ARCHIVUM IMMUNOLOGIAE ET THERAPIAE EXPERIMENTALIS
2000, 48, 547

Bacteriophage therapy of bacterial infections: an update of our Institute`s experience

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*Supported by grant 4P05B01219 from KBN

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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%) BP treatment was found to be ineffective. The results confirm the high effectiveness of BP therapy in combating bacterial infections which do not respond to treatment with all available antibiotics.

Key words: phage therapy, drug resistance, bacterial infections

Bacteriophages (BP) are the viruses that attack bacteria, multiply within and cause disruption of bacterial cell (lysis). Their lytic action is highly specific. After the discovery of BP 85 years ago it was hoped that they will be useful in the treatment of bacterial infections. BP therapy was initiated in 1921 by Bruynoghe and Maisin (4) in the treatment of staphylococcal infections. Although the results were promising little was accomplished in this field during next years. The idea of potential applications of BP therapy was abandoned after introduction into medical practice sulphonamides and then antibiotics. However, lytic action of BP in vitro enabled some investigators to use specific BP for differentiation of various species of bacteria. Many phage typing schemes were elaborated. These methods of differentation are still used worldwide and are very useful in epidemiological investigation (1). 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 drug including antibiotic of last resort - vancomycin are spreading all over the world (6,7,10,12-15,27). This resistance is mainly dissiminated 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 multidrug resistant bacteria is ineffective, and 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 become important alternative to antibiotics in the treatment of bacterial infections. In many cases successful results were obtained in combating infections in humans and animals (1-3,5,8,9,11,17,18,28-30, 32).

BP therapy has been extensively used in Bacteriophage Institute, Tbilisi, Georgia (for rev. see Kutter 9). It was found that specific BP are effective in both prophylaxis and treatment of bacterial infections caused by drug resistant bacteria of different origin. Extensive studies on BP therapy was also carried out in the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroc?aw, Poland. In the years 1981-1986 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 followed ineffective treatment with all available antibiotics. Positive therapeutic effect was obtained in 508 cases i.e. 92,4% (range 75-100%). It was found that BP therapy effectively controls the infections process irrespectively of its localization, age, 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 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 multidrug resistant bacteria. The majority of cases were long, persisting infections in which antibiotic therapy failed. The age of patients ranged from 4 weeks to 86 years. Our studies included isolation and identification of bacterial strains from specimens of patients, determination of sensitivity of the isolated strains to specific BP, and preparation of crude sterile BP lysates for therapy, as described in details earlier (20). In each case, BP were administered orally 3 times daily in the amount of 10 ml (children 5 ml) 30 min before meal after neutralization of the gastric juice. Local administration depended upon a localization of suppurative process. BP were applied directly to the wounds, ear and nose drops, infusions to the fistulas, washing of the nasal cavity, suppurative lesions of pleura and peritoneum, decubitus, fistulas, intraperitoneally during the washing of peritoneal cavity and topically in the cases of multiple skin abscesses.

The BP therapy was carried out at university clinics or hospital departments. The clinical results of BP therapy were evaluated by physicians responsible for patients' care. BP treatment run for 1-12 weeks with an average of 32 days. The results of BP therapy applied in bacterial infections 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 - *Escherichia, Klebsiella, Proteus, Enterobacter, Pseudomonas* and *Staphylococcus aureus (furunculosis)*. It must be stressed that 2738 (69,2%) strains were isolated from infections caused by one species of bacteria, in great majority by *Staphylococcus aureus* (1674 strains) - monoinfections. The remaining 1218 (30,8%) strains were isolated from infections caused by several species of bacteria (polyinfections). *Staphylococcus* and *Pseudomonas* occured more frequently in monoinfections; *Klebsiella, Escherichia, Enterobacter* and *Proteus* occured more frequently in polyinfections. In 1123 (85,9%) patients treated with BP a complete recovery or healing of the local lesions was obtained (range 64-100%), according to etiologic factor and type of infections. 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 *Staphylococcus aureus* and *Pseudomonas 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% respectively (Table 2). Fig.1 and 2 depict representative results of BP therapy.

Our results extend and confirm our earlier data showing the effectiveness of BP therapy in combating of antibiotics-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 only the success of BP therapy is associated with the sensitivity of a 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 consequence of BP upregulation of the immune response.While monitoring of the immune status of patients receiving BP we noted that effective BP therapy is associated with normalization of cytokine production by blood cell cultures (31). Moreover, our preliminary data indicate that purified BP may induced intracytoplasmatic cytokine synthesis in human lymphocytes and monocytes (Górski et al. unpublished observations). One may assume that BP also have immunoregulatory properties by interacting with immunocompetent cells. Further studies on immunoregulatory effect of BP are underway. In addition, a double-blind placebo-controlled clinical trial on effectiveness of BP therapy should be completed within the next 6 months.

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

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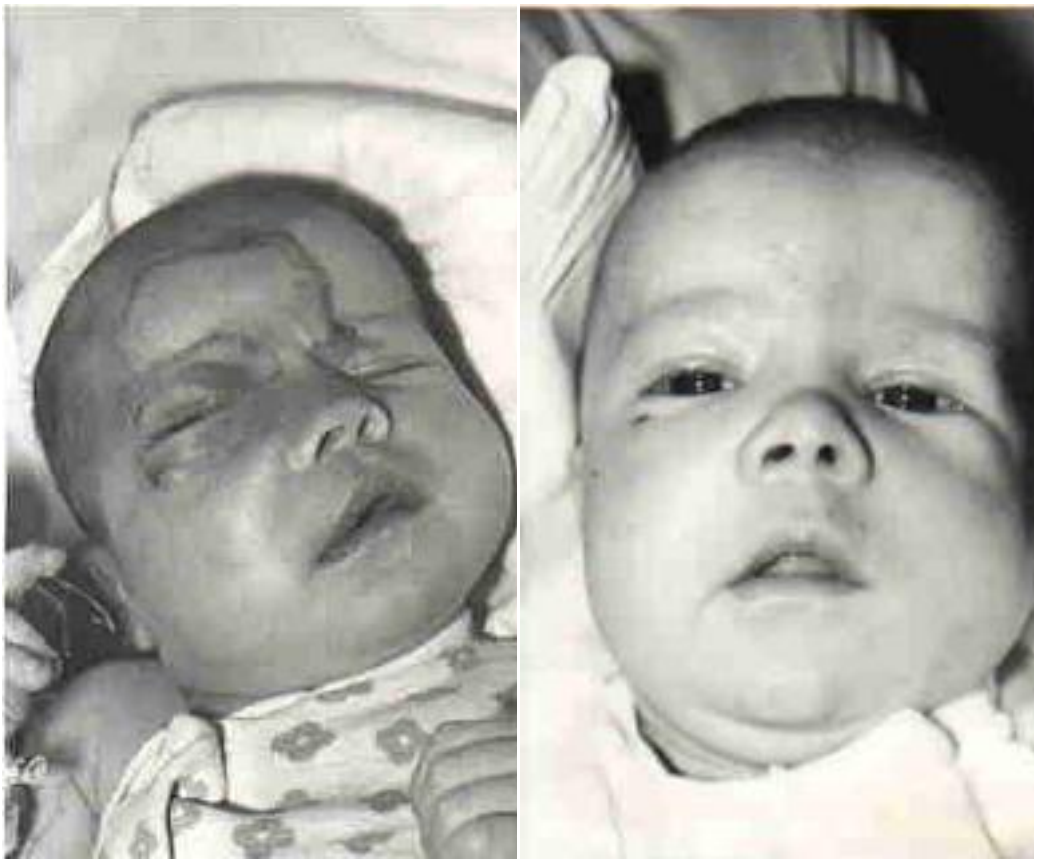
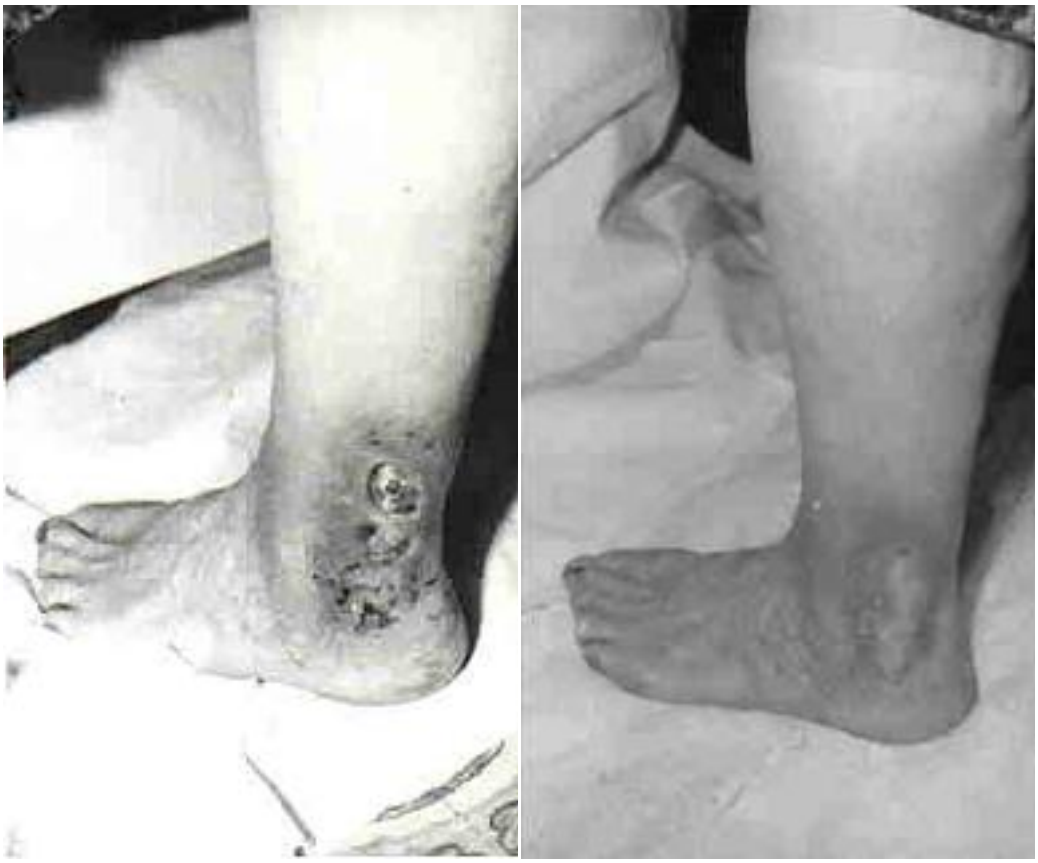
Figure 1. Abscess of nasal area.



A: prior to BP therapy, B: following BP therapy

Figure 2. Infected ulcer.



A: prior to BP therapy, B: following BP therapy

Table 1. Results of bacteriophage treatment (1307 cases).

		Number of cases			
Clinical diagnosis	Etiology	Subjected to phage therapy	Full recovery*	Marked improve-ment**	No effect
Septicemia	*Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Pseudomonas*	106	93 (87.7%)	8 (7.5%)	5 (4.7%)
Purulent otitis media	*Staphylococcus aureus, Klebsiella, Pseudomonas*	33	28 (88.4%)	3 (9.09%)	2 (6.06%)
Purulent meningitis	*Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Pseudomonas*	10	10 (100%)		
Varicose ulcers of lower extremities	*Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Pseudomonas*	77	47 (61.03%)	21 (27.2%)	9 (11.6%)
Mucopurulent chronic bronchitis, laryngitis, rhinitis	*Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Pseudomonas*	271	224 (82.6%)	46 (16.9%)	1 (0.3%)
Bronchopneu-monia, empyema	*Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Pseudomonas*	57	47 (82%)		10 (18%)
Pleuritis with fistula	*Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Pseudomonas*	49	42 (86%)	5 (10%)	2 (4%)
Suppurative peritonitis	*Staphylococcus aureus, Escherichia coli, Klebsiella, Enterobacter, Proteus, Pseudomonas*	66	60 (91%)	5 (8%)	1 (0.15%)
Urinary tract infections	*Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Pseudomonas*	78	59 (75.6%)	9 (11.5%)	10 (12.8%)
Furunculosis	*Staphylococcus aureus*	90	90 (100%)		
Decubitus with infection	*Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Pseudomonas*	16	13 (81%)		3 (19%)
Pyogenic arthritis and myositis	*Staphylococcus aureus, Escherichia coli,*	19	17 (89%)		2 (11%)

	Klebsiella, Proteus, Pseudomonas				
Osteomyelitis of the long bones	*Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Pseudomonas*	40	38 (95%)	2 (5%)	
Suppurative osteitis after bone fractures	*Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Pseudomonas*	41	37 (90%)	4 (10%)	
Pyogenic infections of burns	*Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Pseudomonas*	49	42 (86%)	7 (14%)	
Pyogenic postoperative infection	*Staphylococcus aureus, Escherichia coli, Klebsiella, Pseudomonas*	35	29 (83%)	6 (17%)	
Chronic suppurative fistulas	*Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Pseudomonas*	180	168 (93%)	12 (7%)	
Suppurative sinusitis	*Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Pseudomonas*	46	38 (83%)	3 (7%)	5 (11%)
Purulent mastitis	*Staphylococcus aureus, Escherichia coli*	44	41 (93.1%)	3 (6.8%)	
		1307	1123 (85.9%)	134 (10.2%)	50 (3.8%)

*Full recovery and complete elimination of bacteria
** Improvement, bacteria still detectable

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

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