Bangladesh J Med Microbiol 2013; 07 (01): 25-28
Bangladesh Society of Medical Microbiologists

Review Article

Bacteriophage Therapy: Issues and Controversies

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Introduction:

Introduced in the early 1900s, phage therapy is the application of bacteria-specific viruses to combat uncontrolled and undesired bacteria such as those associated with infections[1]. The emergence of bacterial resistant to most of the currently available antimicrobial drugs has become a critical problem in modern medicine, because of the significant increase in immunocompromised patients in particular[2]. The concern that mankind is re-entering the preantibiotics era has become very real, and the development of alternative anti-infection modalities has become one of the highest priorities of medicine and biotechnology. [3]Bacteriophages are viruses that invade bacterial cells and, in the case of lytic phages, disrupt bacterial metabolism and cause the bacteria to lyse. The history of bacteriophage discovery has been the subject of lengthy debates, including a controversy over claims for priority. Ernest Hankin, a British bacteriologist, reported in 1896 on the presence of marked antibacterial activity (against *Vibrio cholerae*) which he observed in the waters of the Ganges and Jumna rivers in India[4]. He suggested that an unidentified substance was responsible for this phenomenon and for limiting the spread of cholera epidemics. Two years later, the Russian bacteriologist Gamaleya observed a similar phenomenon while working with *Bacillus subtilis*, and the observations of several other investigators are also thought to have been related to the bacteriophage phenomenon[5]. However, Frederick Twort, a clinical bacteriologist from England, reintroduced the subject almost 20 years after Hankin's observation by reporting a similar phenomenon and advancing the hypothesis that it may have been due to, among other possibilities, a virus[6]. Owing to their host specificity which can range from an ability to infect only a few strains of a bacterial species to, more rarely, a capacity to infect more than one relatively closely related bacterial genusphages only minimally impact health-protecting normal bacterial flora[7]. By contrast, many antibiotics, which tend to have broader spectrums of activity, are prone to inducing superinfections, such as antibiotic-associated *Clostridium difficile* colitis or *Candida albicans* yeast infections[8].

Phages against many pathogenic bacteria are easily discovered, often from sewage and other waste materials that contain high bacterial concentrations. Isolation can be more technically demanding, however, if host bacteria themselves are difficult to culture and bacteria may differ in terms of the number of phage types to which they are susceptible[9].

Early research on phage therapy:

Not long after his discovery, d'Herelle used phages to treat dysentery, in what was probably the first attempt to use bacteriophages therapeutically[10]. The phage preparation was ingested by d'Herelle, Hutinel, and several hospital interns in order to confirm its safety before administering it the next day to a 12-year-old boy with severe dysentery. The patient's symptoms ceased after a single administration of d'Herelle's anti-dysentery phage, and the boy fully recovered within a few days[11]. The efficacy of the phage preparation was confirmed shortly afterwards, when three additional patients having bacillary dysentery and treated with one dose of the preparation started to recover within 24 hours of treatment. However, the results of these studies were not immediately published and, therefore, the first reported application of phages to treat infectious diseases of humans came in 1921 from Richard Bruynoghe and Joseph Maisin[12], who used bacteriophages to treat staphylococcal skin infections. The bacteriophages were injected into and around surgically opened lesions, and the authors reported regression of the lesions within 24 to 48 hours[13]. Several similarly promising studies followed, and encouraged by these early results[14].

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Prophylaxis and treatment of bacterial infections in humans:
Since phages consist mostly of nucleic acids and proteins, they are inherently nontoxic[15]. However, phages can interact with immune systems, at least potentially resulting in harmful immune responses, though there is little evidence that this actually is a concern during phage treatment[16]. The international literature contains several hundred reports on phage therapy in humans, with the majority of recent publications coming from researchers in Eastern Europe and the former Soviet Union and only a few reports published in other countries[17]. The most detailed reports on phage therapy in humans were by Slopek *et al*., who published a series of reports on the effectiveness of phages against infections caused by several bacterial pathogens, including multidrug-resistant mutants[18]. They have reported that five hundred fifty patients having bacterial septicemia and ranging in age from 1 week to 86 years were treated at a total of 10 hospitals located in three different cities. Antibiotic treatment was reported to be ineffective in 518 of the patients, leading to the decision to use phage therapy. The etiologic agents in the studies were staphylococci, *Pseudomonas, Escherichia, Klebsiella,* and *Salmonella,* and treatment was initiated after isolating the etiologic agents and selecting specific, highly potent phages from a collection of more than 250 lytic phages[19]. Phages were administered as follows: (i) orally, three times a day before eating and after neutralizing gastric acid by oral administration of baking soda or bicarbonate mineral water a few minutes prior to phage administration; (ii) locally, by applying moist, phage-containing dressings directly on wounds and/or pleural and peritoneal cavities; and (iii) by applying a few drops of phage suspension to the eye, middle ear, or nasal mucosa. During the course of treatment, the etiologic agents were continuously monitored for phage susceptibility, and if phage resistance developed, phages were replaced with different bacteriophages lytic against the newly emerged, phage-resistant bacterial mutants.

Comparison of phages and antimicrobial drugs:
Advantages of phage therapy over the use of antibiotics can be framed in terms of phage properties. Researchers paid more attention to those properties that can contribute substantially to phage therapy utility[20].

Lytic phages are similar to antibiotics in that they have remarkable antibacterial activity. However, therapeutic phages have some at least theoretical advantages over antibiotics, and phages have been reported to be more effective than antibiotics in treating certain infections in humans and experimentally infected animals[21]. In one study *Staphylococcus aureus* phages were used to treat patients having purulent disease of the lungs and pleura[22]. The patients were divided into two groups; the patients in group A (223 individuals) received phages, and the patients in group B (117 individuals) received antibiotics. Also, this clinical trial is one of the few studies using i.e. phage administration. The results were evaluated based on the following criteria: general condition of the patients, X-ray examination, reduction of purulence, and microbiological analysis of blood and sputum. No side effects were observed in any of the patients, including those who received phages intravenously. Moreover, the complete recovery was observed in 82% of the patients in the phage-treated group as opposed to 64% of the patients in the antibiotic-treated group. Because phages infect and kill using mechanisms that differ from those of antibiotics, specific antibiotic resistance mechanisms do not translate into mechanisms of phage resistance. Thus, scientists gave the opinion that phages consequently can be readily employed to treat antibiotic-resistant infections[23].

Good therapeutic phages should have a high potential to reach and then kill bacteria in combination with a low potential to otherwise negatively modify the environments to which they are applied. These characteristics can be reasonably assured so long as phages are obligate lytic, stable under typical storage conditions and temperatures, subject to appropriate efficacy and safety, and, ideally, fully sequenced to confirm the absence of undesirable genes which can produce toxins[24].

Mechanism of action of phages:
Despite the large number of publications on phage therapy, there are very few reports in which the pharmacokinetics of therapeutic phage preparations is delineated. The publications available on the subject, suggest that phages get into the bloodstream of laboratory animals (after a single oral dose) within 2 to 4 hours and that they are found in the internal organs (liver, spleen, kidney) in approximately 10 to 25 hours. Also, data concerning the persistence of administered phages indicate that phages can remain in the human body for relatively prolonged periods of time, i.e., up to several days[26]. However, additional research is needed in order to obtain substantial pharmacological data concerning lytic phages, including full-scale toxicological studies, before lytic phages can be used therapeutically[27]. As for their bactericidal activity, therapeutic phages were assumed to kill their target bacteria by replicating inside and lysing the host cell. However, subsequent studies revealed that not all phages replicate similarly and that there are important differences in the replication cycles of lytic and lysogenic phages28. Furthermore, the recent delineation of the full sequence of the

T4 phage and many years of elegant studies of the mechanism of T4 phage replication have shown that lysis of host bacteria by a lytic phage is a complex process consisting of a cascade of events involving several structural and regulatory genes. Since T4 phage is a typical lytic phage, it is possible that many therapeutic phages act via a similar cascade[29]; however, it is also possible that some therapeutic phages have some unique yet unidentified genes or mechanisms responsible for their ability to effectively lyse their target bacteria. Authors identified and cloned an anti-*Salmonella* phage gene responsible, at least in part, for the phage's potent lethal activity against the *Salmonella* typhimurium host strains[30]. In another study, a unique mechanism has been described for protecting phage DNA from the restriction-modification defenses of an *S. aureus* host strain[31]. Further elucidation of these and similar mechanisms is likely to yield information useful for genetically engineering optimally effective therapeutic phage preparations.

Conclusion:

Bacteriophages have several characteristics that make them potentially attractive therapeutic agents. They are highly specific and very effective in lysing targeted pathogenic bacteria. These are rapidly modifiable to combat the emergence of newly arising bacterial threats. There is a large body of evidences and a desperate enough need to find alternative treatment modalities against rapidly emerging, antibiotic-resistant bacteria to warrant further studies in the field of phage therapy. In an era where antibiotic-resistant bacterial infections are on the rise, phages provide numerous advantages, along with relatively few disadvantages.

Reference:

1. Sulakvelidze A,Alavidze Z, Glenn Morris J. Bacteriophage Therapy. Antimicrob Agents Chemother. 2001; 45 (3): 649-659.
2. Kutateladze M, Adamia R. Bacteriophages as potential new therapeutics to replace or supplement antibiotics. Trends Biotechnol. 2010; 28:591-595.
3. Skurnik M, Pajunen M, Kiljunen S. Biotechnological challenges of phage therapy. BiotechnolLett. 2007; 29: 995-1003.
4. Carlton RM. Phage therapy: past history and future prospects. Arch ImmunolTherExp 1999; 47: 267-274.
5. Hankin EH. L'action bactericide des eaux de la Jumna et du Gangesur le vibrion du cholera. Ann Inst Pasteur. 1896; 10: 511.
6. Twort FW. An investigation on the nature of ultramicroscopic viruses. Lancet 1915; 1241.
7. Van Helvoort T. Bacteriological and physiological research styles in the early controversy on the nature of the bacteriophage phenomenon. Med Hist. 1992; 3: 243-270.
8. Hyman P, Abedon ST. Bacteriophage host range and bacterial resistance. AdvApplMicrobiol. 2010; 70: 217-248.
9. Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S, et al. Phage therapy in clinical practice: treatment of human infections. Curr Pharm Biotechnol. 2010; 11: 69-86.
10. d'Hérelle F. The bacteriophage. Sci News (Harmondsworth, London) 1949; 14: 44-59.
11. d'Hérelle F. Preparation of Therapeutic Bacteriophages, Appendix 1 from: Le Phénomène de la Guérisondans les maladies infectieuses: Masson et Cie, 1938, Paris-OCLC 5784382 (translation by Kuhl SJ, Mazure H) Bacteriophage. 2011; 1: 55-65.
12. Bruynoghe R, Maisin J. Essais de thérapeutique au moyen du bacteriophage. C R Soc Biol. 1921; 85:1120-1121.
13. Gupta R, Prasad Y. Efficacy of polyvalent bacteriophage p-27/HP to control multidrug resistant Staphylococcus aureus associated with human infections. CurrMicrobiol. 2011; 62: 255-260.
14. Skurnik M, Pajunen M, Kiljunen S. Biotechnological challenges of phage therapy. BiotechnolLett. 2007; 29: 995-1003.
15. Clokie MRJ, Kropinski AM. Bacteriophages Methods and Protocols. Isolation, Characterization and Interactions. New York: Humana Press; 2009.
16. Fischbach MA, Walsh CT. Antibiotics for emerging pathogens. Science. 2009; 325: 1089-1093
17. Summers WC. Felix d'Herelle and the origins of molecular biology. New Haven, Conn: Yale University Press; 1999.
18. Slopek S, Durlakowa I, Weber-Dabrowska B, Kucharewicz-Krukowska A, Dabrowski M, Bisikiewicz R. Results of bacteriophage treatment of suppurative bacterial infections. I. General evaluation of the results. Arch ImmunolTher Exp. 1983; 31: 267-291
19. Eaton M D, Bayne-Jones S. Bacteriophage therapy. Review of the principles and results of the use of bacteriophage in the treatment of infections. JAMA. 1934; 23: 1769-1939.

20. Abdul-Hassan HS, El-Tahan E, Massoud B, Gomaa R. Bacteriophage therapy of pseudomonas burn wound sepsis. Ann Medit Burn Club. 1990; 3: 262-264.

21. Slopek S, Durlakowa I, Weber-Dabrowska B, Kucharewicz-Krukowska A, Dabrowski M, Bisikiewicz R. Results of bacteriophage treatment of suppurative bacterial infections. II. Detailed evaluation of the results. Arch ImmunolTher Exp. 1983; 31: 293-327

22. Andriashvili IA, Kvachadze LI, Vashakidze RP, Adamia RS, Chanishvili TG. Molecular mechanisms of DNA protection from restriction endonucleases in Staphylococcus aureus cells. Mol Gen MicrobiolVirusolol. 1986; 8: 43-45.

23. Kochetkova VA, Mamontov AS, Moskovtseva RL, Erastova EI, Trofimov EI, Popov MI, *et al*. Phagotherapy of postoperative suppurative-inflammatory complications in patients with neoplasms. Sov Med. 1989; 6: 23-26.

24. Fischbach MA, Walsh CT. Antibiotics for emerging pathogens. Science. 2009; 325: 1089-1093.

25.Carlton RM. Phage therapy: Past history and future prospects. Arch Immunol TherExp (Warsz) 1999; 47: 267-274.

26. Skurnik M, Pajunen M, Kiljunen S. Biotechnological challenges of phage therapy. BiotechnolLett. 2007; 29: 995-1003.

27. Gill JJ, Hyman P. Phage choice, isolation and preparation for phage therapy. Curr Pharm Biotechnol. 2010; 11: 2-14.

28. Abedon ST, Thomas-Abedon C. Phage therapy pharmacology. Curr Pharm Biotechnol. 2010; 11: 28-47.

29. Alisky J, Iczkowski K, Rapoport A, Troitsky N. Bacteriophages show promise as antimicrobial agents. J Infect. 1998; 36: 5-15.

30. Capparelli R, Nocerino N, Iannaccone M, Ercolini D, Parlato M, Chiara M, et al. Bacteriophage therapy of Salmonella enterica: a fresh appraisal of bacteriophage therapy. J Infect Dis. 2010; 201: 52-61.

31. Kramberger P, Honour RC, Herman RE, Smrekar F, Peterka M. Purification of the Staphylococcus aureus bacteriophages VDX-10 on methacrylate monoliths. J Virol Meth. 2010; 166: 60-64.