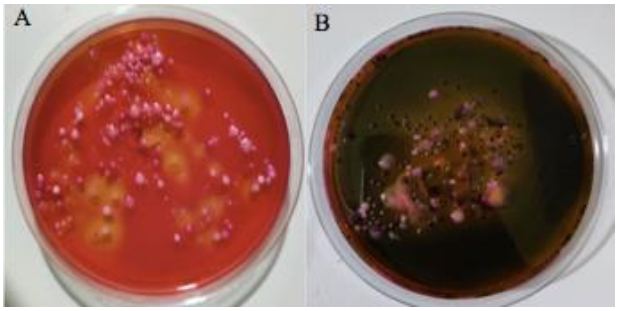


**Figure 1.** Different bacterial colonies on MacConkey and EMB agar. Contamination of sewage water with different gram-negative bacteria, A: bacterial colonies on MacConkey agar; B: bacterial colonies on EMB agar.

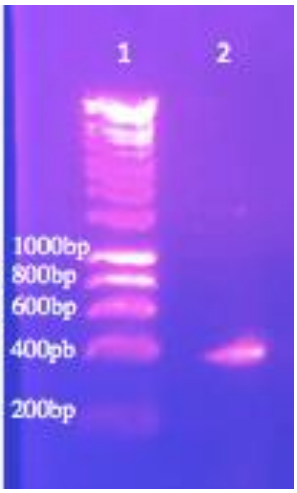


**Figure 2.** Result of 16srRNA gene amplification for host bacterium. Lane 1: Marker (10 kbp), lane 2: host bacterium *E. coli*.

The detection of 16S rRNA gene is a reliable method for detection of bacteria to species or genus level confirmation since this gene is one of the most conserved bacteria genes and rarely affect by horizontal gene transfer (HGT). In addition, this gene is used to study bacterial taxonomy and phylogeny [22,23]. The 16S rRNA PCR is considered a highly sensitive, rapid and accurate method to detect bacteria in clinical and non-clinical samples [24-25].

### Phage plaque morphology

Antibiotic-resistant *E. coli* is one of the biggest problems threatening people around the world and becoming resistant to almost all antibiotics, especially among Iraqi patients. For that, finding a

new safe alternative is necessary and phage therapy is one of the promising alternatives [26-29]. Different phages produce different plaques sizes and shapes that are useful in phage characterization. In this study, we further characterized the AAA1 phage by determining plaque morphology over 48 hrs of incubation and compared it with other phage plaques. The AAA1 phage produced a clear plaque surrounded by a translucent halo after 18 hrs incubation. After 24 hrs incubation, the center and the halo expanded while after 28 hrs the translucent halo progressed in expansion and the clear center became clearer as shown in Figure 3.

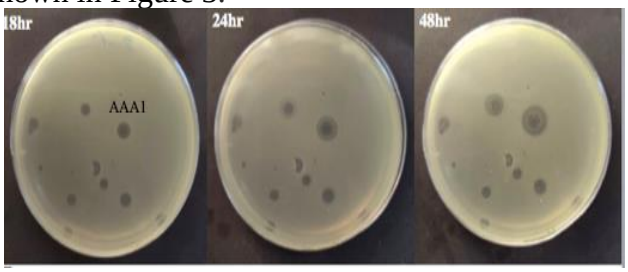


**Figure 3.** The morphology of AAA1 plaques and halos. Different plaques shape is compared with AAA1 plaque shape. The AAA1 plaques were observed at 18,24 and 48 hrs respectively. The halos were expanded after prolonged incubation.

## Transmission Electron Microscopy

The TEM photomicrograph revealed that the AAA1 head is an icosahedral head (~70 nm) and the tail is long, non-contractile, approximately 400 nm, belonging to the *Siphoviridae* family that belongs to the order *Caudovirales* (dsDNA) viruses as described by [30] and shown in Figure 4. Surface water, sewage water and animal faeces are sources for *Siphoviridae* and *Myoviridae* members that could be isolated from as indicated by several studies [31-37].

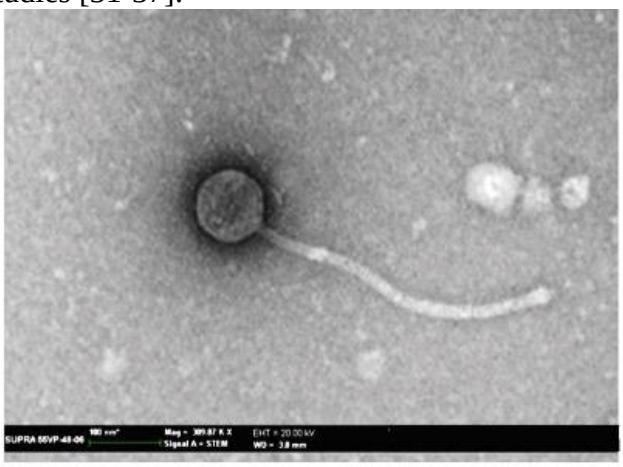

**Figure 4.** Morphology of AAA1 phage under transmission electron microscopy. Icosahedral head and non-contractile tail and the phage have *Siphoviridae* morphology.

## Multiplicity of infection

Phage titre and bacteria at infection determine MOI, which is very important in phage therapy development. MOI is known as the ratio of the number of phages infecting a number of bacterial host cells to reach the maximum phage titer [21]. To evaluate optimal MOI, host *E. coli* was infected with AAA1 phage at different MOI (0.001, 0.01, 0.1, 1, and 10) and the result showed that AAA1 optimal MOI was 0.1 as shown in Figure 5. The results indicate that AAA1 reduced host *E. coli* by 0.1 MOI; therefore, high MOI is not necessary for host lysis. According to Poisson distribution (uninfected, single and multiple infected cells), the number of cells infected will not be directly proportional to the number of phages added. One possibility is that the high titer of phage would block the receptor and thus decrease the rate of bacterial lysis due to an increase in MOI [38]. Another possibility is that the high titer of phage stimulates the immune system of the host as they are considered immunogenic at high titre and inactivated by the host immune system [39,40]. Changing environmental factors may affect MOI effectiveness and thus affect infection efficiency [41].

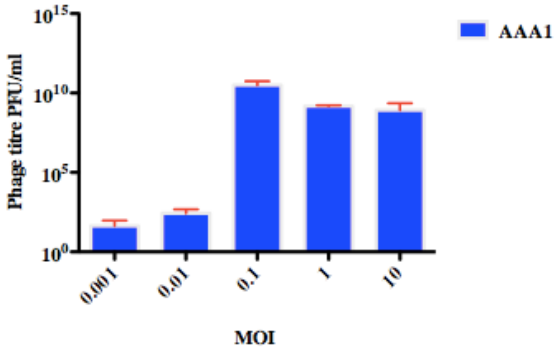


**Figure 5.** The multiplicity of infection (MOI) for AAA1 phage on *E. coli* host.

## One-step growth curve

The one-step growth curve for AAA1 phage was analyzed by infecting the exponential growth of the host *E. coli* with the phage AAA1 at MOI of 0.1. Figure 6 shows that the latent period of phage AAA1 was almost 10 min and the burst size was 93 PFU/cell before entering into the plateau phase, while the burst period was 30 min.

A study in 2018 showed that the latent phase for the phage vB_EcoS_HSE2 that infected

clinical *Escherichia coli* isolates including several MDR *E. coli* was 30 min and 86 particles/infected cell was the burst size depending on the final concentration of the phage and bacteria cell infected by the phage [42].

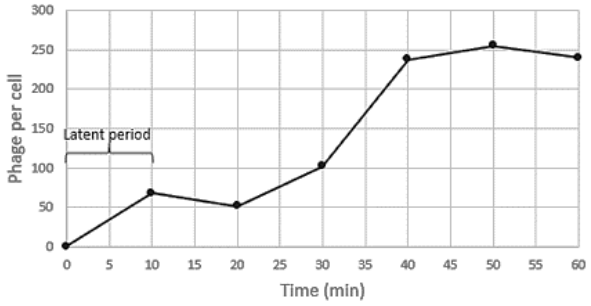


**Figure 6.** One-step growth curve of AAA1 on *E. coli* host phage AAA1 with a burst size of 93 PFU/cell, 10 min latent period and 30 min burst period. AAA1 was mixed with host *E. coli* at MOI 0.1. The titre PFU/ml of each point represents the mean of three independent experiments at different time points.

However, another study showed that the Phage ØCJ19 infecting Enterotoxigenic *Escherichia coli* latent phase was 5 min and less and the burst size was ~ 20 phages/infected cell [43]. A recent study revealed different latent phases 10 and 30 min respectively for two newly isolated phages infecting *E. coli* isolates and the average burst size was 112 and 124 PFU/infected cell respectively [22]. The density of the phage and host bacteria affects the adsorption rate [44]. It has been hypothesized that single phages can grow rapidly by decreasing the latent period even with a small burst size [45]. Incubation temperature, media composition and bacterial growth rate are factors that may affect latent period and phage burst size. For antibacterial agents, a large burst size is preferred because in a relatively short time several hundred folds of the phage would increase [46]. Therefore, short latent phase and large burst size are preferred traits. In the last decades bacterial infections have become one of the most biggest threats to human health and food security globally [47,48]. Several studies indicated a rapid and uncontrolled spread of antibiotic resistant bacteria among Iraqi patients [49–51]. Local studies identified the contamination of Iraqi rivers water with antibiotic-resistant bacteria [52]. To overcome antimicrobial resistant problems, bacteriophages are a promising alternative. On the other hand, bacteria have the ability to reduce phage therapy effectiveness by evolving resistance to bacteriophages with multiple mechanisms such as modifying their cell surface including receptor masking or mutation, blocking the entry of phage DNA, restriction or modification of the phage's nucleic acid or abortive infection system and the clustered regularly interspaced palindromic repeats (CRISPER) [53-58]. One of the best solutions remains nature as the only source that novel phage can be obtained from. Another promising approach is directed evolution, when phage specificity and improved qualities are engineered.

## CONCLUSIONS

The AAA1 novel phage has the potential to be used as a successful alternative antimicrobial agent to control MDR *E. coli*. The AAA1 belonged to the *Siphoviridae* family under electron microscopy examination with optimal MOI of 0.1 and with 10 min latent period and burst size of 93 PFU/ml. Further evaluation is required to identify AAA1 phage genotypically by carrying out genome sequencing to confirm the identity of the phages and role out the presence of undesirable elements such as bacterial toxins, lysogenic components, antibiotic resistance or virulent-related genes. Further study of the co-evolution of phages interactions between the phage and their host.

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**Disclosure and conflict of interest:** The authors declare that they have no conflicts of interest.

## REFERENCES

[1] J. Jang, H.-G. Hur, M. J. Sadowsky, M. N. Byappanahalli, T. Yan, and S. Ishii, "Environmental Escherichia coli : ecology and public health implications-a review," J. Appl. Microbiol., vol. 123, no. 3, pp. 570-581, Sep. 2017. https://doi.org/10.1111/jam.13468

[2] V. M. Vaughn, A. L. Hersh, and E. S. Spivak, "Antibiotic Overuse and Stewardship at Hospital Discharge: The Reducing Overuse of Antibiotics at Discharge Home Framework," Clin Infect Dis, vol. 3;74(9):1696-702, May 2022. https://doi.org/10.1093/cid/ciab842

[3] A. Ousaid, J. Akrim, and Y. Khayati, "Overuse of antibiotics as a key driver to antibiotic resistance in Morocco: A short review with potential solutions," Int

Arab J Antimicrob Agents Internet, 2020, [Online]. Available: http://imed.pub/ojs/index.php/IAJAA/article/view/2368
https://doi.org/10.3823/843

[4] A. H. Pishtiwan and K. M. Khadija, "Prevalence of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing Klebsiella pneumoniae and Escherichia coli isolated from thalassemia patients in Erbil, Iraq," Mediterr J Hematol Infect Dis, vol. 11, 2019.
https://doi.org/10.4084/mjhid.2019.041

[5] D. R. Al-Guranie and S. M. Al-Mayahie, "Prevalence of E. coli ST131 among Uropathogenic E. coli isolates from Iraqi patients in Wasit Province, Iraq," Int J Microbiol, 2020.
https://doi.org/10.1155/2020/8840561

[6] H. H. Al-Hasnawy, M. R. Judi, and H. J. Hamza, "The dissemination of multidrug resistance (MDR) and extensively drug resistant (XDR) among uropathogenic E. coli (UPEC) isolates from urinary tract infection patients in babylon province, Iraq," Baghdad Sci J, vol. 16, pp. 986-992, 2019.
https://doi.org/10.21123/bsj.2019.16.4(Suppl.).0986

[7] L. Poirel et al., "Antimicrobial resistance in Escherichia coli," Microbiol Spectr, vol. 6, pp. 6-4, 2018.
https://doi.org/10.1128/microbiolspec.ARBA-0026-2017

[8] S. Saputra et al., "Antimicrobial resistance in clinical Escherichia coli isolated from companion animals in Australia," Vet Microbiol, vol. 211, pp. 43-50, 2017.
https://doi.org/10.1016/j.vetmic.2017.09.014

[9] B. Fathima and A. C. Archer, "Bacteriophage therapy: recent developments and applications of a renaissant weapon," Res Microbiol, vol. 172, no. 6, 2021.
https://doi.org/10.1016/j.resmic.2021.103863

[10] P. Wintachai and S. P. Voravuthikunchai, "Characterization of Novel Lytic Myoviridae Phage Infecting Multidrug-Resistant Acinetobacter baumannii and Synergistic Antimicrobial Efficacy between Phage and Sacha Inchi Oil," Pharmaceuticals, vol. 15, no. 3, 2022.
https://doi.org/10.3390/ph15030291

[11] K. Hon, S. Liu, S. Camens, G. S. Bouras, A. J. Psaltis, and P.-J. Wormald, "APTC-EC-2A: A Lytic Phage Targeting Multidrug Resistant E. coli Planktonic Cells and Biofilms," Microorganisms, vol. 10, no. 1, 2022.
https://doi.org/10.3390/microorganisms10010102

[12] J. Vera-Mansilla, P. Sánchez, C. A. Silva-Valenzuela, and R. C. Molina-Quiroz, "Isolation and Characterization of Novel Lytic Phages Infecting Multidrug-Resistant Escherichia coli," Microbiol Spectr, vol. 10, no. 1, 2022.
https://doi.org/10.1128/spectrum.01678-21

[13] K. Namdee, M. Khongkow, S. Boonrungsiman, N. Nittayasut, P. Asavarut, and S. Temisak, "Thermoresponsive Bacteriophage Nanocarrier as a Gene Delivery Vector Targeted to the Gastrointestinal Tract. Mol Ther - Nucleic Acids," vol. 12. pp. 33-44, Sep. 2018.
https://doi.org/10.1016/j.omtn.2018.04.012

[14] Z. D. Moye, J. Woolston, and A. Sulakvelidze, "Bacteriophage Applications for Food Production and Processing," Viruses, vol. Apr;10(4):205, 2018.
https://doi.org/10.3390/v10040205

[15] S. Royer, A. P. Morais, and D. W. Fonseca Batistão, "Phage therapy as strategy to face post-antibiotic era: a guide to beginners and experts," Arch Microbiol, vol. May; 203(4):1271-9, 2021.
https://doi.org/10.1007/s00203-020-02167-5

[16] C. Sieiro, L. Areal-Hermida, Á. Pichardo-Gallardo, R. Almuiña-González, T. Miguel, and S. Sánchez, "A hundred years of bacteriophages: Can phages replace antibiotics in agriculture and aquaculture?," Antibiotics, vol. 9, no. 8, 2020.
https://doi.org/10.3390/antibiotics9080493

[17] S. Aslam and R. T. Schooley, "What's Old Is New Again: Bacteriophage Therapy in the 21st Century," Antimicrob Agents Chemother, Dec. 2019.
https://doi.org/10.1128/AAC.01987-19

[18] V. Fanaei, M. Validi, B. Zamanzad, and A. Karimi, "Isolation and identification of specific bacteriophages against methicillin-resistant Staphylococcus aureus, extended-spectrum beta-lactamases-producing Escherichia coli, extended-spectrum beta-lactamases-producing Klebsiella pneumoniae, and multidrug-resistant Acinetobacter baumanniiin vitro," FEMS Microbiol Lett, vol. 1;368(19):fnab139, Oct. 2021.
https://doi.org/10.1093/femsle/fnab139

[19] A. A. Abdulhussein and B. O. Abdulsattar, "Isolation and characterization of two novel phages as a possible therapeutic alternative against multi-drug resistant E. coli," Gene Rep., vol. 28, p. 101644, Sep. 2022.
https://doi.org/10.1016/j.genrep.2022.101644

[20] B. O. Abdulsattar, N. A. Al-Saryi, M. H. Abbas, and A.-R. A. Abdulhussein, "Isolation and purification of Escherichia coli bacteriophage from Tigris river, Baghdad, Iraq," Gene Rep, vol. 19, p. 100591, 2020.
https://doi.org/10.1016/j.genrep.2020.100591

[21] C. Wei, J. Liu, A. N. Maina, F. B. Mwaura, J. Yu, and C. Yan, "Developing a bacteriophage cocktail for biocontrol of potato bacterial wilt," Virol Sin, vol. 32, no. 6, pp. 476-84, 2017.
https://doi.org/10.1007/s12250-017-3987-6

[22] P. Fu, Q. Zhao, L. Shi, Q. Xiong, Z. Ren, and H. Xu, "Identification and characterization of two bacteriophages with lytic activity against multidrug-resistant Escherichia coli," Virus Res, vol. 291, no. 198196, 2021.
https://doi.org/10.1016/j.virusres.2020.198196

[23] R. Urwin and M. C. Maiden, "Multi-locus sequence typing: a tool for global epidemiology," Trends Microbiol, vol. 11, pp. 479-487, 2003.
https://doi.org/10.1016/j.tim.2003.08.006

[24] W. G. Weisburg, S. M. Barns, D. A. Pelletier, and D. J. Lane, "16S ribosomal DNA amplification for phylogenetic study," J Bacteriol, vol. 173, pp. 697-703,

1991.
https://doi.org/10.1128/jb.173.2.697-703.1991

[25] M. I. El-Amir, M. A. El-Feky, D. A. A. Elwafa, and E. A. Abd-Elmawgood, "Rapid diagnosis of neonatal sepsis by PCR for detection of 16S rRNA gene, while blood culture and PCR results were similar in E. coli-predominant EOS cases," Infect Drug Resist, vol. 12, p. 2703, 2019.
https://doi.org/10.2147/IDR.S213958

[26] M. Allami, M. Bahreini, and M. R. Sharifmoghadam, "Antibiotic resistance, phylogenetic typing, and virulence genes profile analysis of uropathogenic Escherichia coli isolated from patients in southern Iraq," J Appl Genet, vol. 63, no. 2, pp. 401-12, 2022.
https://doi.org/10.1007/s13353-022-00683-2

[27] G. A.-M. SM, A.-G. DRT, H. AA, and B. ZA, "Prevalence of common carbapenemase genes and multidrug resistance among uropathogenic Escherichia coli phylogroup B2 isolates from outpatients in Wasit Province/Iraq," Plos One, vol. 17, no. 1, 2022.
https://doi.org/10.1371/journal.pone.0262984

[28] R. N. Hasan, S. A. Jasim, and Y. H. Ali, "Detection of fimH, kpsMTII, hlyA, and traT genes in Escherichia coli isolated from Iraqi patients with cystitis," Gene Rep, vol. 26, no. 101468, 2022.
https://doi.org/10.1016/j.genrep.2021.101468

[29] R. K. Mohammed and A. A. Ibrahim, "Distribution of dfrA1 and cat1 antibiotic resistance genes in uropathogenic Escherichia coli isolated from teens pregnant women in Iraq," Iraqi J Sci, vol. 3340-53, 2022.
https://doi.org/10.24996/ijs.2022.63.8.9

[30] H.-W. Ackermann, "5500 Phages examined in the electron microscope," Arch Virol, vol. 152, pp. 227-243, 2007.
https://doi.org/10.1007/s00705-006-0849-1

[31] F. Abdelrahman, N. Rezk, M. S. Fayez, M. Abdelmoteleb, R. Atteya, and M. Elhadidy, "Isolation, Characterization, and Genomic Analysis of Three Novel *E. coli* Bacteriophages That Effectively Infect E. coli O18," Microorganisms, vol. 10, no. 3, 2022.
https://doi.org/10.3390/microorganisms10030589

[32] E. W. Bumunang, T. A. McAllister, K. Stanford, H. Anany, Y. D. Niu, and C. N. Ateba, "Characterization of Non-O157 STEC infecting bacteriophages isolated from cattle faeces in North-West South Africa," Microorganisms, vol. 7, no. 12, 2019.
https://doi.org/10.3390/microorganisms7120615

[33] N. Chaudhary, C. Narayan, B. Mohan, and N. Taneja, "Characterization and in vitro activity of a lytic phage RDN37 isolated from community sewage water active against MDR Uropathogenic E. coli," Indian J Med Microbiol, vol. 39, no. 3, pp. 343-8, 2021.
https://doi.org/10.1016/j.ijmmb.2021.04.011

[34] M. M. Esmat, A. G. Abdelhamid, S. A. Abo-ELmaaty, M. A. Nasr-Eldin, M. G. Hassan, and A. A. Khattab, "Antibiotics and phage sensitivity as interventions for controlling Escherichia coli isolated from clinical specimens," J Pure Appl Microbiol, vol. 11, no. 4, pp. 1749-55, 2017.
https://doi.org/10.22207/JPAM.11.4.13

[35] Y.-K. Li, H. Chen, M. Shu, C. Zhong, Y. Bi, and H.-H. Yang, "Isolation, characterization and application of an alkaline resistant virulent bacteriophage JN01 against Escherichia coli O157: H7 in milk and beef," LWT, vol. 144, no. 111266, 2021.
https://doi.org/10.1016/j.lwt.2021.111266

[36] M. Raj JR, V. R, H. P, B. U, and K. I, "T4-like Escherichia coli phages from the environment carry blaCTX-M," Lett Appl Microbiol, vol. 67, no. 1, pp. 9-14, 2018.
https://doi.org/10.1111/lam.12994

[37] M. A. Nasr-Eldin, S. A. EL-Maaty, K. A. EL-Dougdoug, M. M. Hazaa, and A. H. Abdel-mageed, "Characterization and development of a phage cocktail for Escherichia coli causing gastrointestinal diseases," J Bas Env Sci, vol. 5, pp. 115-22, 2011.

[38] A. Vieira, Y. J. Silva, A. Cunha, N. C. M. Gomes, H.-W. Ackermann, and A. Almeida, "Phage therapy to control multidrug-resistant Pseudomonas aeruginosa skin infections: in vitro and ex vivo experiments," Eur J Clin Microbiol Infect Dis, vol. 31, pp. 3241-3249, 2012.
https://doi.org/10.1007/s10096-012-1691-x

[39] C. Kocharunchitt, T. Ross, and D. L. McNeil, "Use of bacteriophages as biocontrol agents to control Salmonella associated with seed sprouts," Int J Food Microbiol, vol. 128, pp. 453-459, 2009.
https://doi.org/10.1016/j.ijfoodmicro.2008.10.014

[40] J. Majewska et al., "Oral application of T4 phage induces weak antibody production in the gut and in the blood," Viruses, vol. 7, pp. 4783-4799, 2015.
https://doi.org/10.3390/v7082845

[41] S. T. Abedon, "Phage therapy dosing: The problem (s) with multiplicity of infection (MOI," Bacteriophage, vol. 6, p. 1220348, 2016.
https://doi.org/10.1080/21597081.2016.1220348

[42] Q. Peng and Y. Yuan, "Characterization of a newly isolated phage infecting pathogenic Escherichia coli and analysis of its mosaic structural genes," Sci Rep, vol. 8, pp. 1-10, 2018.
https://doi.org/10.1038/s41598-018-26004-4

[43] G.-H. Kim, J.-W. Kim, J. Kim, J. P. Chae, J.-S. Lee, and S.-S. Yoon, "Genetic analysis and characterization of a bacteriophage ØCJ19 active against enterotoxigenic Escherichia coli," Food Sci Anim Resour, vol. 40, p. 746, 2020.
https://doi.org/10.5851/kosfa.2020.e49

[44] S. T. Abedon, P. Hyman, and C. Thomas, "Experimental examination of bacteriophage latent-period evolution as a response to bacterial availability," Appl Env. Microbiol, vol. 69, no. 12, pp. 7499-506, 2003.
https://doi.org/10.1128/AEM.69.12.7499-7506.2003

[45] S. T. Abedon, T. D. Herschler, and D. Stopar, "Bacteriophage latent-period evolution as a response to resource availability," Appl Env. Microbiol, vol. 67, no. 9, pp. 4233-41, 2001. https://doi.org/10.1128/AEM.67.9.4233-4241.2001

[46] A. S. Nilsson, "Phage therapy-constraints and possibilities," J Med Sci, vol. 119, no. 2, pp. 192-8, 2014. https://doi.org/10.3109/03009734.2014.902878

[47] A. J. Browne, M. G. Chipeta, G. Haines-Woodhouse, E. P. Kumaran, B. H. K. Hamadani, and S. Zaraa, "Global antibiotic consumption and usage in humans, 2000-18: a spatial modelling study," Lancet Planet Health, vol. 5, no. 12, 2021. https://doi.org/10.1016/S2542-5196(21)00280-1

[48] E. Y. Klein et al., "Global increase and geographic convergence in antibiotic consumption between 2000 and 2015," Proc Natl Acad Sci, vol. 115, pp. 3463-3470, 2018. https://doi.org/10.1073/pnas.1717295115

[49] M. Alzaidi JR and A.S., "First Record of Dissemination of BLBLI-Resistant from Public Hospitals in Baghdad, Iraq," Open Microbiol J, vol. 16, no. 1, 2022. https://doi.org/10.2174/18742858-v16-e2201310

[50] M. H. G. Kanaan and H. T. Khashan, "Molecular typing, virulence traits and risk factors of pandrug-resistant Acinetobacter baumannii spread in intensive care unit centers of Baghdad city, Iraq," Rev Med Microbiol, vol. 33, no. 1, pp. 51-5, 2022. https://doi.org/10.1097/MRM.0000000000000282

[51] H. A. Salman, S. M. S. Alsallameh, G. Muhamad, and Z. Taha, "Prevalence of Multi-Antibiotic Resistant Bacteria Isolated from Children with Urinary Tract Infection from Baghdad, Iraq," Microbiol Biotechnol Lett, vol. 50, no. 1, pp. 147-56, 2022. https://doi.org/10.48022/mbl.2110.10011

[52] F. M. Abbas, "Genetic characterization of OXA-1 β-lactamase producing carbapenem-resistant Klebsiella pneumoniae from Al-Hillah river water, Babylon Province, Iraq," J Genet Env. Resour Conserv, vol. 9, no. 1, pp. 197-201, 2021.

[53] R. Barrangou, C. Fremaux, H. Deveau, M. Richards, P. Boyaval, and S. Moineau, "CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes," Science, Mar. 2007. https://doi.org/10.1126/science.1138140

[54] T. Goldfarb, H. Sberro, E. Weinstock, O. Cohen, S. Doron, and Y. Charpak-Amikam, "BREX is a novel phage resistance system widespread in microbial genomes," EMBO J, Jan. 2015. https://doi.org/10.15252/embj.201489455

[55] P. Hyman and S. T. Abedon, "Bacteriophage Host Range and Bacterial Resistance," in Advances in Applied Microbiology, Elsevier, 2010, pp. 217-48. https://doi.org/10.1016/S0065-2164(10)70007-1

[56] S. J. Labrie, J. E. Samson, and S. Moineau, "Bacteriophage resistance mechanisms," Nat Rev Microbiol, May 2010. https://doi.org/10.1038/nrmicro2315

[57] C. Richter, J. T. Chang, and P. C. Fineran, "Function and Regulation of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) / CRISPR Associated (Cas) Systems," Viruses, Oct. 2012. https://doi.org/10.3390/v4102291

[58] I. Yosef, M. Manor, R. Kiro, and U. Qimron, "Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria," Proc Natl Acad Sci, Jun. 2015. https://doi.org/10.1073/pnas.1500107112

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