

EFFICACY OF COMMERCIAL BACTERIOPHAGE PRODUCTS AGAINST ESKAPE PATHOGENS

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The ever-rising prevalence of multidrug-resistant bacteria necessitates the search for a therapeutic alternative to antibiotics. Using therapeutic products based on virulent bacteriophages might provide such an alternative. The aim of our study was to evaluate the efficacy of commercial phage products and natural bacteriophage monoisolates recovered from environmental sources against clinical strains of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. We compiled a collection of 147 strains that were subsequently genotypes using the MLST method. The efficacy of bacteriophages was evaluated in spot tests. The highest efficacy was demonstrated by "Staphylococcal bacteriophage" (86%, effective against *S. aureus*), "Purified polyvalent pyobacteriophage" (87.8%, effective against *K. pneumoniae*), and a group of phage products against *P. aeruginosa*, including "Pseudomonas aeruginosa bacteriophage" (87.5%), "Complex pyobacteriophage" (79.5–90%) and "Purified polyvalent pyobacteriophage" (90–92.5%). The efficacy of "Intesti bacteriophage", which targets *E. faecium*, was 4.2%. The efficacy of commercial phage products against *S. aureus* and *K. pneumoniae* was higher than the efficacy of individual phage monoisolates (60% for the *S. aureus* phage vB_SauP-436-3w and 5.9% for the *K. pneumoniae* phage vB_Kp_M_Seu621). Thus, all tested commercial phage products were highly effective against *P. aeruginosa*, *K. pneumoniae* and *S. aureus*. There are no commercial phage products on the market against other ESKAPE pathogens, including *Acinetobacter baumannii* and *Enterobacter cloacae*. Besides, there are no effective phage products against *E. faecium*. This dictates the need for new effective bacteriophages against these species.

Keywords: bacteriophages, phage therapy, microbiology, ESKAPE pathogens, bacteria

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Compliance with ethical standards: the study was carried out in strict compliance with the sanitary norms and epidemiological safety standards specified in the guidelines on the work with microorganisms belonging to hazard groups III–IV and causative agents of parasitic diseases (Guidelines 1.3.2322-08); supplementary guidelines № 1 to the guidelines on the work with microorganisms belonging to hazard groups III–IV and causative agents of parasitic diseases (Guidelines 1.3.2518-09), sanitary and epidemiological requirements for the handling of medical waste (Sanitary norms and regulations 2.1.7.2790-10), and Federal clinical recommendations on the rational use of bacteriophages in clinical and epidemiological practice.

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ЭФФЕКТИВНОСТЬ ПРЕПАРАТОВ БАКТЕРИОФАГОВ ПРОТИВ ПАТОГЕНОВ ГРУППЫ ESKAPE

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Ежегодный рост числа случаев выявления бактерий с множественной лекарственной устойчивостью делает актуальной задачу поиска альтернативы применяемым антибиотикам. Такой альтернативой могут быть препараты на основе вирулентных бактериофагов. Целью работы было оценить эффективность коммерческих фаговых препаратов и моноизолятов бактериофагов, выделенных из природных источников, против клинических штаммов *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae* и *Pseudomonas aeruginosa*. Была собрана коллекция из 147 штаммов, типированных методом МЛСТ. Оценка эффективности бактериофагов проводили методом спот-тестирования. Наиболее эффективными оказались препараты против *S. aureus* («Бактериофаг стафилококковый», 86%), *K. pneumoniae* («Пиобактериофаг поливалентный очищенный», 87,8%) и *P. aeruginosa* («Бактериофаг псевдомонас аеругиноза», 87,5%; «Пиобактериофаг комплексный», 79,5–90%; «Пиобактериофаг поливалентный очищенный», 90–92,5%). Для *E. faecium* эффективность препарата «Интести-бактериофаг» составила лишь 4,2%. При этом эффективность терапевтических препаратов, активных против *S. aureus* и *K. pneumoniae*, была выше эффективности отдельных моноизолятов бактериофагов (фаг *S. aureus* vB_SauP-436-3w — 60%, фаг *K. pneumoniae* vB_Kp_M_Seu621 — 5,9%). Таким образом, исследуемые препараты обладают высокой активностью против штаммов *P. aeruginosa*, *K. pneumoniae* и *S. aureus*. В свою очередь препаратов, действующих против остальных членов группы ESKAPE-патогенов (*Acinetobacter baumannii* и *Enterobacter cloacae*), а также эффективных против *E. faecium*, не представлено на рынке, что подчеркивает необходимость поиска новых бактериофагов.

Ключевые слова: бактериофаги, бактериофаговая терапия, микробиология, ESKAPE-патогены, бактерии

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Every year, multidrug resistant (MDR) bacteria are becoming more prevalent. MDR strains are defined as having resistance to three or more antibacterial drugs [1]. Bacterial infections caused by MDR strains pose a critical threat to global healthcare. Most MDR strains are found among the so called ESKAPE pathogens (an acronym for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*). These bacteria cause life-threatening nosocomial infections and are especially dangerous for individuals with compromised immunity and chronic conditions [2–4].

According to the World Health Organization, pathogenic bacteria can be classified in terms of threat prioritization as having critical, high or medium priority [1]. Carbapenem-resistant *A. baumannii*, *P. aeruginosa*, *Enterobacteriaceae spp.*, as well as *K. pneumoniae*, are critical priority pathogens. In some countries, the proportion of carbapenem-resistant isolates among *P. aeruginosa* and *K. pneumoniae* can be as high as 50 and 64%, respectively [5]. Methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *E. faecium* belong to the high-priority group. In some countries, MRSA strains amount to 43% of all *S. aureus* isolates, whereas vancomycin-resistant *E. faecium* makes up 59.1% [5]. The number of antibiotic-resistant isolates is constantly increasing.

Infections caused by drug-resistant ESKAPE pathogens dictate the need for novel therapeutic approaches. One of them involves using virulent bacteriophages as a complement or an alternative to antibacterial therapy. The first attempts to exploit bacteriophages in clinical practice were made in the early 20th century. So far, phages have proved to be effective antibacterial agents [6, 7]. Using virulent bacteriophages as therapeutic agents has several advantages. Most importantly, their interaction with a bacterial cell does not depend on the resistance profile of the latter. Phages co-evolve with their bacterial hosts and thereby learn to overcome the host's defenses.

Phage products available on Russia's pharmaceutical market are cocktails composed of several virulent phages. Such cocktails allow targeting an array of different bacterial strains. In Russia, most commercial phage products are manufactured by two companies: Microgen Scientific and Production Association and Micromir Research and Production Center. The manufacturers claim that their phage cocktails are effective against ESKAPE pathogens, including *E. faecium*, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa*. At present, there are no commercial phage preparations on the Russian market exerting activity against *A. baumannii* and *Enterobacter spp.* This emphasizes the importance of their development.

The aim of this work was to evaluate the efficacy of commercial phage cocktails and monoisolates of bacteriophages from environmental sources against clinical strains of *E. faecium*, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa*.

METHODS

Bacterial isolates

Isolates of *E. faecium*, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* ($n = 147$) were obtained from the inpatients of the Federal Research and Clinical Center of Physical-Chemical Medicine of the Federal Medical Biological Agency in 2018–2019. The cultures were grown on Columbia agar or soya broth (both by Oxoid; UK) at 37 °C for 18–24 h.

Bacterial species were identified by means of direct mass spectrometry profiling of bacterial lysates as described in

[8]. A saturated solution of α -cyano-4-hydroxycinnamic acid (Bruker Daltonics; Germany) in 50% acetonitrile and 2.5% trifluoroacetic acid was used as a matrix solution. Mass spectra were recorded on a Microflex MALDI TOF mass spectrometer (Bruker Daltonics; Germany). A bacterial test standard (Bruker Daltonics; Germany) was used for calibration. Mass spectra were recorded, processed and analyzed in flexControl 3.0 and flexAnalysis 3.0 (Bruker Daltonics; Germany). Species identification was aided by MALDI Biotyper 3.0 (Bruker Daltonics; Germany).

Determining bacterial sensitivity to antibiotics

Sensitivity of bacterial strains to antibiotics was evaluated by disk diffusion as recommended by the international Performance Standards for Antimicrobial Susceptibility Testing (Clinical and Laboratory Standards Institute) (CLSI) published in 2019 [9]. Gram-negative *K. pneumoniae* and *P. aeruginosa* were tested for sensitivity to ceftriaxone, gentamicin, ciprofloxacin, and meropenem. Gram-positive *S. aureus* and *E. faecium* were tested for sensitivity to erythromycin, ciprofloxacin and tetracycline. Additionally, *S. aureus* isolates were tested for resistance to oxacillin and gentamicin. Sensitivity of *E. faecium* to vancomycin was evaluated using a method of serial dilutions following CLSI recommendations [9].

Molecular genetic testing of bacterial strains

K. pneumoniae, *P. aeruginosa* and *E. faecium* strains were genotyped using multilocus sequence typing (MLST) following standard schemes [10–14]. For *S. aureus*, spa-typing was applied according to the standard protocol; this technique allows determining the sequence of the *Staphylococcus* protein A gene [15].

Bacterial DNA was isolated using a DNA-express kit (Lytech; Russia) following the manufacturer's protocol. DNA samples were stored at –20 °C. Genes targeted by genetic typing were amplified in a TETRAD DNA ENGINE thermocycler (MJ Research; USA). Amplification was carried out in 25 μ l of the reaction mix containing 66 mM Tris-HCl (pH 9), 16.6 mM $(\text{NH}_4)_2\text{SO}_4$, 2.5 mM MgCl_2 , 250 μ M of each dNTP, 1 Taq DNA polymerase unit (Lytech; Russia), and 10 pmol of primers. Amplification products were separated in 2% agarose gel stained with ethidium bromide for DNA visualization.

Sanger sequencing was performed in a 3730 DNA Analyzer (Thermo Fisher Scientific; UK). Gene sequences were analyzed in the Ridom StaphType TM software (Ridom GmbH; Würzburg, Germany) and Vector NTI Suite 9 (Thermo Fisher Scientific; UK). Allelic profiles and MLST types were determined by comparing the obtained nucleotide sequences to the sequences stored in the international PubMLST database [11].

Commercial phage products

In this study, we evaluated the efficacy of 14 commercial products of virulent bacteriophages manufactured by Microgen (Table 1). All phage products were bought at Moscow pharmacies and are approved for clinical use.

Isolation of bacteriophages from environmental sources

Bacteriophages capable of infecting some *K. pneumoniae* and *S. aureus* strains were isolated from water samples collected in different water reservoirs; isolation was performed using the enrichment culture method. Briefly, a 50 ml water sample was

Table 1. Commercial bacteriophage products used in the study

Name	Activity spectrum	Batch number	Manufactured in
"Staphylococcal bacteriophage"	<i>Staphylococcus aureus</i> and some other coagulase-negative staphylococci	N33	Nizhny Novgorod
		P332	Perm
"Pseudomonas aeruginosa bacteriophage"	<i>Pseudomonas aeruginosa</i>	N7	Nizhny Novgorod
"Klebsiella pneumoniae purified bacteriophage"	<i>Klebsiella pneumoniae</i>	P252	Perm
		P251	
"Klebsiella pneumoniae purified polyvalent bacteriophage"	<i>Klebsiella pneumoniae</i> , <i>Klebsiella ozaenae</i> , <i>Klebsiella rhinoscleromatis</i>	U27	Ufa
"Purified polyvalent pyobacteriophage"	<i>Staphylococcus spp</i> , <i>Streptococcus spp</i> , <i>Proteus spp</i> , <i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumoniae</i> , <i>Escherichia coli</i>	U1	Ufa
		U25	
"Complex pyobacteriophage"	<i>Staphylococcus spp</i> , <i>Enterococcus spp</i> , <i>Streptococcus spp</i> , enteropathogenic <i>Escherichia coli</i> , <i>Proteus vulgaris</i> , <i>Proteus mirabilis</i> , <i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumoniae</i> , <i>Klebsiella oxytoca</i>	N74	Nizhny Novgorod
		N45	
"Intesti bacteriophage"	<i>Shigella flexneri</i> , <i>Shigella sonnei</i> , <i>Salmonella typhimurium</i> , <i>Salmonella spp</i> , <i>Escherichia coli</i> , <i>Proteus spp</i> , <i>Enterococcus spp</i> , <i>Staphylococcus spp</i> , <i>Pseudomonas aeruginosa</i>	N101	Nizhny Novgorod
		N123	
		N86	
		N175	

filtered through a 0.45 µm Millipore filter (Merck Millipore; USA). A 2x lysogeny broth (LB) (Oxoid; UK) was combined with the water sample; 300 µL of the overnight bacterial culture were added to the mixture and incubated on a rocking shaker at 37 °C for 18 h. Then, bacterial cells were centrifuged at 3,500 g, and the supernatant was filtered through a 0.22 µm Millipore filter (Merck Millipore; USA). Monoisolates were obtained through a series of 3 sequential isolations from negative colonies. The obtained bacteriophages were grown in 50 ml of LB containing 300 µl of the overnight bacterial culture. Bacteriophage concentrations in the phage lysate were measured using a classic double layer agar method proposed by A. Gratia [16].

Evaluating the efficacy of commercial phage products and monobacteriophage lysates

The efficacy of lytic phages (titers of over 10^7) was evaluated in a spot test. Briefly, 0.1 ml of the overnight culture was combined with 0.6% semi-liquid LB agar. The resulting suspension was applied onto Petri dishes coated with 1.5% LB agar. After the top LB agar layer hardened, 5 µl of the studied phage was applied onto it and incubated at 37 °C for 18–24 h. In 24 h, either individual negative colonies or a transparent lysis zone were observed where the agar drop had been applied. If this was the case, the bacterial strain was considered sensitive to the tested phage. In the absence of a lysis zone, the

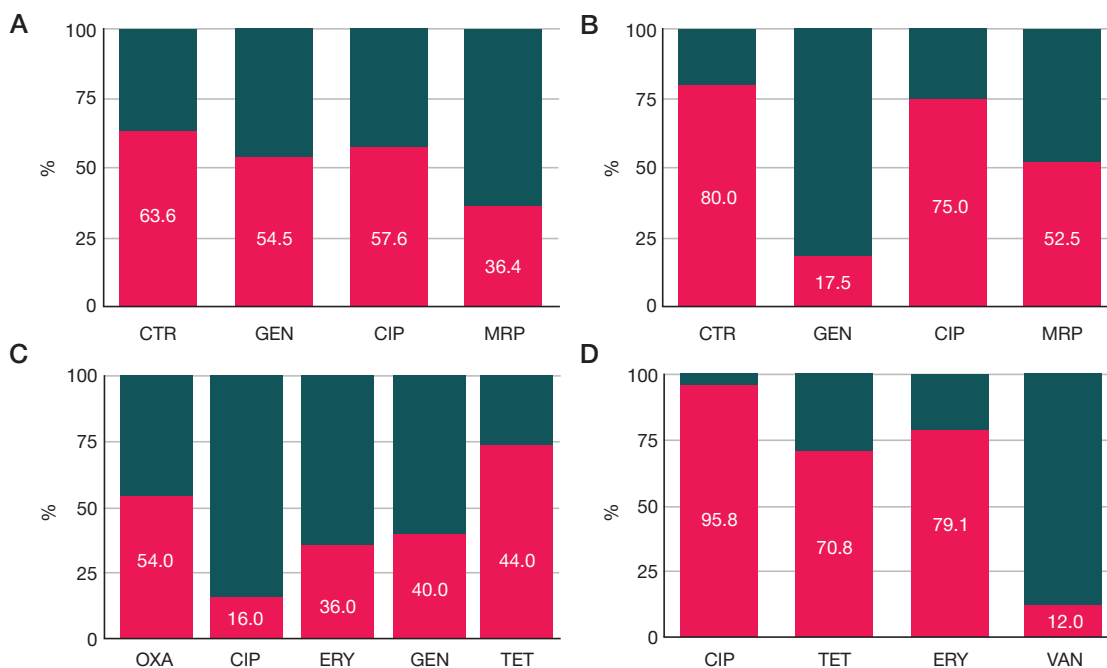


Fig. 1. Resistance to antibiotics among the strains of *K. pneumoniae* (A), *P. aeruginosa* (B), *S. aureus* (C), and *E. faecium* (D). The pink shows the proportion of resistant strains. CIP — ciprofloxacin, TET — tetracycline, ERY — erythromycin, MRP — meropenem, VAN — vancomycin, OXA — oxacillin, CTR — ceftriaxone, GEN — gentamicin

bacterial strain was considered resistant to the tested phage. The efficacy of a phage against a certain bacterial strain was determined as percentage of susceptible bacterial strains of a given species in the total pool of strains of this species included in our collection.

RESULTS

We compiled a collection of 147 bacterial strains, which included 33 strains of *K. pneumoniae* (22.5%), 40 strains of *P. aeruginosa* (27.2%), 50 strains of *S. aureus* (34%), and 24 strains of *E. faecium* (16.3%). Susceptibility profiles were obtained for all strains included in the collection (Fig. 1).

Of 33 *K. pneumoniae* strains, 9 (27.3%) were sensitive to all antibiotics they were tested against, 4 (12.1%) strains were resistant to only one antibacterial drug, and 17 (51.5%) strains exhibited multidrug resistance. Of 40 *P. aeruginosa* strains included in the collection, 7 (17.5%) were sensitive to all antibiotics they were tested against, 15 (37.5%) were resistant to one antibacterial drug, and 6 (15%) strains fell into the MDR category.

Of 50 *S. aureus* strains included in the collection, 19 (38%) were sensitive to all antibiotics they were tested against, 7 (14%) were resistant to one antibacterial drug, and 22 (44%) were classified as MDR. Twenty-seven (54%) *S. aureus* strains were resistant to oxacillin. There were no susceptible strains among *E. faecium* isolates; 3 (12.5%) of 24 *E. faecium* strains were resistant to one antibacterial drug, and 19 (19.2%) were multidrug-resistant. Vancomycin-resistant *E. faecium* strains amounted to 12%.

Using MLST, we identified 15 sequence types among *K. pneumoniae* strains (Fig. 2A). The most common of them were ST395 and ST23 represented by 14 (42.4%) and 5 (15.2%) strains, respectively. In addition, two unique sequence types were identified in this group of pathogens (2-1-1-1-9-4-1 and 2-1-1-1-9-4-18). According to MLST, *P. aeruginosa* strains fell into 26 different sequence types (Fig. 2B). ST12 was the most common sequence type among *P. aeruginosa* strains (5 out of total 40 strains; 12.5%). In addition, 3 unique sequence types were identified: type 15-5-11-8-4-4-1 represented by 2 strains, type 15-2-11-3-3-38-3 represented by 2 strains and type 17-5-12-3-14-4-7 represented by 1 strain. *E. faecium* strains belonged to 12 different sequence types, the most common being ST18 (4 out of 24 strains; 16.7%), ST17 (3 of 24 strains; 12.5%), ST78 (3 of 24 strains; 12.5%) and ST192 (3 of 24 strains; 12.5%) (Fig. 2C).

Spa-typing revealed the diversity of *S. aureus* strains (Fig. 2D) in our collection. This species was represented by 18 spa-types; the types t008 and t308 prevailed, accounting for 20 (40%) and 6 (12%) of the total 50 *S. aureus* strains.

The efficacy of 14 commercial phage products (see Table 1; Fig. 3) was tested on the compiled collection of characterized ESKAPE pathogens. The best effect against *K. pneumoniae* was observed for "Purified polyvalent pyobacteriophage", batch number U1, which killed 29 (87.9%) of 33 *K. pneumoniae* strains (Fig. 3A). The efficacy of the commercial phage products against *P. aeruginosa* varied from 76.9 to 92.5% (Fig. 3B). "Staphylococcal bacteriophage" was effective against 43 (86%) of 50 *S. aureus* strains (Fig. 3C). "Intesti bacteriophage", batch number P86, was the only

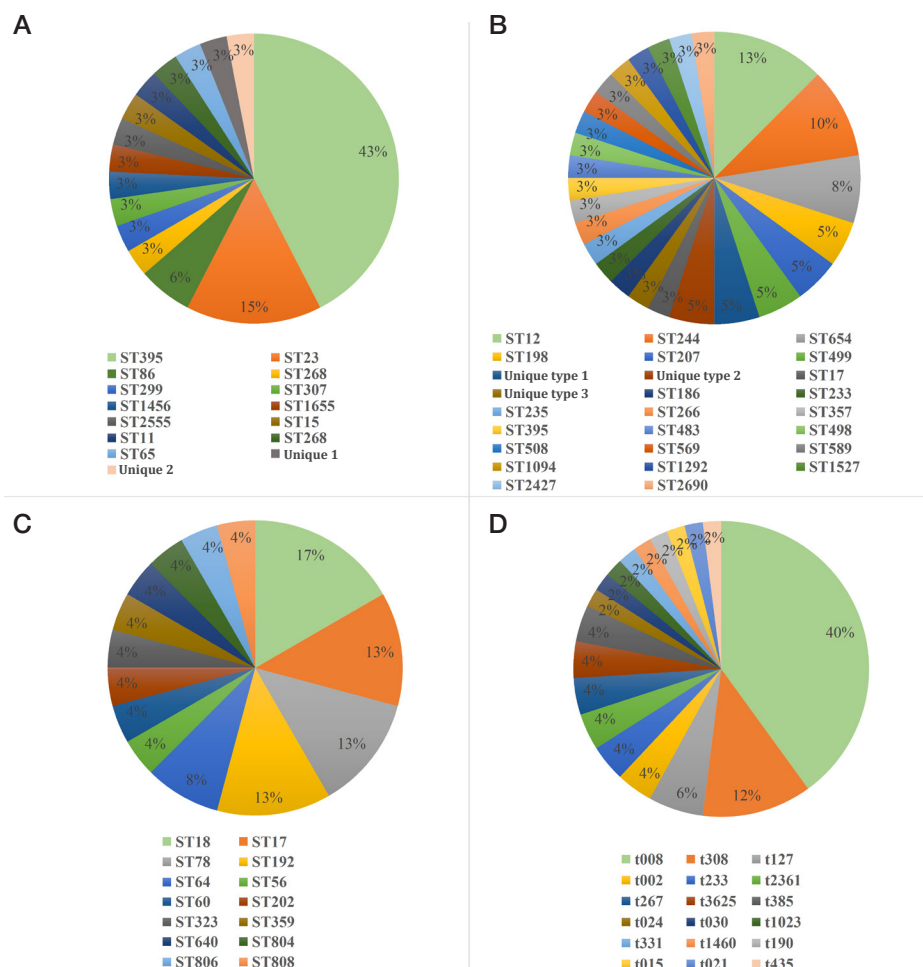


Fig. 2. Results of molecular genetic typing for *K. pneumoniae* (A) *P. aeruginosa* (B) *E. faecium* (C), and *S. aureus* (D)



Fig. 3. The efficacy of commercial phage products against *K. pneumoniae* (A), *P. aeruginosa* (B) and *S. aureus* (C). The green shows the proportion of strains sensitive to the tested phage products. Batch numbers represent the tested products: "Purified polyvalent pyobacteriophage" (U1, U25); "Complex pyobacteriophage" (N74, N45); "Klebsiella pneumoniae purified bacteriophage" (P252, P251); "Klebsiella pneumoniae purified polyvalent bacteriophage" (U27); "Pseudomonas aeruginosa bacteriophage" (N7); "Staphylococcal bacteriophage" (P332, N33).

available bacteriophage against *E. faecium*; it successfully infected 24 (4.2%) *E. faecium* strains.

To compare the efficacy of commercial phage products with that of natural phages, bacteriophage monoisolates exhibiting activity against *K. pneumoniae* and *S. aureus* were recovered from natural reservoirs (vB_Kp_M_Seu621 and vB_Saup-436-3w, respectively). Their titers were 10^{12} PFU/ml (for vB_Kp_M_Seu621) and 10^{11} PFU/ml (for vB_Saup-436-3w), respectively. The efficacy of the vB_Kp_M_Seu621 and vB_Saup-436-3w monoisolates was 5.9 and 60%, respectively (see Fig. 3A and 3C).

DISCUSSION

The efficacy of polyvalent phage products against *K. pneumoniae* varied from 42.4 to 87.9%; for monoisolates, this range was narrower: from 33.3 to 78.1% (see Fig. 3A). This suggests that the phage cocktails used in the study differed in their composition and should be updated and tested against currently circulating bacterial strains. The efficacy of the phage vB_Kp_M_Seu621 (5.9%) isolated from environmental sources was much lower than the efficacy of the tested commercial phage products which might be associated with the diversity of *K. pneumoniae* capsule types. The capsule can serve as a receptor for bacteriophages and determine the efficacy of interaction between the phage and its host [17].

It should be noted that almost all strains of *K. pneumoniae* included in the collection (32 of 33; 97.9%) were sensitive to at least one of the tested phage products. There was no significant difference in the efficacy of lysis between MDR and susceptible strains. The majority of MDR strains belonged to the sequence type ST395. Strains of this sequence type are very common among nosocomial pathogens and are associated with the spread of the *bla*OXA-48 gene, which confers resistance to β -lactams [18]. MDR strains representing this sequence type were susceptible to "Purified polyvalent pyobacteriophage" (U1); the efficacy of this phage product against ST395 strains was 81.8% (9 of 11). It also caused lysis of other MDR strains of *K. pneumoniae* belonging to the types ST15, ST23, ST268.

The highest efficacy of virulent phages was observed for *P. aeruginosa* strains. The efficacy of polyvalent phage products against this pathogen was 76.2–90%, whereas the efficacy of monovalent phage products was 87.5% (see Fig. 3B). These findings correlate with previously published data. A Turkish study carried out on a small sample of 10 *P. aeruginosa* strains demonstrated that the efficacy of "Complex pyobacteriophage" and "Intesti bacteriophage" was 90 and 80%, respectively [19].

Similar to their effect on *K. pneumoniae*, the tested products caused lysis of almost all *P. aeruginosa* strains included in our collection (39 of 40; 97.5%). MDR strains represented by the types ST235, ST357 and ST654 were successfully lysed by the majority of the tested phage preparations.

Monovalent bacteriophage products demonstrated 86% efficacy against *S. aureus* ("Staphylococcal bacteriophage", Fig. 3C). High efficacy of the phage product was earlier reported by other researchers. For example, the efficacy of the phage vB_SauM-fRuSau02 isolated from this commercial product was previously evaluated against 135 staph strains, including 30 strains of coagulase-negative staphylococci [20]. Notably, *S. aureus* strains used in the study had different origins: 51 strains were isolated from humans, whereas 54 strains, from pigs. The efficacy of the phage vB_SauM-fRuSau02 was very high (96%) against *S. aureus* isolated from humans. In turn, the efficacy of this phage against coagulase-negative staphylococci species and *S. aureus* strains isolated from animals was lower (50 and 33%, respectively) [20]. Another study investigated the efficacy of the commercial phage product "Stafal phage" (Bohemia Pharmaceuticals; Czech Republic). The study revealed that bacteriophages isolated from this preparation effectively killed 83% of MRSA and 99% of MSSA (methicillin susceptible *Staphylococcus aureus*) [21].

In our study, all MRSA, as well as MDR strains, were sensitive to "Staphylococcal bacteriophage" (batch number N33). One more MRSA strain from the MDR group was sensitive to another batch of this commercial product (P332). This strain was represented by the spa-type t127.

The efficacy of the phage monoisolate vB_SauP-436-3w against the strains included in our collection was lower (30 of 50; 60%) than the efficacy of the commercial product "Staphylococcal bacteriophage" (43 of 50; 86%), but still significantly higher than the efficacy of the phage vB_Kp_M_Seu621, which effectively killed *K. pneumoniae*. This can be explained by the fact that receptors for staphylophages are represented by teichoic acids of bacterial cells [22], whose variability is much lower than that of gram-negative bacteria capsules.

The efficacy of all tested commercial phage products against *E. faecium* was poor (1 of 24; 4.2%). The only strain sensitive to the tested phages was represented by the type

ST-17. Bacteriophages that exert activity against this species are listed as ingredients of commercial phage products, which are claimed to have a broad activity spectrum. Monovalent lytic phage products against *E. faecium* are not available on the Russian market.

A possible correlation between a bacterial strain's resistance to a phage and its resistance to antibacterial agents might have serious clinical implications. Another important finding would be a correlation between the resistance of a bacterial strain to a phage and the clonal complex the bacterium belonged to. In this study, we conducted a search for such correlations. We established that phage products induced lysis of both susceptible and sensitive (in terms of antibiotic resistance) bacteria. This is a crucial factor in deciding whether phages can be used as a clinical alternative to antibiotics. We established no correlations between the sensitivity of bacterial strains to the tested phages and their sensitivity to antibacterial agents ($p > 0.05$). We also found that bacterial strains representing one sequence type could be sensitive or resistant to a phage. This was true for all tested bacterial species. Thus, there was no clear correlation between the type of interaction between a phage and a bacterial cell, and a bacterial MLST sequence type ($p > 0.05$).

CONCLUSION

We found that strains included in our collection belonged to different genetic groups and have increased resistance to antimicrobial drugs, which makes them suitable for investigating the efficacy of commercial phage products. Commercial phage products available on the Russian market are highly effective against such ESKAPE pathogens as *P. aeruginosa* and *S. aureus*. However, not all tested phage products were equally effective against *K. pneumoniae*. Phage cocktails should be preferred to monovalent phages in the therapy of infections caused by gram-negative microorganisms, including *K. pneumoniae*.

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