

Review

Bacteriophage Therapy of Bacterial Infections: an Update of Our Institute's Experience

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Abstract. 1307 patients with suppurative bacterial infections caused by multidrug-resistant bacteria of different species were treated with specific bacteriophages (BP). BP therapy was highly effective; full recovery was noted in 1123 cases (85.9%). In 134 cases (10.9%) transient improvement was observed and only in 50 cases (3.8%) was BP treatment found to be ineffective. The results confirm the high effectiveness of BP therapy in combating bacterial infections which do not respond to treatment with the available antibiotics.

Key words: phage therapy; drug resistance; bacterial infections.

Bacteriophages (BP) are viruses that attack bacteria, multiply within and cause disruption of bacterial cells (lysis). Their lytic action is highly specific. After the discovery of BP 85 years ago, it was hoped that they would be useful in the treatment of bacterial infections. BP therapy was initiated in 1921 by BRUYNOGHE and MAISIN⁴ in the treatment of staphylococcal infections. Although the results were promising, little was accomplished in this field during the following years. The idea of potential applications of BP therapy was abandoned after the introduction of sulphonamides and then antibiotics into medical practice. However, the lytic action of BP *in vitro* enabled some investigators to use specific BP for the differentiation of various species of bacteria. Many phage-typing schemes were elaborated. These methods of differentiation are still used worldwide and are very useful in epidemiological investigation¹.

Renewed interest in BP therapy emerged again with the appearance of drug-resistant bacteria. In the recent years bacteria highly resistant to most or all drugs, including the antibiotic of last resort, vancomycin, have been spreading all over the world^{6, 7, 10, 12–15, 27}. This resistance is mainly disseminated by plasmids, transposons and insertion elements. Resistance markers may be transmitted between cells of different species of bacteria. Thus, antibiotic treatment of infections caused by multi-drug-resistant bacteria is ineffective and the growing resistance of pathogenic bacteria is of great importance in medical practice. During last two decades data have been accumulated to show that BP therapy has become an important alternative to antibiotics in the treatment of bacterial infections. In many cases, successful results were obtained in combating infections in humans and in animals^{1–3, 5, 8, 9, 11, 17, 18, 28–31}.

Author's fees were financed by the Association for the Joint Administration of Copyright KOPIPOL (Kielce, Poland) from funds collected on the basis of the Law on Author's Rights.

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BP therapy has been extensively used at the Bacteriophage Institute, Tbilisi, Georgia (for rev. see KUTER¹⁰). It was found that specific BP are effective in both the prophylaxis and treatment of bacterial infections caused by drug-resistant bacteria of different origin. Extensive studies on BP therapy have also been carried out at the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland. Between 1981 and 1986 BP therapy was applied in 550 cases of suppurative bacterial infections caused by staphylococci and Gram-negative bacteria (*Klebsiella*, *Escherichia*, *Proteus* and *Pseudomonas*). In 518 cases, BP therapy followed ineffective treatment with all available antibiotics. Positive therapeutic effects were obtained in 508 cases, i.e. 92.4% (range 75–100%). It was found that BP therapy effectively controls the infections process irrespective of its localization, patient age and sex, and type of infection (monoinfections, polyinfections). The highest effectiveness of BP was noted in furunculosis (100% cured). High effectiveness (over 90% cured) was also observed in osteomyelitis, infections of connective tissue and lymphatic vessels, as well as chronic suppurative fistulas^{19–26}.

In this paper we present our results of the BP treatment of bacterial infections in the years 1987–1999. During that period BP were applied in 1307 patients with different suppurative infections caused by multi-drug-resistant bacteria. The majority of cases were long, persisting infections in which antibiotic therapy had failed. The age of patients ranged from 4 weeks to 86 years. Our studies included isolation and identification of bacterial strains from patient specimens, determination of the sensitivity of the isolated strains to specific BP, and preparation of crude sterile BP lysates for therapy, as described in detail earlier²⁰. In each case, BP were administered orally 3 times daily in the amount of 10 ml (children 5 ml) 30 min before eating, after neutralization of the gastric juice. Local administration depended upon a localization of the suppurative process. BP were applied directly to the wounds, as ear and nose drops, as infusions to the fistulas, washing of the nasal cavity suppurative lesions of pleura and peritoneum, decubitus and fistulas, intraperitoneally during the washing of the peritoneal cavity and topically in the cases of multiple skin abscesses.

The BP therapy was carried out at university clinics and hospital wards. The clinical results of the BP therapy were evaluated by the physicians responsible for the patients' care. BP treatment lasted 1–12 weeks, with an average of 32 days.

The results of BP therapy applied to bacterial infec-

tions are depicted in Table 1. As may be seen, BP therapy was highly effective in the treatment of infections caused by different species of bacteria, as *Escherichia*, *Klebsiella*, *Proteus*, *Enterobacter*, *Pseudomonas* and *Staphylococcus aureus* (furunculosis). It must be stressed that 2738 strains (69.2%) were isolated from infections caused by one species of bacteria (monoinfections), the great majority by *S. aureus* (1674 strains). The remaining 1218 strains (30.8%) were isolated from infections caused by several species of bacteria (polyinfections). *Staphylococcus* and *Pseudomonas* occurred more frequently in monoinfections; *Klebsiella*, *Escherichia*, *Enterobacter* and *Proteus* occurred more frequently in polyinfections. In 1123 patients (85.9%) treated with BP, a complete recovery or healing of the local lesions was obtained (range 64–100%), according to the etiologic factor and type of infection. Noteworthy is that BP therapy was most effective in purulent meningitis and furunculosis (100% cured). High effectiveness was also noted in septicemia of different origin, purulent otitis media, suppurative peritonitis, pyogenic arthritis and myositis, osteomyelitis of the long bones, suppurative osteitis after bone fractures, pyogenic infections of burns, purulent mastitis and chronic suppurative fistulas. In 134 cases (10.4%) transient improvement was observed and in 50 cases (3.8%) BP therapy was found to be ineffective. Of particular importance is that two dangerous pathogens, *S. aureus* and *P. aeruginosa* (which frequently cause serious infections), were highly sensitive to our sets of specific phages (95 and 89%, respectively). Other pathogens, *E. coli* and *Klebsiella*, were inhibited by specific phages in 81 and 60% of cases, respectively (Table 2). Figures 1 and 2 depict representative results of BP therapy.

Our results extend and confirm our earlier data showing the effectiveness of BP therapy in the combating of antibiotic-resistant bacterial infections. In fact, our results suggest that BP therapy is more effective than antibiotic treatment. In many cases, specific BP therapy constituted the only means of eliminating life-threatening infections. It must be stressed, however, that the success of BP therapy is associated only with the sensitivity of the causative bacteria to its specific phage.

It should be highlighted that in many cases following BP therapy an increased protection against subsequent bacterial and viral infections has been observed. Thus, it may be that the BP therapeutic effect (disappearance of clinical symptoms and negative bacteriologic tests) is not only a result of the destruction of bacterial cells in the infections sites, but also a con-

Table 1. Results of bacteriophage treatment (1307 cases)

Clinical diagnosis	Etiology	Number of cases			
		subjected to phage therapy	full recovery*	marked improvement**	no effect
Septicemia	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>	106	93 (87.7%)	8 (7.5%)	5 (4.7%)
Purulent otitis media	<i>S. aureus</i> , <i>Klebsiella</i> , <i>Pseudomonas</i>	33	28 (88.4%)	3 (9.09%)	2 (6.06%)
Purulent meningitis	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>	10	10 (100%)		
Varicose ulcers of lower extremities	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>	77	47 (61.03%)	21 (27.2%)	9 (11.6%)
Mucopurulent chronic bronchitis, laryngitis, rhinitis	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>	271	224 (82.6%)	46 (16.9%)	1 (0.3%)
Bronchopneumonia, empyema	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>	57	47 (82%)		10 (18%)
Pleuritis with fistula	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>	49	42 (86%)	5 (10%)	2 (4%)
Suppurative peritonitis	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Enterobacter</i> , <i>Proteus</i> , <i>Pseudomonas</i>	66	60 (91%)	5 (8%)	1 (0.15%)
Urinary tract infections	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>	78	59 (75.6%)	9 (11.5%)	10 (12.8%)
Furunculosis	<i>S. aureus</i>	90	90 (100%)		
Decubitus with infection	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>	16	13 (81%)		3 (19%)
Pyogenic arthritis and myositis	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>	19	17 (89%)		2 (11%)
Osteomyelitis of the long bones	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>	40	38 (95%)	2 (5%)	
Suppurative osteitis after bone fractures	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>	41	37 (90%)	4 (10%)	
Pyogenic infections of burns	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>	49	42 (86%)	7 (14%)	
Pyogenic postoperative infection	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Pseudomonas</i>	35	29 (83%)	6 (17%)	
Chronic suppurative fistulas	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>	180	168 (93%)	12 (7%)	
Suppurative sinusitis	<i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>	46	38 (83%)	3 (7%)	5 (11%)
Purulent mastitis	<i>S. aureus</i> , <i>E. coli</i>	44	41 (93.1%)	3 (6.8%)	
Total		1307	1123 (85.9%)	134 (10.2%)	50 (3.8%)

* Full recovery and complete elimination of bacteria.

** Improvement, bacteria still detectable.

sequence of BP up-regulation of the immune response. While monitoring the immune status of patients receiving BP we noted that effective BP therapy is associated with a normalization of cytokine production by blood

cell cultures³². Moreover, our preliminary data indicate that purified BP may induce intracytoplasmatic cytokine synthesis in human lymphocytes and monocytes (GÓRSKI et al., unpublished observations). One may as-

Table 2. Sensitivity of bacterial strains within different species to specific bacteriophages

Set of phages against	Number of bacterial isolates	
	tested	phage sensitive (%)
<i>Staphylococcus</i>	2433	2311 (95)
<i>Pseudomonas</i>	422	376 (89)
<i>Escherichia</i>	465	380 (81)
<i>Klebsiella</i>	210	125 (60)

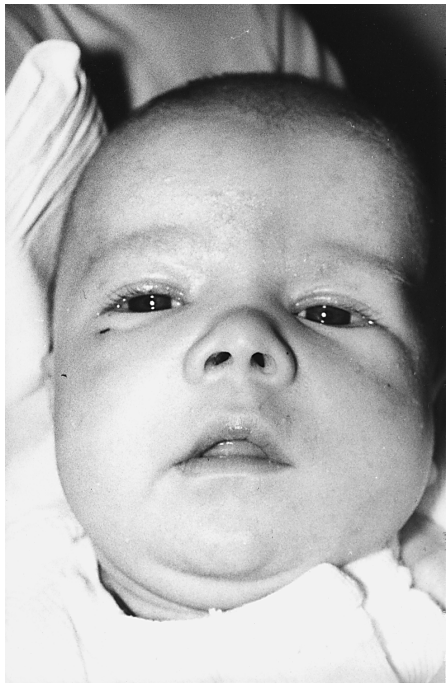


Fig. 1. Abscess of nasal arca. A – prior to BP therapy, B – following BP therapy



Fig. 2. Infected ulcer. A – prior to BP therapy, B – following BP therapy

sume that BP also have immunoregulatory properties by interacting with immunocompetent cells. Further studies on the immunoregulatory effect of BP are underway. In addition, a double-blind placebo-control-

led clinical trial on the effectiveness of BP therapy should be completed within the next 6 months.

We hope that our data open new perspectives for BP therapy and its worldwide application in the treatment and eradication of bacterial infections.

Acknowledgment. This work was supported by grant 4PO5BO1219 from the State Committee for Scientific Research (KBN).

References

1. ACKERMAN H. W. and DUBOW M. (1987): Viruses of prokaryotes. I. General properties of bacteriophages. CRC Press, Boca Roton, Florida.
2. BARROW R. A. and SOOTHILL J. S. (1997): Bacteriophage therapy and prophylaxis: rediscovery and renewed assessment of potential. *Trends Microbiol.*, **5**, 258–271.
3. BERCHIERI A. JR., LOVELL M. A. and BARROW P. A. (1991): The activity in the chicken alimentary tract of bacteriophages lytic for *Salmonella typhimurium*. *Res. Microbiol.*, **142**, 541–549.
4. BRUYNOGHE R. and MAISIN J. (1921): Essais de therapeutique au moyem du bactériophage. *C. R. Soc. Biol.*, **85**, 1120–1121.
5. CARLTON R. M. (1999): Phage therapy: past history and future prospects. *Arch. Immunol. Ther. Exp.*, **47**, 267–274.
6. CISŁO M., DĄBROWSKI M., WEBER-DĄBROWSKA B. and WOJTOŃ A. (1987): Bacteriophage treatment of suppurative skin infection. *Arch. Immunol. Ther. Exp.*, **35**, 175–183.
7. COHEN M. L. (1992): Epidemiology of drug resistance: implications for a post-antimicrobial era. *Science*, **257**, 1050.
8. GILMORE M. S. and HOCH J. A. (1999): A vancomycine surprise. *Nature*, **399**, 524–527.
9. HOLZMAN D. (1988): Phage as antibacterial tool. *Genet. Eng. News*, 15 Oct., pp. 1,12, 41, 48.
10. KUTTER E. (1997): Phage therapy. Bacteriophages as antibiotics. Evergreen State College. Olympia WA 98505 Nov. 15.
11. LORCH A. (1999): Bacteriophages an alternative to antibiotics. *Biotech. Develgo Monitor*, **39**, 14–17.
12. LEDERBERG J. (1996): Commentary. *Proc. Acad. Sci. USA*, **93**, 3167–3168.
13. NEU H. C. (1992): The crisis in antibiotic resistance. *Science*, **257**, 1064–1071.
14. NOVAK R., HENRIQUES B., CHARPENTIER E., NORMARK S. and TUOMANEN E. (1999): Emergence of vancomycin tolerance in *Streptococcus pneumoniae*. *Nature*, **399**, 590–593.
15. SIERADZKI K., ROBERTS R. B., HABER S. W. and TOMASZ A. (1999): The development of vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus*. *N. Engl. J. Med.*, **340**, 517–523.
16. SMITH T. L., PEARSON M. L., WILCOX K. R., CRUZ C., LANCASTER M. V., ROBINSON-DUNN B., TENOVER F. C., ZERVOS M. J., BAND J. D., WHITE E. and JARVIS W. R. (1999): Emergence of vancomycin resistance in *Staphylococcus aureus*. *N. Engl. J. Med.*, **340**, 493–501.
17. SMITH H. W. and HUGGINS R. B. (1983): Effectiveness of phages in treating experimental *E. coli* diarrhoea in calves, piglets and lambs. *J. Gen. Microbiol.*, **129**, 2659–2675.
18. SMITH H. W. and HUGGINS R. B. (1987): The control of experimental *E. coli* in calves by means of bacteriophage. *J. Gen. Microbiol.*, **133**, 1111–1126.
19. STRÓJ L., WEBER-DĄBROWSKA B., PARTYKA K., MULCZYK M. and WÓJCIK M. (1999): Successful bacteriophage treatment in purulent cerebrospinal meningitis in a newborn (in Polish). *Neur. Neuroch. Pol.*, **33**, 693–698.
20. ŚLOPEK S., DURLAKOWA I., WEBER-DĄBROWSKA B., KUCHARIEWICZ-KRUKOWSKA A., DĄBROWSKI M. and BISIKIEWICZ R. (1983): Results of bacteriophage treatment of suppurative bacterial infections. I. General evolution of the results. *Arch. Immunol. Ther. Exp.*, **31**, 267–291.
21. ŚLOPEK S., DURLAKOWA I., WEBER-DĄBROWSKA B., KUCHARIEWICZ-KRUKOWSKA A., DĄBROWSKI M. and BISIKIEWICZ R. (1983): Results of bacteriophage treatment of suppurative bacterial infections. II. Detailed evaluation of the results. *Arch. Immunol. Ther. Exp.*, **31**, 293–327.
22. ŚLOPEK S., DURLAKOWA I., WEBER-DĄBROWSKA B. and KUCHARIEWICZ-KRUKOWSKA A. (1984): Results of bacteriophage treatment of suppurative bacterial infections. III. Detailed evaluation of the results obtained in further 150 cases. *Arch. Immunol. Ther. Exp.*, **32**, 317–335.
23. ŚLOPEK S., KUCHARIEWICZ-KRUKOWSKA A., WEBER-DĄBROWSKA B. and DĄBROWSKI M. (1985): Results of bacteriophage treatment of suppurative bacterial infections. IV. Evaluation of the results obtained in 370 cases. *Arch. Immunol. Ther. Exp.*, **33**, 219–240.
24. ŚLOPEK S., KUCHARIEWICZ-KRUKOWSKA A., WEBER-DĄBROWSKA B. and DĄBROWSKI M. (1985): Results of bacteriophage treatment of suppurative bacterial infections. V. Evaluation of the results obtained in children. *Arch. Immunol. Ther. Exp.*, **33**, 241–260.
25. ŚLOPEK S., KUCHARIEWICZ-KRUKOWSKA A., WEBER-DĄBROWSKA B. and DĄBROWSKI M. (1985): Results of bacteriophage treatment of suppurative bacterial infections. VI. Analysis of treatment of suppurative *Staphylococcus* infections. *Arch. Immunol. Ther. Exp.*, **33**, 261–273.
26. ŚLOPEK S., WEBER-DĄBROWSKA B., DĄBROWSKI M. and KUCHARIEWICZ-KRUKOWSKA A. (1987): Results of bacteriophage treatment of suppurative bacterial infections in the years 1981–1986. *Arch. Immunol. Ther. Exp.*, **35**, 569–583.
27. VALDVOGEL F. (1999): New resistance in *Staphylococcus aureus*. *N. Engl. J. Med.*, **340**, 556–557.
28. VIEU J. F. (1975): Les Bacteriophages. In FABRE J. (ed.): *Fraite de Therapeutique*, Vol. Serums et Vaccins. Flammarion, Paris, 337–400.
29. VIEU J. F. et al. (1979): Donnees actuelles sur les applications therapeutiques des bacteriophages. *Bull. Natl. Med.*, 103–161.
30. WEBER-DĄBROWSKA B., KOŹMIŃSKA J., MULCZYK M. and KACZKOWSKI H. (1996): The use of bacteriophages in the treatment of chronic suppurative sinusitis (in Polish). *Post. Med. Klin. Dośw.*, **5**, 291–293.
31. WEBER-DĄBROWSKA B., MULCZYK M. and GÓRSKI A. (2000): Therapy of infections in cancer patients with bacteriophages. *Clin. Appl. Immunol. Rev.* (in press).
32. WEBER-DĄBROWSKA B., ZIMECKI M. and MULCZYK M. (2000): Effective phage therapy is associated with normalization of cytokine production by blood cell cultures. *Arch. Immunol. Ther. Exp.*, **48**, 31–37.

Received in August 2000

Accepted in August 2000