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Review Article

Resistant urinary tract infection during post antibiotic era - Is it time for revival of phage therapy?

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Abstract

Treatment of antibiotic resistant uropathogenic *Escherichia coli* (UPEC) infection is currently a major challenge in the field of antimicrobial therapeutics. In addition, the evolution of Extended Spectrum Beta Lactamase (ESBL) and bio-film producing UPEC pose further hurdles in treating recurrent urinary tract infection (UTI) due to UPEC. The last decade witnessed emergence of various alternative treatment measures to tide over this crisis, including non-antibiotic agents and vaccines. However, in terms of efficacy, none of these agents fulfil our needs adequately. It appears that we are slowly and surely entering into the post-antibiotic era. This has prompted researchers to turn their attention to re-discover bacteriophage therapy as an alternative for treatment of resistant uropathogens. Phage Bacteriophages (phages) are tiny viruses, which are highly specific to their biological hosts and have been known to the researchers for more than two decades. Despite its demonstrated utility long ago, phage therapy was not in the lime light due to various reasons. These tiny members of the extensive microbiome in the human body are now being reconsidered as alternatives to antibiotics in resistant situations. The phages possess the potential of being used as natural phages (or as cocktail), genetically engineered phages, phage lytic enzymes or phage antibiotic synergy. In this paper, we attempt to review the current status of phage therapy in UPEC and assess whether it is time to incorporate it into treatment practices during this post antibiotic era with widespread chemical therapeutic resistance.

Keywords: urinary tract infection (UTI), uropathogenic *Escherichia coli*, bacteriophages, phage therapy, resistant UTI, lysins

1 Introduction

Urinary Tract Infection (UTI) is considered as the most common bacterial infection especially in primary care hospitals [1]. The occurrence of UTI is higher in females due to inherent anatomical and physiological reasons. The estimated incidence of UTI in school-aged girls is 1%, 4% in women of childbearing age and 15% in elderly population. UTI also accounts for 6.2% of deaths over the age of 65 years [2-4]. It is well known that UTI is caused mainly by *Escherichia coli* (*E. coli*) bacterial strains in over 80% of cases. Though *E. Coli* is regarded as a harmless commensal bacteria occurring in the human intestine, it becomes pathogenic in immunocompromised individuals and by undergoing genetic changes. Extra intestinal *E. coli* (Uropathogenic *E. coli*, UPEC) is more pathogenic compared to Intestinal Pathogenic *E. coli* (InPEC). Recurrent UTI, defined as at least 3 episodes of UTI within 12 months or at least 2 episodes within 6 months is found to occur in a staggering 20-30% of women with UTI [5]. Recurrent UTI can be a relapse caused by the same microorganism after adequate treatment or a reinfection caused by a different microorganism or by the same organism after complete treatment, with subsequent negative urine culture.

1.1 Rationale for review

Low dose antibiotic prophylaxis has been widely practised for the treatment of recurrent UTI. However there has been a significant upsurge in UTI cases recently which are due to organisms showing resistance to several antibiotics. WHO has included *E. coli* belonging to Enterobacteriaceae family in the critical category of resistant pathogens needing advanced antimicrobial agents [6]. The emergence of antibiotic resistant strains of *E. coli*, Extended Spectrum Beta Lactamase (ESBL) producing strains and those which produce biofilms further add to the hurdles in managing pathogenic *E. coli* infections. The burden of antimicrobial resistance (AMR) is alarmingly increasing worldwide and it is estimated that 10 million people will die every year due to AMR by 2050 unless the problem of AMR is addressed by a collective global response [7]. Though many therapeutic measures including non-antibacterial agents and vaccines have been researched and popularised for the treatment of recurrent UTI, none of these have established themselves as fool-proof methods as of now [8]. This has

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renewed the interest among researchers for exploring the use of bacteriophages as potential alternative for treatment of recurrent and resistant UTI. Bacteriophages (phages, bacterial viruses) are tiny forms of highly heterogenous viruses having DNA/RNA as their genetic material. The phages specifically recognise receptors present on the bacteria, enter into bacterial host and release their genetic material. This results in host cell lysis by overtaking the replication process of bacteria and releasing the progeny of lytic phages. In some instances, the phage genetic material can get integrated into the bacterial genome resulting in the formation of Temperate Phages which ultimately control the bacterial infection [9, 10].

The identification of bacteriophages as bacterial eaters has been credited to Frederick Twort, a bacteriologist in London in 1915 and Felix d' Herelle, A French-Canadian microbiologist in Paris in 1917 [11, 12]. However the widespread use of bacteriophages to treat bacterial infections took a backseat except in former Soviet Union Countries like Georgia and Russia. The lack of interest in phage therapy was partly due to the emergence of popular and lucrative antibiotics and other therapeutic molecules. However, the irrational use of antibiotics, poor regulations and surveillance, unhealthy clinical practice and lack of new therapies contributed to antibiotic resistance seen in the present time [14, 15]. Surprisingly, it is now identified that there is significant bacterial resistance among Enterobacteriaceae family even to the most recent of available antimicrobials, namely carbapenams [13]. The emergence of Multi Drug Resistance (MDR), Extensively Drug Resistant (XDR) and Pan-Drug Resistant (PDR) strains of bacteria has raised the alarm for scientists to renew their interest in the use of alternate therapeutic molecules against resistant bacteria. The failure of non-antibacterial measures which emerged with hype during the last decade to successfully treat resistant bacterial infection, has now forced scientists to redirect research towards using bacteriophages and lysins as possible alternatives, with potential benefits towards treatment of MDR and XDR *E. coli* strains [16-18]. The present review is an effort to compile the latest information on the subject of bacteriophage therapy and to assess whether the present situation demands an immediate revival of this novel therapeutic approach using this technique in recurrent and resistant UTI.

2 Methods

This was a systematic review of articles published in Pubmed and Google Scholar on Phage Therapy for various bacterial infections during the period 2010 to 2021 obtained through Google Search Engine.

2.1 Results of Search

Out of the 143 articles, excluding those which had repetitions, 49 articles were included in the final analysis, which were relevant to the subject of discussion. The data available in these articles were reviewed and presented in this paper.

3 Discussion

3.1 Types of phage therapy

There are four types of phage therapy useful for UTI. These include the use of Natural Phage Cocktails, Genetically Engineered Phages, Phage Lytic Enzymes/Proteins in natural and engineered forms and Phages Synergestic with Antibiotics.

3.2 Natural phage cocktail therapy

The use of phage therapy can be either with a single phage type (monophage therapy) or with more than two phage types (polyphage or cocktail therapy). Polyphage therapy helps to increase the host range since the phages in the cocktail have different host specificities and thereby overcome phage resistance caused by receptor mutation [19]. The phage receptors of *E. coli* strains can be present either on the surface or on the pili [20, 21]. Nishikawa et al. had proved the efficacy of a cocktail made of T4 phage and newly isolated KEP10 phages obtained from sewage water on lysis of MDR *E. coli* in mouse [22]. Sillankorva et al. used a cocktail containing 3 phages (T4, T1 and Phi X 174 like phages) for effective lysis of UPEC [23].

Phage cocktails could be effective in treating infections by uropathogens producing biofilm where antibiotics are less effective due to lowered metabolic activity of bacteria in the biofilms [24]. This was confirmed by Chibeu et al. who found out that polyphage therapy could be effective in more than 80% of a subset of biofilm forming UPEC strains in his study [25]. Subsequent studies by several other researchers have also confirmed that a cocktail of lytic phages developed by them could effectively delay the formation of phage resistant bacterial cells [26-28]. In yet another study conducted by Sybesma et al., it was observed that the lytic activity of Pyobacterophage cocktail was 66% and Enkobacterophage cocktail was 93% on *E. coli* isolated from the urine of patients suffering from UTI [29]. The phage cocktails were obtained from commercially available preparations in Georgia or from phage collection of the George Eliava Institute of Bacteriophage. However, it is interesting to note that the lytic activity was less marked for the cocktail when used against *Klebsiella pneumonia* strains of bacteria. Single type of phages that identify more than one bacterial receptor has also been isolated by some researchers which possess potential advantages over single receptor phages [30, 31].

Combining phage cocktails with antibiotics/antiseptics or polyscaccharide degrading enzyme, depolymerase has also been reported to be a novel approach for treatment of biofilm forming *E. coli* residing on urinary catheters [32].

3.3 Genetically engineered phages

Narrow host range, emergence of phage resistant bacterial strains, technical difficulties in procuring regulatory approval, problems of manufacturing and deleterious effects of bacterial lysins on host immunogenicity all pose potential limitations for the use of natural phages in hospital settings. To address these issues, researchers are exploring the feasibility of genetically modified or engineered phages for treatment of uropathogens [33, 34]. Techniques for synthesising genetically modified phages include homologous recombination between phage and bacterial plasmid DNA, phage recombineering of electroporated DNA, *in vivo* recombineering, CRISPR-CaS (Clustered Regulatory Interspaced Short Palindromic Repeats-CaS) Mediated genome engineering and various other methods [35]. Such modified bacteriophages show greater efficacy in reducing bacterial biofilms compared to natural lytic phages.

3.4 Phage lytic proteins

With advancement of genomics, phage lytic proteins or enzymes extracted from phages have been explored as agents for control of resistant uropathogens. The phages produce two types of cell wall lytic proteins or enzymes. i. Endolysins produced internally by phages during the late states of infection causing lysis of bacteria from within [36-38]; ii. Virion associated lysins or peptidoglycan hydrolases which cause lysis of bacterial cells from without when they attach to the bacterial surface [37-41]. Though the application of phage lytic proteins was earlier restricted to only gram positive bacteria, some researchers later reported that gram negative bacterial cells having outer membrane (OM) could also be lysed by using membrane disrupting peptides or chelating agents like ethylene diamine tetra acetic acid (EDTA) in combination with phage lytic proteins [38, 42]. There are also studies using endolysins fused with Outer Membrane Permiabilizers (OMP) effective against gram negative bacteria including *E. coli*, *Klebsiella* and *Acinetobacter* strains [43, 44].

There are various techniques to facilitate endolysin-OM penetration in gram negative uropathogens. The first strategy involves coupling of endolysins with compounds that target them to specific receptors present on OM of gram-negative bacteria [45,46,47]. The other strategy involves the use of artilysins wherein the endolysins are engineered to fuse with OMPs or peptides having the potential to distort the outer leaflet of OM [48]. An artilysin named Art-175 was made by Briers et al which could kill *Pseudomonas aeruginosa* strains which is now extrapolated for the lysis of colistin resistant *E. coli* strains also [49, 50].

3.5 Phage antibiotic synergy (PAS)

PAS is a process in which sub-inhibitory concentration of an antibiotic agent, stimulates the production of virulent phages by host bacteria [51]. When the biomass of host bacteria increases, the biosynthetic capacity of bacterial cell also increases resulting in an increase in the number of virulent phages. Such phages spread at much faster rate when the host cells lyse due to antibiotics [52]. Elongated or filamentous cells make the phage receptors more accessible to phages causing cell lysis [53]. A sub-inhibitory concentration of stress inducer like an antibiotic agent, initially causes a low concentration of phages, delaying the bacterial lysis. This in turn leads to increased phage production in the bacterial host. This mechanism was first explained by Kim et al. which has been found out to provide better long-term benefits including avoidance of emergence of antibiotic resistant bacterial strains [54]. Though *in vitro* and *in vivo* studies of PAS have been carried out using various animal models and some human cell lines, this therapy is yet to be available specifically for UPEC [55].

3.6 The status of phage therapy in current clinical practice scenario

Though current status of phage therapy through all the four different techniques seems to be highly promising in the treatment of recurrent and resistant UTI, not many human trials have reached beyond phase 2. Ujmajuridze et al. have shown positive results with no adverse effects in using bacteriophage named Pyo for the treatment of clinical UTI [56]. Pyophage cocktails have also been effectively utilized in certain other clinical trials [57]. Oral *E. coli* phage cocktail has been used in a study conducted in Bangladesh for the treatment of diarrhoea caused by InPEC with minor efficacy [58]. A clinical phase 2 trial of phagoburn designed for treatment of burn wounds caused by *E. coli* and *Pseudomonas aeruginosa* by the same researchers also didn't find satisfactory endpoint. Gutierrez et al. reported no harmful effects upon intravenous injection of endolysin SAL 200 in the treatment of *Staphylococcus aureus* in a phase 1 trial [59]. However, another clinical trial using lytic protein P128 was unsuccessful in treatment of nasal strains of *Staphylococcus aureus*. Successful treatment with Compassionate phage therapy (CPT) in patients on whom antibiotic has failed has also been recently reported and holds promise in future [60, 61, 62].

Though the results of animal studies correspond well with those of *in vitro* studies, the use of phage therapy as alternative to chemical-based treatment of human bacterial infections will require much greater impetus in future demanding further research and development [63]. Further studies are also required to investigate the dose, ideal route of administration, frequency and duration of phage therapy in the treatment of clinical UTI [64]. There has been development of some novel approaches employed in phage therapy recently. An approach named Magistral phage has been introduced in Belgium where customised phages would be prepared in the laboratory as per doctor's prescription [61]. In the USA, the host phage therapy centre has been established which reported remarkable success in the treatment of several patients using phage therapy [60]. How these new models could be adopted and implemented by other countries crippling under Covid-19 pandemic is a million-dollar question to be answered.

A critical analysis of 37 articles on or related to the use of lytic phages as antibacterial agents and the current state of

phage therapy implementation is available in an article by Abedon et al [65]. It is interesting to note that many bacteriophages exist as members of the microbiota in lower urinary tract [66]. These phages, while modulating the composition of the commensal microbiota in healthy status, persist throughout the course of dominant infections in the individuals. It is prudent that phage therapy should ideally cause minimal or no disturbance to this commensal bacterial community and should probably be directed very narrowly towards a specific pathogen in the community. Therefore, it is important to understand the relevance and role of phages within the urinary tract when adopting phage therapy against UPEC infection [67].

It is discerning to note that in the middle of SARS-CoV-2 pandemic, MDR bacterial infections are emerging with increased frequency, possibly due to widespread prophylactic administration of antibiotics in ICU patients against secondary bacterial infection. This is expected to cause significant global public health concerns in future that could lead to heavy human and economic losses. Therefore, there is an urgent need to seek alternative treatment against MDR bacteria within various human systems in the immediate future. Bacteriophage therapy could be a potential new tool in this scenario as well [68, 69].

4 Conclusion

Bacteriophage therapy is evolving as a very promising alternative to antibiotics in the treatment of recurrent and resistant UPEC. In it, the scientists have found that the old proverb “The enemy of my enemy is my friend” fits their context beautifully and it is time to fill new bottles meant for antibiotics with the old wine called the phages. Though the research in this field has shown promise, there is no singular proved approach for satisfactory clinical use of this modality of treatment. However, the diversity and adaptability of phage therapy will soon pave the way for an effective treatment of recurrent and resistant UPEC in the near future. Majority of data on phage therapy currently is obtained from *in vitro* studies and studies on lab animals, with a major limitation in the lack of appropriate clinical research on human volunteers. There is an urgent need to speed up the availability of phage therapy in resistant UPEC in clinical practice overcoming regulatory and legal hurdles, before the bacteria become smarter than humans.

As an epilogue, researchers should also keep in mind the possibility of bacteria adopting phage resistance and therefore should identify other strategies to overcome this inevitability too, while the research on phage therapy is in progress.

References

1. Hotchandani R, Aggarwal KK. Urinary tract infections in women. Indian J Clin Pract. 2012;23:187-192.
2. Pushpalatha KS. Urinary tract infection and management . J Nighting Nursing Times. 2008;4:28-32.
3. Detweiler K, Mayers D, Fletcher SG. Bacteruria and urinary tract infections in the elderly. Urol Clin North Am. 2015;42:561-568.
4. Kline KA, Bowdish DM. Infection in an aging population. Curr Opin Microbiol. 2016;29:63-67. doi.org/10.1016/j.mib.2015.11.003.
5. Albert X, Huertax J, Pereiro II, Sanfelix J, Gosalbes V, Perotta C. Antibiotics for preventing recurrent urinary tract infection in non-pregnant women. Cochrane Database Sys Rev. 2004;CD001209.
6. Tacconelli E, Magrini N, Kahlmeter G, Sing N. Global priority list of antibiotic – resistant bacteria to guide research, discovery and development of new antibiotics. World Health Organization. 2017;27:318-327.
7. de-Kraker MEA, Stewardson AJ, Harbarth S. Will 10 million people die a year due to antimicrobial resistance by 2050? PLOS Medicine. 2016;13(11):e1002184. doi.org/10.1371/journel.pmed.1002184.
8. Beerepoot M, Geerlings S. Non – antibiotic prophylaxis for urinary tract infections. Pathogens. 2016;5:36. doi:10.3390/pathogens5020036.
9. Pirnay JP, De Vos D, Verbeken G, Merabishvili M, Chanishvili N, Vaneechoutte M et al. The phage therapy paradigm: pret-a- porter or sur-mesure? Pharm Res. 2011;28:934-937. doi.org/10.1007/s11095-010-0313-5.
10. Lin DM, Koskella B, Lin HC. Phage therapy: an alternative to antibiotics in the age of multi-drug resistance. World J Gastroinstest Pharmacol Ther. 2017;8:162-173. doi.org/10.4292/wjgpt.v8.i3.162.
11. Twort FW. An investigation on the nature of the ultramicroscopic viruses. Lancet. 1915;186:1241-1243. doi.org/10.1016/S01406736(01)20383-3.
12. D’Herelle F. On an invisible microbe antagonistic towards dysenteric bacilli: brief note by Mr. F. D’Herelle, presented by Mr. Roux. Res Microbiol. 1917;158:553-554. doi.org/10.1016/j.resmic.2007.07.005.
13. Aminov RI. A brief history of the antibiotic era: lessons learned and challenges for the future. Front Microbiol. 2010;1:1-7. doi.org/10.3389/fmicb.2010.00134.
14. Akova M. Epidemiology of antimicrobial resistance in blood stream infections. Virulence. 2016;7:252-266. doi.org/10.1080/21505594.1159366.
15. Frieri M, Kumar K, Boutin A. Antibiotic resistance. J Infect Public Health. 2017;10:369-378.
16. Rama JLR, Sieiro C, DC’ Miguel T, Sanchez S, Villa TG. Bacteriophages and lysins as possible alternatives to treat antibiotic resistant urinary tract infections. Antibiotics. 2020;9(8):466.
17. Malik S, Sidhu PK, Rana JS, Nehra K. Managing urinary tract infections through phage therapy: a novel approach. Folia Microbiol. 2020;65:217-231. doi.org/10.1007/s12223-019-00750-y.
18. Piatek BZ, Piatek R. Phage therapy as a novel strategy in the treatment of urinary tract infections caused by E-coli. Antibiotics. 2020;9:304. doi:10.3390/antibiotics9060304.
19. Goodridge LD. Designing phage therapeutics. Curr Pharm Biotechnol. 2010;11:15-27. doi.org/10.2174/138920110790725348.
20. Montag D, Hashemolhosseini S, Henning U. Receptor recognizing proteins of T-even type bacteriophages. The receptor recognizing area of proteins 37 of phages T4 Tula and Tulb. J Mol Biol. 1990;216:327-334. doi.org/10.16/S0022-2836(05)80324-9.
21. Lindberg AA. Bacteriophage receptors. Ann Rev Microbiol. 1973;27:205-241. doi.org/10.1146/annurev.mi.27.100173.001225.
22. Nishikawa H, Yasuda M, Uchiyama J, Rashel M, Maede Y, Takemura I et al. T- even-related bacteriophages as candidate for treatment of Escherichia coli urinary tract infections. Arch Virol. 2008;153:507-515. doi.org/10.1007/s00705-007-0031-4.

23. Sillankorva S, Oliveira D, Moura A, Henriques M, Faustino A, Nicolau A et al. efficacy of a broad host range lytic bacteriophage against E- coli adhered to urothelium. Curr Microbiol. 2010;62:1128-1132. doi.org/10.1007/s00284-010-9834-8.
24. Lehman SM, Donlan RM. Bacteriophage- mediated control of a two-species biofilm formed by micro- organisms causing catheter associated urinary tract infections in an in vitro urinary catheter model. Antimicrob Agents Chemother. 2015;59:1127-1137. doi.org/10.1128/AAC.03786-14.
25. Chibeu A, Lingohr EJ, Masson L, Manges A, Harel J, Ackermann HW et al. Bacteriophages with the ability to degrade uropathogenic Escherichia coli biofilms. Viruses. 2012;4:471-478. doi.org/10.3390/v4040471.
26. Tanji Y, Shimada T, Yoichi M, Miyanaga K, Hori K, Unno H. Towards rational control of Escherichia coli 0157:H7 by a phage cocktail. Appl Microbiol Biotechnol. 2004;64:270-274.
27. Gu J, Liu X, Li Y, Han W, Lei I, Yang Y et al. A method for generation phage cocktail with great therapeutic potential. PLoS one. 2012;7:1-8. doi.org/10.1371/journal.pone.0031698.
28. Chadha P, Katare OP, Chibber S. In vivo efficacy of single phage versus phage cocktail in resolving burn wound infection in BALB/ c mice. Microb Pathog. 2016;99:68-77.
29. Sybesma W, Zbinden R, Chanishrill N, Kntateladze M, Chikotua A, Ujmajuridze A et al. Bacteriophages as potential treatment for urinary tract infections. Front Microbiol. 2016;7:article:465. doi.org/10.3389/fmicb.2016.00465.
30. Ackermann HW, Krisch HM. A catalogue of T4 – type bacteriophage. Arch Virol. 1997;142:2329-2345.
31. Tetart F, Desplats C, Kutateladze M, Monod C, Ackermann H-W, Krisch HM. Phylogeny of the major head and tail genes of the wide ranging T4 – type bacteriophages. J Bacteriol. 2001;183:358-366. doi.org/10.1128/JB.183.1.358-366.2001.
32. Pires DP, Melo LDR, Boas VD, Sillankorva S, Azeredo J. Phage therapy as an alternative or complimentary strategy for prevent and control biofilm- related infections. Curr Opin Microbiol. 2017;39:48-56. doi.org/10.1016/j.mib.2017.09.004.
33. Lu TK, Koeris MS. The next generation of bacteriophage therapy. Curr Opin Microbiol. 2011;14:524-531. doi.org/10.1016/j.mib.2011.07.028.
34. Moller-olsen C, Ho SFS, Shukla RD, Feher T, Sagona AP. Engineered K1F bacteriophages kill intracellular Escherichia coli K1 in human epithelial cells. Sci Rep. 2018;08:17559. doi.org/10.1038/s41598-018-35859-6
35. Pires BP, Clieto S, Sillankorva S, Azeredo J, Lu TK. Genetically engineered phages: a review of advances over the last decade. Microbiol Mol Biol Rev. 2016;80:523-543. doi.org/10.1128/MMBR.00069-15.
36. Fischetti VA. Bacteriophage lytic enzymes: novel anti-infective. Trends Microbiol. 2005;13:491-496.
37. Rodriguez-Rubio L, Gutierrez D, Donovan DM, Martinez B, Rodriguez A, Garcia P. Phage lytic proteins: biotechnological applications beyond clinical anti microbials. Crit Rev Biotechnol. 2016;36:542-552. doi.org/10.3109/07388551.2014.993587.
38. Schmelcher M, Donvan DM, Loessner MJ. Bacteriophage endolysin as novel anti microbials. Future Microbiol. 2012;07:1147-1171. doi.org/10.2217/fmb.12.97.
39. Oliveira H, Sao- jose C, Azeredo J. Phage –derived peptidoglycan degrading enzymes: challenges and future prospects for in vivo therapy. Viruses. 2018;10:292. doi.org/10.3390/V10060292.
40. Delbruck M. The growth of bacteriophage and lysis of the host. J Gen Physiol. 1940;23:643-660.
41. Rodriguez L, Martinez B, Zhou Y, Rodriguez A, Donovan DM, Garcia P. Lytic activity of virion –associated peptidoglycan hydrolase HydH5 of staphylococcus aureuse bacteriophage vb_sauSphiiPLA88.BMC Microbiol. 2011;11:138. doi.org/10.1186/1471-2180-11-138.
42. Salazar O, Asenjo JA. Enzymatic lysis of microbial cells. Biotechnol Lett. 2007;29:985-994. doi.org/10.1007/s10529-007-9345-2.
43. Wang S, Gu J, Lv M, Guo Z, Yan G, Yu L et al. The anti bacterial activity of E-coli bacteriophage lysine lysep3 is enhanced by fusing the bacillus amyloliquefaciens bacteriophage endolysing binding domine D8 to the C-terminal region. J Microbiol. 2017;55:403-408. doi.org/10.1007/s12275-017-6431-6.
44. Gu N, Feng C, Ren J, Zhuang X, Zhang Y, Zhu Y et al. A novel anti microbialEndolysin, LysPA26, against pseudomonas aerugenosa. Front Microbiol. 2017;8:293. doi.org/10.3389/fmicb.2017.0029.
45. Briers Y, Lavigne R. Breacking barriers: expansion of the use of endolysin as novel anti bacterials against gram- negative bacteria. Future Microbiol. 2015;10:377-390. doi.org/10.22117/fmb.15.8.
46. Sao-Josc C. Engineering of phage- derived lytic enzymes: improving their potential as anti microbials. Antibiotics. 2018;7:29. doi.org/10.3390/antibiotics7020029.
47. Lukacik P, Barnard T, Keller PW, Chaturvedi KS, Seddiki N, Fairman JW et al. Structural engineering of a phage lysine that target gram – negative pathogens. Proc Natl Acad Sci USA. 2012;109:9857-9862. doi.org/10.1073/pnas.1203472109.
48. Briers Y, Walmagh M, Puyenbroeck V, Cornelisen A, Cenens W, Aertsen A, J et al. Engineered endolysin –based "artilysin" to compact multi drug resistant gram negative pathogens. mBio. 2014;05:e01379-14. doi.org/10.1128/nBio.01379-14.
49. Briers Y, Walmagh M, Grymonprez B, Biebl M, Pirnay JP, Defraine V et al. Art 175 is a highly efficient anti bacterial against multidrug- resistant strains and persisters of pseudomonas aeruginosa. Anti Micro Agents Chemother. 2014;58:3774-3784. doi.org/10.1128/AAC.02668-14.
50. Schirmeier E, Zimmermann P, Hofmann V, Biebl M, Jerstmans H, Maervoet VE et al. Inhibitory and bacteriocidal effect of artilysin ART-175 against colistin -resistant mcr-1 positive Escherichia coli isolates. Int J Antimicro Agents. 2017;51:528-529. doi.org/10.1016/j.ijantimicag.2017.08.027.
51. Ryan EM, Mahmoud Y, Alkawareek RS, Donnelly GBF. Synergistic phage-antibiotic combinations for the control of Escherichia coli biofilms in vitro. FEMS Immunol Med Microbiol. 2012;65:395-398. doi.org/10.1111/j.1574-695X.2012.00977.S.
52. Comeau AM, Tetart F, Trojet SN, Prere MF, kirsch HM. Phage antibiotic synergy (PAS) β- lactam and quinolone antibiotics stimulate virulent phage growth. PLoS One. 2017;2:e799. doi.org/10.1371/journal.pone.0000799.
53. Kamal F, Dennis JJ. Burkholderia cepacia complex phage antibiotic synergy (PAS): antibiotics stimulate lytic phage activity. Appl Environ Microbiol. 2015;81:1132-1138. doi.org/10.1128/AEM.02850-14.
54. Kim M, Jo Y, Hwang YJ, Hong HW, Hong SS, Park K et al. Phage antibiotic synergy via delayed lysis. Appl Environ Microbiol. 2018;84:e02085-e02018. doi.org/10.1128/AEM.02085-18.
55. Letrado P, Corsini B, Diez-martinez R, Bustamante N, Yuste JE, Garcia P. Bactericidal synergism between antibiotics and phage endolysin CpL-711 to kill multi drug resistant pneumococcus. Future Microbiol. 2018;13:1215-1223. doi.org/10.2217/fmb-2018-0077.
56. Ujmajuridze A, Chanishvili N, Joderdzishvili M, Leitner L, Mehnert U, Chkhotua A et al. Adapted bacteriophage for treating urinary tract infections. Front Microbiol. 2018;9:1832. doi.org/10.3389/fmicb.2018.01832.
57. Furfaro LL, Payne MS, Chang BJ. Bacteriophage therapy:clinical trials and regulatory hurdles. Front Cell Infect Microbiol. 2018;8:376. doi.org/10.3389/fcimb.2018.00376.
58. Abdelkader K, Gerstmans H, Saafan A, Dishisha T, Briers Y. The pre-clinical and clinical progress of bacteriophages and

their lytic enzymes: the parts are easier than the whole. Viruses. 2019;11:96. doi.org/10.3390/v11020096.
59. Gutierrez D, Fernandez L, Rodriguez A, Garcia P. Are phage lytic proteins the secret weapon to kill staphylococcus aureus? M Bio. 2018;9:e01923-17. doi.org/10.1128/mBio.01923-17.
60. Gorski A, Miedzybrodzki R, Wegrzyn G, Matysiak JE, Borysowski J, Dabrowska WB. Phage therapy: current status and perspectives. Med Res Rev. 2019;1-5. doi.org/10.1002/med.21593.
61. McCallin S, Sacher JC, Zheng J, Chan BK. Current state of compassionate phage therapy. Viruses. 2019;11:343. doi.org/10.3390/v11040343.
62. Fish R, Kutter E, Wheat G, Blasdel B, Kutateladze M, Kuhl S. Compassionate Use of Bacteriophage Therapy for Foot Ulcer Treatment as an Effective Step for Moving Toward Clinical Trials. In: Azeredo J., Sillankorva S. (eds) Bacteriophage Therapy. Methods in Molecular Biology. Humana Press, New York, NY. 2018;1693. doi.org/10.1007/978-1-4939-7395-8_14
63. Moghadam MT, Amirmozafari N, Shariati A, Hallajzadeh M, Mirkalantari S, Khoshbayan A et al. How phages overcome the challenges of drug resistant bacteria in clinical infections. Infection and Drug Resi. 2020;13:45-61.
64. Chegini Z, Khoshbayan A, Vesal S, Moradabadi A, Hashemi A, Shariati A. Bacteriophage therapy for inhibition of multidrug resistance uropathogenic bacteria – a narrative review. Ann Clin Microbiol Antimicrob. 2021;20:30. doi.org/10.1186/s12941-021-00433-y.
65. Abedon SJ, Garcia P, Mullany P, Aminov R. Phage therapy: past, present and future. Front Microbiol. 2017;8:article:98. doi.org/10.3389/fmicb.2017.00981.
66. Garretto A, Miller–Ensminger T, Wolfe AJ, Putonti C. Bcteriophages of the lower urinary tract. Nature Reviews Urology. 2019;16:422-432.
67. Zaczek M, Dabrowska WB, Miedzybrodzki R, Gorski A. Phage prevalence in the human urinary tract- current knowledge and therapeutics implication. Microorganism. 2020;8:1802. doi.org/10.3390/microorganisms8111802.
68. Alsaadi A, Beamnd B, Easwaran M, Abdelrahman F, El-Shibing A, Alghoribi MF et al. Learning from mistakes: The role of phage in pandemics. Front Microbiol. 2021;12:653107. doi.org/10.3389/fmicb.2021.653107.
69. Moeilling K, Broecker F, Willy C. A wake up call: we need phage therapy now. Viruses. 2018;10:688. doi.org/10.3390/v10120688.