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Adaptive Phage Therapy in the Treatment of Patients with Recurrent Pneumonia (Pilot Study)

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Адаптивная фаготерапия пациентов с рецидивирующими пневмониями (пилотное исследование)

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Summary

Aim. To evaluate the safety and efficacy of the adaptive phage therapy technique in patients with recurrent pneumonia in neurological critical care.

Material and methods. The clinical study included 83 chronically critically ill patients with severe brain damage. The bacteriophage cocktail selected against specific hospital strains was administered by inhalation to 43 patients. The control group included 40 patients who received conventional antimicrobial therapy. The changes in clinical, laboratory and instrumental parameters, levels of biomarkers, microbiological and PCR tests of bronchoalveolar lavage fluid were assessed, including those in the «phage therapy with antibiotics» (*n*=29) and «phage therapy without antibiotics» (*n*=14) subgroups.

Results. The groups were comparable in terms of basic parameters (age, sex, diagnosis, organ dysfunction according to APACHE II, use of vasoactive drugs) and the level of airway colonization with antibiotic-resistant bacterial strains. Good tolerability and absence of clinically significant side effects were observed during inhalational administration of the bacteriophage cocktail. Computed tomography on day 21 showed a significant reduction in lung damage in patients who received bacteriophages. Patients treated with bacteriophages without antibiotics had significantly lower need for mechanical ventilation. The mortality rate on day 28 did not differ significantly and was 4.7% (2/43) in the bacteriophage-treated group vs 5% (2/40) in the control group.

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Conclusion. The first experience of using the adaptive phage therapy technique in chronically critically ill patients in neurological intensive care demonstrated the safety of inhalational administration of the bacteriophage cocktail. The efficacy of the technique was confirmed by the treatment results obtained in the phage therapy group, which were not inferior to those in the group with conventional antibiotic therapy, while several clinical and laboratory parameters tended to improve even in patients who received bacteriophages and did not receive antibiotics.

Keywords: antibiotics; resistance; pneumonia; bacteriophages; personalization; phage therapy

Conflict of interest. The authors declare no conflict of interest.

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Introduction

Chronically critically ill patients remain in intensive care units (ICU) for a long time and require comprehensive treatment, including life support. This creates conditions for the development of prolonged and recurrent pneumonia associated with hospital-acquired multidrug-resistant bacterial strains.

Currently, the antibiotic armamentarium for the treatment of patients in chronic critical illness has almost been exhausted. The treatment of infections caused by multidrug-resistant bacteria, including carbapenemase-producing pathogens, is extremely difficult due to the highly limited choice of effective drugs; their treatment is characterized by such negative sequelae as prolonged hospital stay, adverse treatment outcomes, and increased direct and indirect costs [1].

A potentially promising alternative to antibiotics could be the use of bacteriophages to treat and prevent nosocomial infections. Bacteriophages are intracellular obligate parasites of bacteria and play an important role in the natural regulation of bacterial populations. In the 1930s and 1940s, bacteriophages were actively used in the Soviet Union in various areas of medicine. However, the beginning of commercial production of penicillin and other antibiotics as well as the need for individual selection of bacteriophages for a particular infectious agent halted large-scale research and widespread use of bacteriophages for a long time. The development of molecular biological methods and genome sequencing technologies contributed to a more rational approach to the selection and use of therapeutic bacteriophages, which allowed scientific research in this area to continue [2, 3].

Accumulated clinical experience demonstrates high efficacy and safety of therapeutic and prophylactic preparations with bacteriophages in the treatment of infections in otorhinolaryngology, surgery, urology [4–6].

A systematic literature review published in 2019 summarizes the results of 13 studies conducted in Russia, the United States, Western Europe, and Asia, in which bacteriophages have been used to treat and prevent infections in humans. Concluding the review, the authors acknowledge that the beneficial effect of phage therapy is undeniable [7].

Traditionally, bacteriophages are used to treat infections caused by so-called «wild» strains with innate antibiotic sensitivity. In recent years, an increasing number of researchers and developers of antimicrobial drugs report the possibility of using bacteriophages active against antibiotic-resistant strains, in particular, a wide range of clinical isolates of *Staphylococcus aureus*. Experimental studies on laboratory animals confirm the effectiveness of bacteriophages against antibiotic-resistant strains of microorganisms causing pneumonia and sepsis [8–11]. In a clinical study by Australian researchers published in Nature Microbiology in 2020, the efficacy of a bacteriophage preparation was evaluated in 13 patients with severe staphylococcal infections, including endocarditis and septic shock [12]. The drug produced from three bacteriophages was administered intravenously twice a day for 14 days under careful monitoring of hematological and biochemical parameters; good tolerability, high safety, and no local and systemic adverse reactions were noted. However, the circulation of several «challenging» Gram-negative and Gram-positive strains simultaneously is characteristic of present-day ICU. Moreover, the continuous use of the latest generations of antibiotics with unpredictable frequency results in the selection of pan-resistant pathogens, which may cause outbreaks of hospital infections. In these circumstances, there is a need for a complex drug that will contain a set of bacteriophages active against the entire list of challenging pathogens in a particular ICU. In addition, periodic monitoring of this drug's efficacy is necessary, and, in case of resistant strain selection, new bacteriophages should be added to the preparation. In other words, the drug should be adapted to the changing microbial landscape.

This challenging issue is being addressed by the collaborative efforts of the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology and the «MicroMir» research and production center. Based on a hospital bacteria collection of 66 antibiotic-resistant strains iso-

lated from 40 ICU patients of the Center of Intensive Care Medicine and Rehabilitology, «cocktail» of bacteriophages for inhalation was developed and proposed as an adaptive phage therapy method.

The preparation included about 50 bacteriophages active against bacterial respiratory pathogens, mainly hospital multidrug-resistant strains, such as *Acinetobacter baumannii, Citrobacter freundii, Enterobacter cloacae, Enterobacter kobei, Enterococcus faecium, Klebsiella pneumoniae* subsp. *ozaenae, Klebsiella pneumoniae, Cutibacterium acnes, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Stenotrophomonas maltophilia, Streptococcus pyogenes*. The preparation contains 3 to 4 virulent bacteriophages against each of the pathogens above.

The aim of the study was to evaluate the safety and efficacy of adaptive phage therapy technique in the treatment of patients with recurrent pneumonia in neurological critical care.

Material and Methods

Type of clinical study: prospective, nonrandomized, open-label parallel group study.

The site of the study: intensive care units (ICU) of the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, where patients with severe brain damage after acute stroke, traumatic brain injury (TBI), brain tumor surgery, anoxia, etc., are treated. This study included chronically critically ill patients transferred from intensive care units of other medical institutions for further intensive treatment.

The term «chronically critically ill» refers to those who have survived acute critical disease of any etiology, but remain long-term patients requiring intensive monitoring and temporary life support [13].

The study protocol was reviewed and approved by the Local Ethics Committee of the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology No. 4/20 dated September 22, 2020.

Patient groups. According to the Protocol, adult patients admitted to the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology were prospectively included in the study if they met the following inclusion criteria:

1. Patient age > 18 years.
2. Chronic critical illness, ICU stay for more than 2 weeks, previous treatment with antibiotics.
3. Previous pneumonia with the risk of recurrence, clinical indications for antimicrobial treatment (CT evidence, etc.).
4. Informed consent of the patients or their close relatives.

Exclusion criteria:

1. Low survival chance (SAPS II score > 65).
2. Treatment with immune suppressive drugs, including steroids.
3. Signs of acute infection/sepsis (according to the Sepsis-3 criteria).
4. Procalcitonin level > 2 ng/ml.
5. Candidemia.

Patients were enrolled gradually.

At the first stage of the study, consecutive enrollment of eligible patients was performed without intervention in the treatment process, with recording of clinical, laboratory and microbiological monitoring parameters at four time points (days 1, 7, 14, and 28 after admission).

At the second stage, the patients included in the study were prescribed a bacteriophage cocktail in addition to conventional antimicrobial therapy (safety assessment).

In the third stage, when the procalcitonin level was less than 0.5 ng/ml, only the bacteriophage cocktail without antibiotics was administered to selected patients.

Eighty-three patients were included in the study.

The main Group 1 consisted of 43 patients who received the bacteriophage cocktail. This group was divided into 2 subgroups: «phage therapy with antibiotics» (antibiotics + bacteriophage cocktail, Group 1A, n=29) and «phage therapy without antibiotics» (bacteriophage cocktail only, Group 1B, n=14).

Group 2 included patients who received conventional antibiotic therapy, n=40.

Patients received comprehensive treatment and rehabilitation including life support, drugs to improve consciousness level, nutritional and metabolic therapy, symptomatic care, etc. Therapeutic and rehabilitation measures were performed by specialists who were not aware of the inclusion of patients in this study.

The «Ground Glass» software for automatic assessment of the ground glass opacity lesions was used to analyze the chest CT scans. Segmentation of the right and left lungs along with trachea was performed with the threshold of -250 HU. The lesions with customized densities (from -785 HU to 150 HU as a default) were outlined inside the lungs. The small vessels which could be mislabeled as «lesions» were excluded from assessment using the morphological closing operation.

During the study, the patients were under continuous clinical and laboratory monitoring with assessment of cardiovascular, neurological, respiratory parameters, liver and kidney function, as well as the organ dysfunction using the SOFA score. Serial measurement of serum biomarkers (C-reactive protein, procalcitonin) was carried out. Levels of albumin, urea, creatinine, and CRP were determined

Table 1. Main characteristics of patients.

Parameter	Values in groups				*P*
	1, *n*=43			2, *n*=40	1A+1B vs. 2
	1A, *n*=29	1B, *n*=14	1A+1B		
Age, median	62 (39–71)	50 (32–74)	60 (38–72)	64 (54–72)	0.177
Sex, male/female, *n*	20/9	10/4	30/13	21/19	0.120
Diagnosis, *n* (%)					
Post:					
stroke	16 (55)	6 (43)	22 (51)	26 (65)	0.267
TBI	9 (31)	4 (29)	13 (30)	7 (18)	0.206
brain surgery	1 (4)	2 (14)	3 (7)	4 (10)	0.706
anoxia	3 (10)	2 (14)	5 (12)	3 (7)	0.714
APACHE II severity score, day 1, median	12 (8–15)	11 (10–16)	12 (10–15)	10 (8–14)	0.287
Lung ventilation required, Day 1, *n* (%)	14 (48)	5 (36)	19 (44)	24 (60)	0.189
Number of antibiotic-resistant bacteria in the initial BAL fluid sample, total (average per 1 patient)	46/29 (1.6)	25/14 (1.8)	71 (1.7)	72 (1.8)	—

Note. For tables 1–6, figures 1, 2: 1A — antibiotics + bacteriophage cocktail; 1B — bacteriophage cocktail alone; 2 — antibacterial therapy without bacteriophage cocktail.

on an automatic biochemical analyzer AU 480 (Beckman Coulter; USA) using original reagents. The procalcitonin concentration was measured on a VIDAS immunoanalyzer (bioMerieux SA, France).

Third-party laboratory physicians performed microbiological studies of bronchoalveolar lavage (BAL) fluid using culture and PCR methods. For microbiological examination a morning sputum sample was taken in sterile tubes in aseptic conditions. The time interval after the last administration of antibiotics and sputum collection was 8–12 hours. The native clinical specimens were immediately transported to the bacteriological laboratory. Microorganisms were identified and antibiotic sensitivity was determined using a BD Phoenix-100 automated system (USA).

To assess the taxonomic composition of BAL fluid a reagent kit for DNA isolation from clinical material «RIBO-prep» and reagent kits for the detection and quantification of *Pseudomonas aeruginosa* DNA, *Enterobacteriaceae* DNA, Staphylococci (*Staphylococcus* spp.) and Streptococci (*Streptococcus* spp.) were used. Qualitative assessment of antibiotic resistance genes was performed using reagent kits for the detection of acquired carbapenemase genes of KPC and OXA48-like groups (types OXA48 and OXA162), acquired carbapenemase genes VIM, IMP and NDM of MBL groups (Amplisens, Russia) by PCR with real-time hybridization-fluorescent detection of amplification products. Measurements were performed on a CFX 96 plate amplifier (BioRad; USA).

Statistical analysis was performed using Statistica 10.0 software. The generally accepted mathematical and statistical methods were used to calculate the main characteristics of sample groups. The Shapiro–Wilk test was used to assess the normality of variable distribution in the groups. Mann–Whitney test was used to analyze non-normally distributed variables. Wilcoxon test was used for comparative analysis of quantitative variables. Fisher's exact test was used to compare proportions (frequencies). To compare proportions at different time points, the McNimar test was used (Table 4). Data were presented as median ±25–75 percentiles (25–75 IQR). The critical level of significance was set at 0.05, and Bonferroni correction was used for multiple pairwise intergroup comparisons.

Results

The main characteristics of patients enrolled in the study are given in Table 1.

The groups were comparable in terms of sex, age, etiology of brain injury, severity of condition according to scales, and ventilation requirement.

The safety and efficacy of the bacteriophage cocktail were assessed using clinical and laboratory monitoring methods.

Assessment of clinical parameters. Neurological status of all chronically critically ill patients did not change over time. There were no differences between the groups in the severity of acute respiratory failure, also there were no significant changes in PaO_2/FiO_2 ratio in the groups. In Group 1A, there were significant positive changes in the severity of lung lesions according to CT (Table 2). No such improvement was observed in group 1B.

During the observation period 1 patient (6.7%) was weaned from mechanical ventilation in group 1B and 1 patient (3.1%) was weaned in group 1A. Organ dysfunction score according to the SOFA scale did not exceed 4–5 points in all groups during the study period. There were no significant differences between the groups in the frequency of vasoactive drug administration. The study did not reveal any clinically significant side effects of the bacteriophage cocktail administration. Mortality by day 28 was comparable in group 1 (2/43, 5%) and in group 2 (2/40, 5%) (*P*=1.0) with no fatal outcomes in subgroup 1B.

Table 2. Changes in lung lesion severity based on chest CT scans.

Parameter	Day	Values in subgroups		*P* (1A vs.1B) Mann–Whitney test for independent groups
		1A, *n*=22*	1B, *n*=12*	
Lung volume, ml[3]	1	3198 (2524–4221)	3125 (2580–3441)	0.510
	21	3844 (2341–4503)	2983 (2336–3705)	0.292
P (day 1 vs day 21) pairwise comparison using the Wilcoxon test		0,485	0,530	—
Lung lesion volume, %	1	33 (16–40)	30 (18–37)	0.683
	21	22 (10–38)	30 (4–56)	0.736
P (day 1 vs day 21) pairwise comparison using the Wilcoxon test		0.027	0.859	—

Note. * — lung CT data are shown only for 34 of 43 (80%) patients due to failure of automatic calculation of lung lesion volume in some scans.

Table 3. Changes in clinical and laboratory parameters during the first two weeks of treatment in the study groups

Clinical and laboratory parameters	Values in the groups				*P*
	1, *n*=43			2, *n*=40	1A+1B vs. 2
	1A, *n*=29	1B, *n*=14	1A+1B		
On mechanical ventilation, day 1, *n* (%)	14/29 (48)	5/14 (36)	19/43 (44)	24/40 (60)	0.189
On mechanical ventilation, day 7, n (%)	18/29 (62)	3/14 (21)	21/43 (49)	25/40 (63)	0.270
On mechanical ventilation, day 14, n (%)	15/27 (56)	2/14 (14)	17/41 (41)	25/39 (64)	0.048
Albumin level, day 1, g/l	30 (27–33)	31 (25–35)	30 (26–32)	28 (24–35)	0.549
Albumin level, day 7, g/l	30 (28–32)	29 (27–37)	29 (27–31)	26 (24–29)	0.001
Albumin level, day 14, g/l	29 (27–31)	31 (25–34)	29 (26–32)	26 (24–30)	0.071

Table 4. Frequency of abnormal liver or renal function parameters by day 14 of treatment compared with the data on day 1 of follow-up.

Patients with abnormal clinical chemistry parameters	Number of patients, *n* (%)							
	Group 1					Group 2		
	1A		1Б		*P**	Day 1	Day 14	*P**
	Day 1	Day 14	Day 1	Day 14				
Bilirubin	0/29	0/27	0/14	0/14	—	1/40 (2)	1/39 (3)	0.900
ALT	7/29 (24)	8/27 (30)	6/14 (43)	2/14 (14)	0.804	10/40 (25)	14/39 (36)	0.424
AST	5/29 (17)	4/27 (15)	2/14 (14)	0/14	0.900	6/40 (15)	7/39 (18)	0.900
Urea	11/29 (38)	5/27 (18)	4/14 (29)	4/14 (29)	0.021	12/40 (30)	9/39 (23)	0.791
Creatinine	5/29 (17)	5/27 (18)	2/14 (14)	0/14	0.625	12/40 (30)	8/39 (20)	0.424

Note. * — McNimar test. The proportions of patients with elevated laboratory values on day 1 and day 14 in the pooled group 1 were compared (day 1 vs. day 14 over time); similarly, for group 2.

Table 3 shows that the need for lung ventilation tended to increase in patients who received antibiotics (group 2 and subgroup 1A) and, conversely, decreased in subgroup 1B, where only bacteriophages were used. Monitoring of biochemical parameters in group 2 also revealed a significant decrease in albumin level on day 7.

Table 4 compares the groups in terms of the number of cases of abnormal values of liver and kidney function parameters.

In group 2 and subgroup 1A, where antibiotics were used for 2 weeks, the proportion of patients with elevated enzyme levels persisted or even increased compared to the baseline. At the same time, in subgroup 1B, where patients were treated with bacteriophages alone without antibiotics, this pattern was not observed; on the contrary, there was a tendency for reduced number of cases with elevated clinical chemistry parameters, such as ALT, AST, and creatinine.

Changes in the levels of biomarkers. Procalcitonin is one of the markers which provides rationale for withholding antimicrobial therapy.

Most chronically critically ill patients had procalcitonin levels below the reference values (0.25–0.50 ng/ml) during the entire follow-up period, only less than a third of patients had minor elevations (0.51–0.80 ng/ml). No significant differences between the subgroups were observed according to the laboratory monitoring data during two weeks after the study initiation (Table 5).

The level of C-reactive protein was dozens of times higher than the reference value (5 mg/l) in all patients. The changes in CRP level were assessed by the percentage of reduction by thirty percent or more compared to the baseline values. In group 1, a decrease in CRP concentration on day 7 was observed in 40% of patients, while in group 2, this parameter was reduced only in 30% of cases (Fig. 1).

Table 5. Procalcitonin (PCT) concentration over time in subgroups 1A and 1B of patients treated with bacteriophages, median (interquartile range).

Day of study	PCT (ng/ml) in subgroups		*P* value for 1A vs. 1B
	1A	1B	
1	0.08 (0.05–0.36)	0.11 (0.05–0.35)	0.771
7	0.14 (0.08–0.56)	0.14 (0.05–0.51)	0.866
14	0.12 (0.05–0.80)	0.1 (0.05–0.43)	0.525

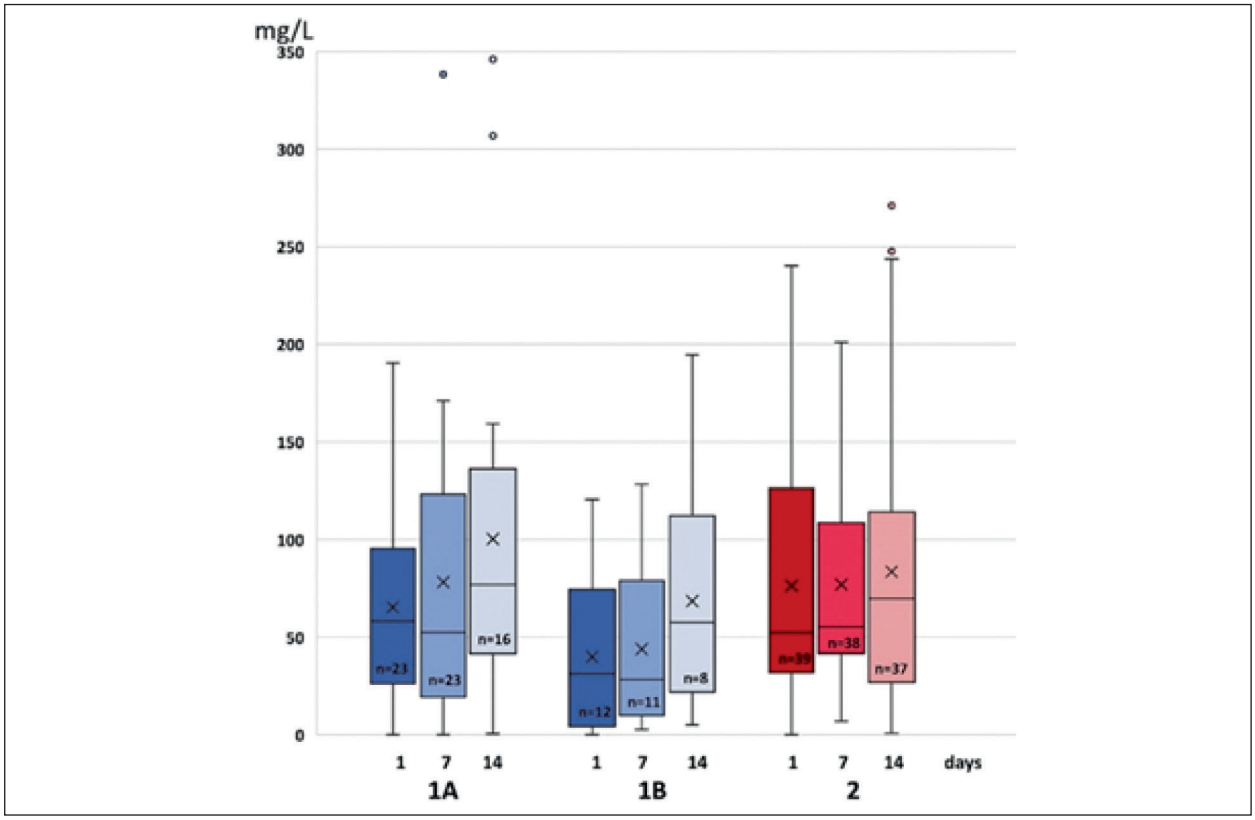

Fig. 1. Levels of C-reactive protein in the subgroups of patients treated with bacteriophages (median and interquartile range).
Note.

Day of study	*P*-values (after Bonferroni correction)		
	1A vs Group 2	1B vs Group 2	1A vs 1B
1	0.696	0.074	0.172
7	0.455	0.049	0.201
14	0.483	0.716	0.452

Results of microbiological monitoring.

The growth of hospital-acquired Gram-negative microorganisms in BAL fluid samples was observed in patients of different groups with approximately equal frequency at the time of inclusion in the study (Table 6).

By the end of the first week after inclusion, in Group 2 patients persistence of antibiotic-resistant strains such as *Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter* spp., and *Escherichia coli* was observed. At the same time, in Group 1 patients who received the bacteriophage cocktail, a decrease in the main challenging Gram-negative microorganisms, such as *Acinetobacter* spp., *Escherichia coli* and *Klebsiella pneumoniae,* was observed. *Serratia* spp. strains were less common, but were less amenable to elimination, and their count even increased in the antibiotic therapy group (Table 6).

Molecular genetic typing by PCR was performed for individual strains of the challenging hospital microorganisms. The results of DNA testing for P*seudomonas aeruginosa* and *Enterobacteriaceae* family (including *Escherichia coli, Klebsiella* spp., *P. mirabilis*) in BAL fluid were comparable with the obtained microbiological data and confirmed the lack of a significant increase in bacterial identification by day 14 after the start of therapy. An increase in DNA content of the Enterobacteriaceae family was noted by day 7, which probably reflected bacterial lysis on airway mucosa (Fig. 2).

Table 6. Microorganisms isolated from BAL fluid samples in patients on days 1 and 7 from the start of treatment in the study groups.

Microorganism	Group					
	1, *n*=43		2, *n*=40	1, *n*=43		2, *n*=40
	1A, *n*=29	1B, *n*=14		1A, *n*=29	1B, *n*=14	
	Day 1			Day 7		
Pseudomonas aeruginosa	10	4	11	4	8	13
Klebsiella pneumoniae	15	10	26	16	6	25
Acinetobacter baumannii/calcoaceticus	8	3	13	3	2	10
Enterococcus faecalis	—	—	3	1	—	1
Staphylococcus aureus/haemolyticus	—	—	5	—	—	4
Escherichia coli	5	1	7	3	—	5
Serratia plymuthica/Serratia marcescens	2	3	3	5	4	6
Stenotrophomonas maltophilia	—	2	2	2	—	3
Proteus mirabilis/vulgaris	2	—	6	5	1	9
Providencia stuartii/alcalifaciens	2	—	4	2	2	2
Others:						
Chryseobacterium meningosepticum	1		—	—	—	
Alcaligenes faecalis		1				2
Morganella morganii		1				

Staphylococcus spp. DNA content exceeding 10^3 copies/ml was detected only in 2 patients on day 1 in group 1A, with subsequent reduction back to reference values by day 7.

DNA of metallobetalactamase (VIM and NDM types) and carbapenemase (KPC and OXA48) genes was detected in 60% of patients in both subgroups at the time of inclusion in the study. The upward trend in antibiotic resistance persisted in both subgroups; by day 14, the KPC gene was detected in 100% of patients in the 1A subgroup, while in patients who received only the bacteriophage combination alone it was found in 90% of cases. A detailed analysis in subgroup 1A showed that in patients treated with carbapenems (*n*=10) there was a relative increase in the acquired carbapenemase genes of KPC and OXA48 groups by 10 and 22%, respectively, by day 7. However, patients in the same group receiving other antibiotics (*n*=13) had a 22% decrease in the detection of OXA48 group acquired carbapenemase genes.

Clinical case 1. Patient P., 38 years old, was injured in a traffic accident driving a motorcycle. He has sustained combined brain and abdominal trauma, the following surgeries were performed: laparotomy, splenectomy, adbdominal drainage. Later, multiple organ failure (respiratory, cardiovascular, hepatic, renal, nutritional) developed. On day 49 after the accident the patient was transferred to the Institute of Rehabilitation of the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology for further rehabilitation. On admission, the patient's general condition was extremely poor, he remained in a vegetative state.

During the stay in the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, the patient had 3 episodes of nosocomial pneumonia, the last one occurred on day 70 after the accident, he received repeated courses of antimicrobial therapy, including the antibiotics of latest generations, and the main pathogen of severe infectious complications was the antibiotic resistant strain of *Klebsiella pneumoniae*.

On day 80, to prevent nosocomial pneumonia recurrence, the bacteriophage cocktail active against antibiotic-resistant bacteria and customized for the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology based on the microbiological data of the biomaterial from the ICU patients was administered. Due to the severity of patients' condition and high risk of pneumonia recurrence, a long-term course of the bacteriophage cocktail inhalation for 4 weeks was prescribed. During this period no recurrent pneumonia episodes were observed. The procalcitonin level remained below the reference values (less than 0.25 ng/ml). In a series of bronchoalveolar lavage examinations, *Klebsiella pneumoniae* titer decreased, furthermore, the initial antibiotic-resistant strain was eliminated, and the strain isolated after the treatment course was sensitive to a wide range of antibacterial agents.

Clinical case 2. An 82-year-old patient underwent brain surgery to remove a tumor in the deep parts of the left frontal and parietal lobes. The postoperative period was complicated by bilateral extensive pneumonia, sepsis, and septic shock. The patient received multiple courses of antibiotic therapy. On day 84 the patient was transferred to the Institute of Rehabilitation of the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology for further rehabilitation. On admission, the patient's general condition was extremely poor, with minimal level of consciousness. Based on clinical assessment and CT scan findings, the patient was diagnosed with recurrent bilateral extensive pneumonia. The patient was prescribed a combination of vancomycin and meropenem. However, despite antibiotic therapy, the patient's

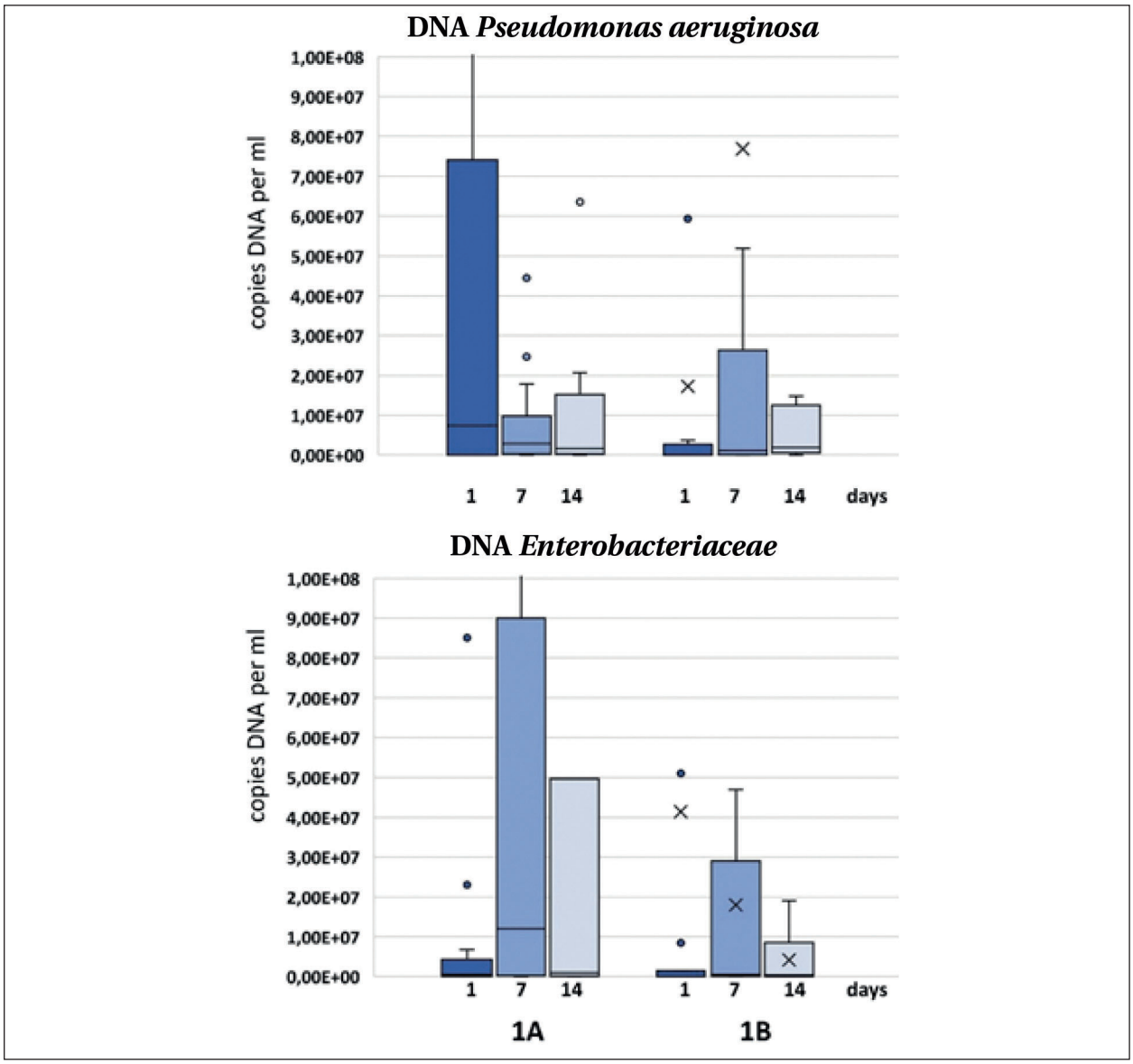

Fig. 2. The DNA content of *Pseudomonas aeruginosa* and *Enterobacteriaceae* in the group of patients receiving bacteriophages (subgroups 1A and 1B) (median, interquartile range).

Notes.

Day of study	*P*-value, Mann–Whitney test	
	1A vs 1B (*P. aeruginosa*)	1A vs 1B (*Enterobacteriaceae*)
1	0.122	0.118
7	0.542	0.309
14	0.837	0.595

condition deteriorated, respiratory failure progressed, and lung ventilation in BIPAP mode was initiated. Microbiological examination of BAL fluid revealed multiresistant strains of *Acinetobacter baumannii* and *Klebsiella pneumoniae*. On day 89, the bacteriophage cocktail active against antibiotic-resistant bacteria (including phage lysates of *Acinetobacter baumannii* and *Klebsiella pneumoniae* among others), customized for the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology based on the patient's microbiological data was added. According to the results of microbiological tests on days 96 and 102 (that is, 7 and 14 days after the start of bacteriophage administration), *Klebsiella pneumoniae* was not found in bronchoalveolar lavage, while the multiresistant *Acinetobacter baumannii* strain was still present. In view of clinical improvement, on day 10 of the treatment with bacteriophages, vancomycin was discontinued, on day 14 meropenem was stopped.

Thus, starting on day 104, the patient received only the bacteriophage cocktail. According to CT scan results, improvement was

found. From day 107 (19th day after the initiation of bacteriophage combination) the patient was weaned from mechanical ventilation. On auscultation improvement was also found with reduction or disappearance of crackles. Fever subsided; the level of inflammatory markers returned to normal values. The change of sputum characteristics was registered: it became more mucous-like and its volume decreased. The procalcitonin level was normal (less than 0.25 ng/ml), which indicated the absence of bacterial infection. On day 109 (21 days after the start of the bacteriophage combination), microbiological test of the BAL fluid showed no bacterial growth, which confirmed the efficacy of the bacteriophage combination.

Discussion

In our study, a preparation for inhalation containing dozens of bacteriophages was administered, which had not been studied before. The data show that the bacteriophage cocktail is more effective than individual phages in broadening the lytic spectrum and significantly reducing the risk of phage-resistant forms generation [14]. During our study, careful clinical and laboratory monitoring revealed no adverse events when using the bacteriophage preparation for inhalation at a dose of 5 ml, 2–3 times per day, for at least 14 days.

There are few publications describing the use of inhaled [15] or intravenous [12] bacteriophages in patients with pneumonia, bronchitis, infectious endocarditis, etc. The authors also confirm safety and claim no adverse reactions when using bacteriophages for therapeutic purposes.

Our study was the first to evaluate the safety of bacteriophage use in a group of patients with chronical critical illness. Along with a good tolerability and an absence of local and general adverse events, an important aspect to note is that the adaptive phage therapy technique has demonstrated safety in terms of the risk of hospital bacterial strains selection. The main clinical outcomes in Group 1 using the bacteriophage cocktail were similar to those of conventional antibiotic therapy. Moreover, in the group of combined use of antibiotics and bacteriophages (1A) reduced severity of lung damage according to CT scan was revealed, which suggests efficacy of this drug combination in the treatment of nosocomial pneumonia. Hyperinflammation, characterized by levels of CRP more than 10 times higher than the reference values, is characteristic of chronic critical illness [13, 16]. Reduced intensity of the inflammatory response associated with bacterial infection, i. e., a decrease in the CRP level, may be one of the effects of bacteriophage use [17]. And our study did reveal a trend toward decreasing the level of CRP in the group receiving the bacteriophage cocktail.

The microbiological and molecular genetic monitoring showed that in some patients the use of the bacteriophage cocktail was associated with elimination of major multidrug-resistant Gram-negative bacteria. However, the recurrent detection of *Serratia* spp., *Acinetobacter baumanii, Stenotrophomonas maltophilia* indicates the need for control and regular adaptation of bacteriophage composition in the preparation.

Involvement of antibiotic-resistant bacteria significantly worsens the course of nosocomial infection and increases morbidity and mortality [18]. Currently, molecular methods are the «gold standard» for detecting carbapenemase producers [19]. Serine carbapenemases of molecular types OXA48 and KPC, as well as MBL of type NDM, are most prevalent in *Enterobacteriaceae. P. aeruginosa* is characterized by the production of MBL types VIM and, to a lesser extent, IMP [20]. Microbiological monitoring using PCR in our study showed that carbapenem resistance genes were detected in more than 60% of patients included in the study starting from the first day. This high prevalence of resistant strains can be explained by the severity of the patients' condition, prolonged hospital stay, and massive antibiotic therapy including several classes of antibiotics. The results of the study showed that the use of the bacteriophage preparation did not associate with increased antibiotic resistance, accumulation and proliferation of carbapenemase and metallobetalactamase genes.

In the human body antibiotics are known to bind to proteins, undergo conjugation, be metabolized and actively eliminated by the organs, which requires energy costs and creates additional burden on the body of a long-term sick person. When assessing the changes in blood clinical chemistry parameters we obtained interesting data: after antibiotic withdrawal in subgroup 1B there were no liver enzyme (ALT and AST) elevations over two weeks. An important advantage of bacteriophages over conventional antibiotic therapy is that when they are used instead of antibiotics, the antimicrobial effect is achieved «passively»: without the participation of cells of the macroorganism, the organism «recovers» that is very important for chronically critically ill patients requiring rehabilitation [21].

We used the adaptive phage therapy technique, which implies strict matching of the bacteriophage set to the needs of a particular ICU. Avoiding the need for individual selection of antibacterial agents based on microbiological testing of every specific patient, when implemented in practice, could reduce the decision time if treatment must be started immediately, thereby increasing the effectiveness of treatment of critically ill patients.

Conclusion

This clinical study presents the first experience in the use of adaptive phage therapy technique in the neurological critical care. Safety, absence of side effects and adverse events were demonstrated for the inhalational administration of the bacteriophage cocktail in the treatment of chronically critically ill patients with recurrent pneumonia. The effectiveness of the technique was confirmed by the treatment outcomes seen in the phage therapy group, which were not inferior to those in the group receiving conventional antibiotic therapy. Several clinical and laboratory parameters tended to improve even in cases of complete withdrawal of antibiotics in favor of bacteriophages.

The microbiological and molecular genetic monitoring showed that the use of the bacteriophage cocktail was not associated with increased antibiotic resistance, accumulation and proliferation of carbapenemase and metallo-beta-lactamase genes. In several patients using the bacteriophage preparation, elimination of the major multiresistant Gram-negative bacteria was observed, while at the same time persistent respiratory tract colonization with some challenging microorganisms indicates the need for control and regular adjustment of bacteriophage composition. Despite the relatively small sample size, the results obtained in this study indicate the feasibility of further study of the effects of adaptive phage therapy as a promising alternative to antibiotics in patients in neurological critical care.

References

1. *Rello J., Kalwaje Eshwara V., Conway-Morris A., Lagunes L., Alves J., Alp E., Zhang Z., Mer M., TOTEM Study Investigators.* Perceived differences between intensivists and infectious diseases consultants facing antimicrobial resistance: a global cross-sectional survey. *Eur J Clin Microbiol Infect Dis.* 2019; 38: 1235–1240. DOI: 10.1007/s10096-019-03530-1. PMID: 30900056
2. *Kutter E.* Phage therapy: Bacteriophages as natural, self-replicating antimicrobials. Practical Handbook of Microbiology, Third Edition. CRC Press; 2015. pp. 883–908.
3. *Merabishvili M., Pirnay J-P., Verbeken G., Chanishvili N., Tediashvili M., Lashkhi N., Glonti T., Krylov V., Mast J., Parys L.V., Lavigne R., Volckaert G., Mattheus W., Verween G., De Corte P., Rose T., Jennes S., Zizi M., De Vos D., Vaneechoutte M.* Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. *PLoS One.* 2009; 4: e4944. DOI: 10.1371/journal.pone.0004944. PMID: 19300511
4. *Perepanova T.S., Merinov D.S., Kazachenko A.V., Khazan P.L., Malova Y.A.* [Bacteriophage Therapy of Urological Infections]. *Urologiia.* 2020; 106–114. [In Russ.].
5. *Ooi M.L., Drilling A.J., Morales S., Fong S., Moraitis S., Macias-Valle L., Vreugde S., Psaltis A.J., Wormald P.-J.* Safety and Tolerability of Bacteriophage Therapy for Chronic Rhinosinusitis Due to Staphylococcus aureus. *JAMA Otolaryngol Head Neck Surg.* 2019; 145: 723–729. DOI: 10.1001/jamaoto.2019.1191. PMID: 31219531
6. *Liu D., Van Belleghem J.D., de Vries C.R., Burgener E., Chen Q., Manasherob R., Aronson J.R., Amanatullah D.F., Tamma P.D., Suh G.A.* The Safety and Toxicity of Phage Therapy: A Review of Animal and Clinical Studies. *Viruses.* 2021; 13. DOI: 10.3390/v13071268. PMID: 34209836
7. *Saperkin N.V., Kovalishena O.V., Kvashnina D.V., Ruizendaal E., Scholten R.* Efficiency of phage therapy in humans: systematic review. *J Infectology.* 2019; 11: 19–30.
8. *Cheng M., Liang J., Zhang Y., Hu L., Gong P., Cai R., Zhang L., Zhang H., Ge J., Ji Y., Guo Z., Feng X., Sun Ch., Yang Y., Lei L., Han W., Gu J.* The Bacteriophage EF-P29 Efficiently Protects against Lethal Vancomycin-Resistant Enterococcus faecalis and Alleviates Gut Microbiota Imbalance in a Murine Bacteremia Model. *Frontiers in Microbiology.* 2017. DOI: 10.3389/fmicb.2017.00837. PMID: 2853657.
9. *Yang X., Haque A., Matsuzaki S., Matsumoto T., Nakamura S.* The Efficacy of Phage Therapy in a Murine Model of Pseudomonas aeruginosa Pneumonia and Sepsis. *Frontiers in Microbiology.* 2021. DOI: 10.3389/fmicb.2021.682255. PMID: 34290683
10. *Takemura-Uchiyama I., Uchiyama J., Osanai M., Morimoto N., Asagiri T., Ujihara T., Daibata M., Sugiura T., Matsuzaki S.* Experimental phage therapy against lethal lung-derived septicemia caused by Staphylococcus aureus in mice. *Microbes Infect.* 2014; 16: 512–517. DOI: 10.1016/j.micinf.2014.02.011. PMID: 24631574
11. *Anand T., Virmani N., Kumar S., Mohanty A.K., Pavulraj S., Bera B.C., Vaid R.K., Ahlawat U., Tripathi B.N.* Phage therapy for treatment of virulent Klebsiella pneumoniae infection in a mouse model. *J Glob Antimicrob Resist.* 2020; 21: 34–41. DOI: 10.1016/j.jgar.2019.09.018. PMID: 31604128
12. *Petrovic Fabijan A., Lin R.C.Y., Ho J., Maddocks S., Ben Zakour N.L., Iredell J.R., Westmead Bacteriophage Therapy Team.* Safety of bacteriophage therapy in severe Staphylococcus aureus infection. *Nat Microbiol.* 2020; 5: 465–472. DOI: 10.1038/s41564-019-0634-z. PMID: 32066959
13. *Parfenov A.L., Petrova M.V., Pichugina I.M., Luginina E.V.* Comorbidity Development in Patients with Severe Brain Injury Resulting in Chronic Critical Condition (Review). *Obshchaya Reanimatologiya=General Reanimatology.* 2020; 16 (4): 72-89. [In Russ.]. DOI: 10.15360/1813-9779-2020-4-72-89

14. *Zurabov F., Zhilenkov E.* Characterization of four virulent Klebsiella pneumoniae bacteriophages, and evaluation of their potential use in complex phage preparation. *Virol J.* 2021; 18: 9. DOI: 10.1186/ s12985-020-01485-w. PMID: 33407669
15. *Slopek S., Weber-Dabrowska B., Dabrowski M., Kucharewicz-Krukowska A.* Results of bacteriophage treatment of suppurative bacterial infections in the years 1981–1986. *Arch Immunol Ther Exp.* 1987; 35: 569–583.
16. *Chernevskaya E.A., Megley A.Yu., Buyakova I.V., Kovaleva N.Yu., Gorshkov K.M., Zakharchenko V.E., Beloborodova N.V.* Taxonomic dysbiosis of microbiota and serum biomarkers as a reflection of the severity of central nervous system damage. *Vestnik Rossijskogo gosudarstvennogo meditsinskogo universiteta.* 2020; 5: 58–63 [In Russ.]. DOI:10.24075/vrgmu. 2020.053
17. *Miedzybrodzki R., Fortuna W., Weber-Dabrowska B., Górski A.* A retrospective analysis of changes in inflammatory markers in patients treated with bacterial viruses. *Clin Exp Med.* 2009; 9: 303–312. DOI: 10.1007/s10238-009-0044-2. PMID: 19350363
18. *van Hecke O., Wang K., Lee J.J., Roberts N.W., Butler C.C.* Implications of Antibiotic Resistance for Patients' Recovery From Common Infections in the Community: A Systematic Review and Meta-analysis. *Clinical Infectious Diseases. Clin Infect Dis.* 2017; 65 (3): 371–382. DOI: 10.1093/cid/cix233. PMID: 28369247
19. *Popov D.A., Bakulev A. N. National Medical Research Center of Cardiovascular Surgery.* Comparative review of the modern methods for carbapenemases detection. *Clinical Microbiology and Antimicrobial Chemotherapy.* 2019; 21 (2): 125–133. DOI: 10.36488/cmac. 2019.2.125-133
20. *Meletis G.* Carbapenem resistance: overview of the problem and future perspectives. *Ther Adv Infect Dis.* 2016; 3: 15–21. DOI: 10.1177/2049936115621709. PMID: 26862399
21. *Górski A., Międzybrodzki R., Żaczek M., Borysowski J.* Phages in the fight against COVID-19? *Future Microbiol.* 2020; 15: 1095–1100. DOI: 10.2217/fmb-2020-0082. PMID: 32845164.

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