EVALUATION OF THE EFFECTIVENESS OF ETIOTROPIC THERAPY WITH LINEZOLID AND BACTERIOPHAGE IN A MOUSE MODEL FOR STAPHYLOCOCCAL INFECTION

Kornienko MA[1] ✉, Kuzin VV[2], Abdraimova NK[1], Gorodnichev RB[1], Shitikov EA[1]

[1] Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow, Russia
[2] State Research Center for Applied Microbiology and Biotechnology, Obolensk, u.d. Serpukhov, Moscow region, Russia

Staphylococcus aureus is the causative agent of a wide range of infections, including severe systemic diseases, which is often multidrug resistant. Given the growing overall antibiotic resistance, a promising approach to treating staphylococcal infections is administration of bacteriophages, especially in combination with antibiotics. This study aimed to evaluate the synergistic effect of linezolid and bacteriophage vB_SauM-515A1 in combating a systemic infection in BALB/c mice. Using 36 animals, we established the optimal way of administration and the infecting dose of the microorganism (5×10^8 CFU/mouse intravenously), and identified the threshold concentrations of antimicrobial agents for monotherapy. The evaluation was based on the revealed contamination of internal organs (kidneys, spleen) and blood. To learn the etiotropic effect of linezolid (10 mg/kg animal weight) combined with the phage (2×10^7 PFU/mouse), we worked with a control group and a test group, 12 mice in each; 2, 8, 18, and 24 hours after infection, the former received the drug only, the latter — the investigated combination. Combined therapy had a more pronounced effect, decreasing the bacterial load in the kidneys by two to three orders of magnitude compared with monotherapy on the first day of treatment. Thus, the combined use of linezolid and bacteriophages is promising for the treatment of infections caused by *S. aureus*, and may increase the effectiveness of treatment and reduce the risk of side effects of high-dose antibiotics.

Keywords: *Staphylococcus aureus*, bacteriophages, phage therapy, combination therapy, linezolid, synergism, synergy between antibiotics and bacteriophages, mouse model, multidrug resistance

Funding: the work was supported by the Russian Science Foundation grant No. 22-15-00443, https://rscf.ru/project/22-15-00443/

Author contribution: Kornienko MA, Kuzin VV — study planning, data collection and processing, article authoring; Abdraimova NK — data collection and processing, Gorodnichev RB — study planning, data collection and processing; Shitikov EA — study planning, data processing, article authoring.

Compliance with ethical standards: the study was approved by the Ethics Committee of the State Research Center for Applied Microbiology and Biotechnology (Veterinary Minutes #3-2024 of June 10, 2024), performed in accordance with the requirements of Federal Law #61-FZ of 12.04.2010 "On the Circulation of Medicines"; Order #708N of the Ministry of Health of the Russian Federation of 23.08.2010 "On Approval of the Rules of Laboratory Practice"; SanPiN 3.3686-21 "Sanitary and epidemiological requirements for prevention of infectious diseases."

✉ **Correspondence should be addressed:** Maria A. Kornienko
Malaya Pirogovskaya, 1a, Moscow, 119435; kornienkomariya@gmail.com

Received: 06.11.2024 **Accepted:** 09.12.2024 **Published online:** 25.12.2024

DOI: 10.24075/brsmu.2024.062

ОЦЕНКА ЭФФЕКТИВНОСТИ ЭТИОТРОПНОЙ ТЕРАПИИ ЛИНЕЗОЛИДОМ И БАКТЕРИОФАГОМ НА МЫШИНОЙ МОДЕЛИ СТАФИЛОКОККОВОЙ ИНФЕКЦИИ

М. А. Корниенко[1] ✉, В. В. Кузин[2], К. Н. Абдраймова[1], Р. Б. Городничев[1], Е. А. Шитиков[1]

[1] Федеральный научно-клинический центр физико-химической медицины имени Ю. М. Лопухина Федерального медико-биологического агентства, Москва, Россия
[2] Государственный научный центр прикладной микробиологии и биотехнологии Роспотребнадзора, Оболенск, Россия

Staphylococcus aureus — возбудитель широкого спектра инфекций, включая тяжелые системные заболевания, и часто характеризуется множественной лекарственной устойчивостью. В условиях растущей антибиотикорезистентности перспективным методом лечения стафилококковых инфекций является применение бактериофагов, особенно в сочетании с антибиотиками. Целью работы было оценить синергетический эффект линезолида и бактериофага vB_SauM-515A1 при лечении системной инфекции у мышей BALB/c. С использованием 36 животных были подобраны оптимальный способ введения и инфицирующая доза микроорганизма (внутривенно 5×10^8 КОЕ/мышь), а также установлены пороговые концентрации антимикробных агентов при монотерапии. Оценку проводили по результатам исследования обсемененности внутренних органов (почки, селезенка) и крови. Для оценки комбинированного эффекта этиотропного действия линезолида (10 мг/кг массы животного) и фага (2×10^7 БОЕ/мышь) эксперимент проводили на контрольной и экспериментальных группах (по 12 особей в группе), получавших внутрибрюшинно монотерапию и комбинированное лечение через 2, 8, 18, 24 ч после заражения. Комбинированная терапия продемонстрировала более выраженный эффект: снижение бактериальной нагрузки в почках на два–три порядка по сравнению с монотерапией в первые сутки терапии. Таким образом, совместное использование линезолида и бактериофагов перспективно для лечения инфекций, вызванных *S. aureus*, и может повысить эффективность лечения и снизить риск побочных эффектов применения высоких доз антибиотиков.

Ключевые слова: *Staphylococcus aureus*, бактериофаги, фаговая терапия, комбинированная терапия, линезолид, синергизм, синергизм антибиотиков и бактериофагов, мышиные модели, множественная лекарственная устойчивость

Финансирование: исследование выполнено за счет гранта Российского научного фонда № 22-15-00443, https://rscf.ru/project/22-15-00443/

Вклад авторов: М. А. Корниенко, В. В. Кузин — план исследований, набор и обработка данных, написание статьи; Н. К. Абдраймова — набор и обработка данных, Р. Б. Городничев — план исследований, набор и обработка данных; Е. А. Шитиков — план исследований, обработка данных, написание статьи.

Соблюдение этических стандартов: исследование одобрено этическим комитетом ФБУН ГНЦ ПМБ (ветеринарный протокол № 3-2024 от 10 июня 2024 г.), выполнено в соответствии с требованиями Федерального закона от 12.04.2010 г. № 61-ФЗ «Об обращении лекарственных средств»; Приказа Минздравсоцразвития России от 23.08.2010 № 708Н «Об утверждении правил лабораторной практики»; СанПиН 3.3686-21 «Санитарно-эпидемиологические требования по профилактике инфекционных болезней».

✉ **Для корреспонденции:** Мария Андреевна Корниенко
ул. Малая Пироговская, д. 1а, г. Москва, 119435, Россия; kornienkomariya@gmail.com

Статья получена: 06.11.2022 **Статья принята к печати:** 09.12.2024 **Опубликована онлайн:** 25.12.2024

DOI: 10.24075/vrgmu.2024.062

Staphylococcus aureus is a major causative agent of both hospital-acquired and community-acquired infections, ranging from mild skin infections to life-threatening systemic diseases [1, 2]. In 2019, *S. aureus* caused more than 1 million deaths worldwide, largely because of the antibiotic resistance of strains of this species [3]. Clinically, the most significant of them are the methicillin-resistant strains of *S. aureus* (MRSA), which are resistant to beta-lactam antibiotics and often exhibit multidrug resistance (MDR) [2].

Recently, virulent bacteriophages, or phages, are increasingly considered as agents against infections caused by resistant bacteria [4]. One of the most promising applications of the phages is in combination with antibiotics. This approach promises incerased effectiveness of etiotropic treatment, smaller doses of antibiotics, minimized side effects, and reduced likelihood of acquired resistance on the part of the pathogens because of the intercomplementary effects of the antimicrobial agents [4]. There are two types of such effects, additive and synergistic. The additive effect is defined as the cumulative action of drugs equal to the sum of their individual effects. Synergism means amplification of the combined antimicrobial effect to the level exceeding that of the additive effect. However, the drugs can also be antagonistic to each other, i.e., their combined efficacy is below the effect achieved when they are use separately [5, 6].

In vitro studies have shown that in most cases, combined use of staphylophages and most antibiotics yields synergy [4]. Combining linezolid and staphylophages of the *Herelleviridae family* is a particularly interesting approach. Phages of this family have a wide lytic range, which supports their potential therapeutic applications [7–9]. Linezolid is a drug used against staphylococcal infections, those resistant to vancomycin in particular [10].

Linezolid inhibits protein synthesis by disrupting the formation of a functionally active complex needed to initiate the translation [10]. However, its use is associated with a number of limitations. First, prolonged administration of the antibiotic can cause serious side effects [11]. Secondly, the use of linezolid against microorganisms that require concentrations upwards of 4 μgr/ml or higher to suppress their growth may lead to deterioration of clinical efficacy [10] due to the peculiarities of its administration and possible fluctuations in blood plasma concentrations [12]. Thus, the combined linezolid-staphylophages therapy can increase the effectiveness of treatment and mitigate the risk of side effects by reducing the dose of the antibiotic, which makes this approach promising for clinical practice.

The synergy in the combination of linezolid and *Herelleviridae* family bacteriophages was previously demonstrated in vitro by us and other researchers [13–15]. The synergistic effect has also been confirmed in mouse models of staphylococcal infection [16–18]. Nevertheless, several studies describe antagonistic interaction between phages and linezolid [19, 20], which probably stems from the concentration of the antibiotic and the sequence of administration of the agents (in case of biofilms).

The purpose of this study was to expand knowledge of the synergy of linezolid and the vB_SauM-515A1 bacteriophage (*Herelleviridae* family) [13] by evaluating the effect of their combined and separate use in the context of treatment of systemic staphylococcal infection in BALB/c mice. The resulting data may be key to optimizing combination therapy for MRSA infections and may boost its effectiveness in clinical practice.

METHODS

Bacterial strains, phages, storage and cultivation conditions

The study used *S. aureus* SA413, a previously described strain, taken from the collection of Yu. M. Lopukhin Federal Research and Clinical Center for Physical-Chemical Medicine. The strain, isolated from purulent discharge of soft tissues, was classified as methicillin-sensitive *S. aureus* sequence type 8 (ST8); the minimum inhibitory concentration of linezolid for it was 8 μg/ml. This strain was selected because a previous in vitro study has shown the bacteriophage and linezolid to produce synergistic effect when acting thereon [13]. The strain was cultured on a meat peptone agar (MPA) nutrient medium (State Research Center for Applied Biotechnology and Microbiology, Obolensk, Russia).

The bacteriophage vB_SauM-515A1 was previously isolated from the commercially available P332 series Staphylococcal bacteriophage preparation (Microgen; Russia). Its detailed description was given earlier. The bacteriophage was grown on the SA413 strain of *S. aureus*, in an LB broth (Miller's modification) (Oxoid; Great Britain), at 37 °C. The phage lysate was then filtered through a 0.22 μm syringe filter with a hydrophilic polyethersulfone membrane (Millipore, USA), and purified by ultracentrifugation in a sucrose gradient as described earlier [7]. After purification, the bacteriophage was resuspended in a sterile saline solution. The titer of the bacteriophage in the preparation was assessed using the standard Grazia titration method [22]. The bacteriophage preparation was stored at 4 °C.

Animals

Female BALB/c mice weighing 18–22 g, 68 weeks old, were used as model animals. They were taken from the laboratory animal nursery of the Stolbovaya branch of the Research Center for Biomedical Technologies (Series Certificate No. 20353 of 30.05.2024). The mice were kept in groups, under standard conditions, as per the international standards and requirements, with unrestricted access to water and feed (Laboratorkorm; Russia). The animals were euthanized through CO_2 inhalation.

Parenchymal organs (spleen, liver) from the dead mice were examined for staphylococcal infection using the dense nutrient surface imprinting method; the medium was *Staphylococcagar* (State Research Center for Applied Microbiology and Biotechnology; Russia).

Modeling of staphylococcal infection in mice

Modeling simulate staphylococcal infection, we tested various infectious doses of the *S. aureus* SA413 strain and two approaches of administration, intravenous and intraperitoneal. The bacterial inoculum was grown in a liquid nutrient medium to an optical density (OD_{620}) of 0.75 (5×10^9 CFU/ml), and diluted with saline to the desired concentration. The animals were divided into six groups, three mice in each: group 1 — intravenous administration of 5×10^6 CFU/mouse; group 2 — intravenous administration of 5×10^7 CFU/mouse; group 3 — intravenous administration of 5×10^8 CFU/mouse; group 4 — intraperitoneal administration of 5×10^6 CFU/mouse; group 5 — intraperitoneal administration of 5×10^7 CFU/mouse; group 6 — intraperitoneal administration of 5×10^8 CFU/mouse. The volumes of the injected inoculum were 200 μl (intraperitoneal) and 100 μl (intravenous). The animals were monitored for three days to account for deaths. On the third day, bacterial

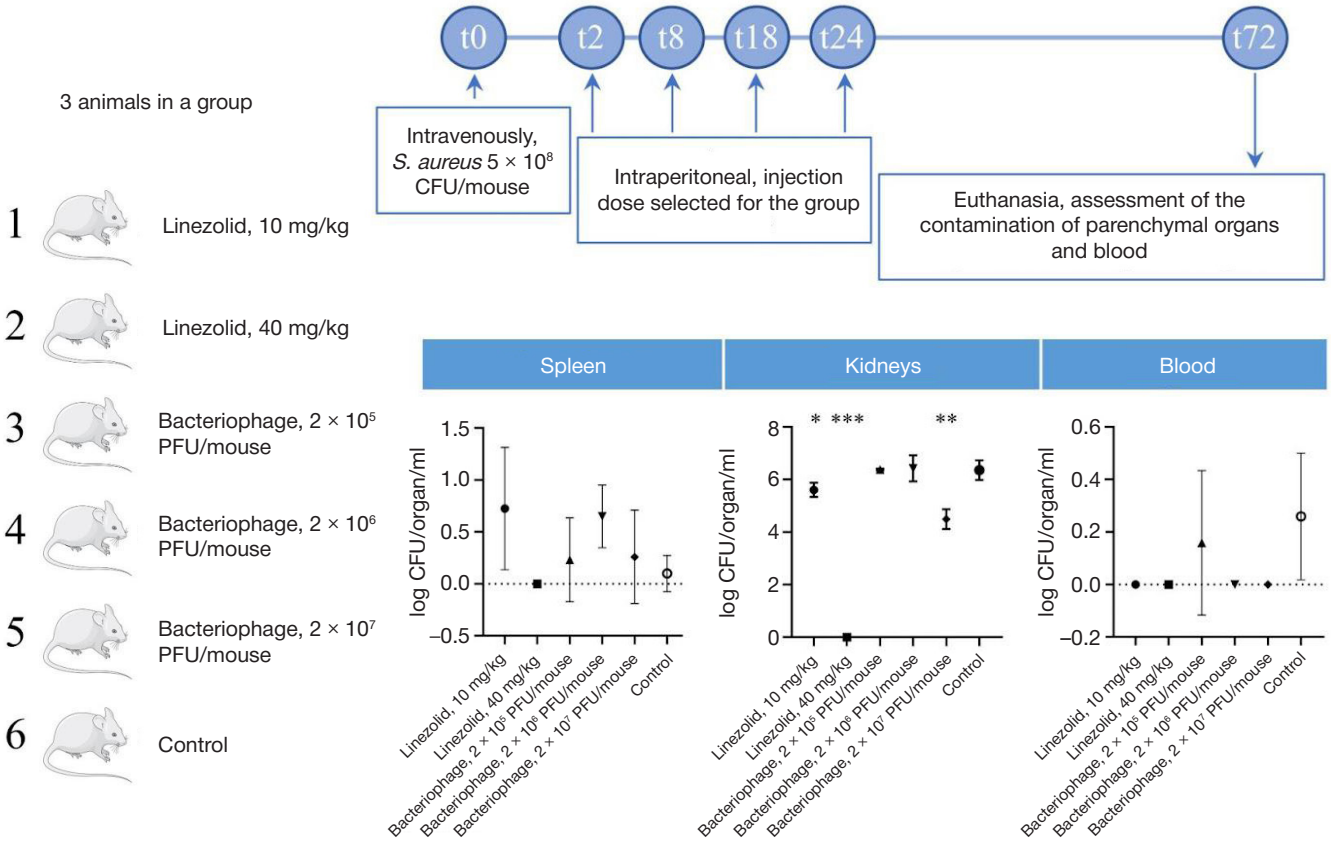

Fig. 1. Identification of the minimum inhibitory and therapeutic doses of linezolid and bacteriophage against staphylococcal infection * — $p < 0.05$; ** — $p < 0.001$; *** — $p < 0.0001$

contamination of parenchymal organs and blood was assessed in the surviving animals.

Selection of doses of antimicrobial agents

To assess the therapeutic and minimum inhibitory doses of linezolid and the bacteriophage vB_SauM-515A1, we divided the mice into 6 groups, three mice in each. Two, eight, eighteen, and twenty-four hours after infection, mice were injected with either linezolid (Sigma-Aldrich; USA) at concentrations of 10 mg/kg of animal weight or 40 mg/kg of animal weight, or the vB_SauM-515A1 bacteriophage at doses of 2×10^5, 2×10^6, and 2×10^7 PFU/mouse. Sterile saline solution was used to dilute the preparations to the necessary concentrations. Control group received saline solution without treatment, similar administration patterns as the test groups. The preparations (200 µl) were injected intraperitoneally. The animals were observed for three days, then euthanized. Parenchymal organs (spleen, kidneys) and blood were collected from them and examined for bacterial contamination and phage content. Blood (1 ml) was sampled from the heart through a puncture into sterile vacuum tubes with sodium heparin (no gel) (Improvacuter; China) designed for blood plasma testing.

Evaluation of the effectiveness of the combined effect of linezolid and bacteriophage

To assess the effectiveness of the combination, we used the antibacterial agents in minimal inhibitory doses. The experiment employed four experimental groups of animals, 12 mice in each, infected with the *S. aureus* SA413 strain. The infectious dose and the pattern of administration were selected based on the results of preliminary experiments. For the monotherapy stage, the animals received 200 µl of drugs intraperitoneally 2, 8, 18 and 24 hours post-infection. For the combined therapy stage (similar to the monotherapy stage time-wise), the mice were first injected with 200 µl of he antibiotic in one side of the peritoneum, then with 200 µl of the phage in the other side of the peritoneum. The first group of mice was treated with linezolid; the second group was treated with bacteriophage; the third group received the combination of the two; the fourth group (control) was injected with saline solution. Subsequently, three mice from each group were euthanized on the first and second days, and six mice on the third day. Their organs anfd blood were collected and examined for bacterial contamination.

Examination for bacterial contamination bacteriophages in parenchymal organs and blood

The organs were homogenized in sterile mortars, with 1 ml of saline solution added per organ. Next, blood samples and suspension samples were diluted tenfold in saline solution and plated on the Staphylococcagar dense nutrient medium (State Research Center for Applied Microbiology and Biotechnology; Russia). In parallel, we measured bacteriophage content in the suspensions using the Grazia titration method and Staphylococcagar medium (State Research Center for Applied Microbiology and Biotechnology; Russia); the samples made for the purpose were 100 µl serial dilutions. The measurements were done in five technical repetitions.

Data presentation and statistical analysis

For statistical analysis, we used Prism software (GraphPad Software 8; USA). The Shapiro-Wilk test allowed assessing the normalcy of data distribution, and the Student's *t*-test was used

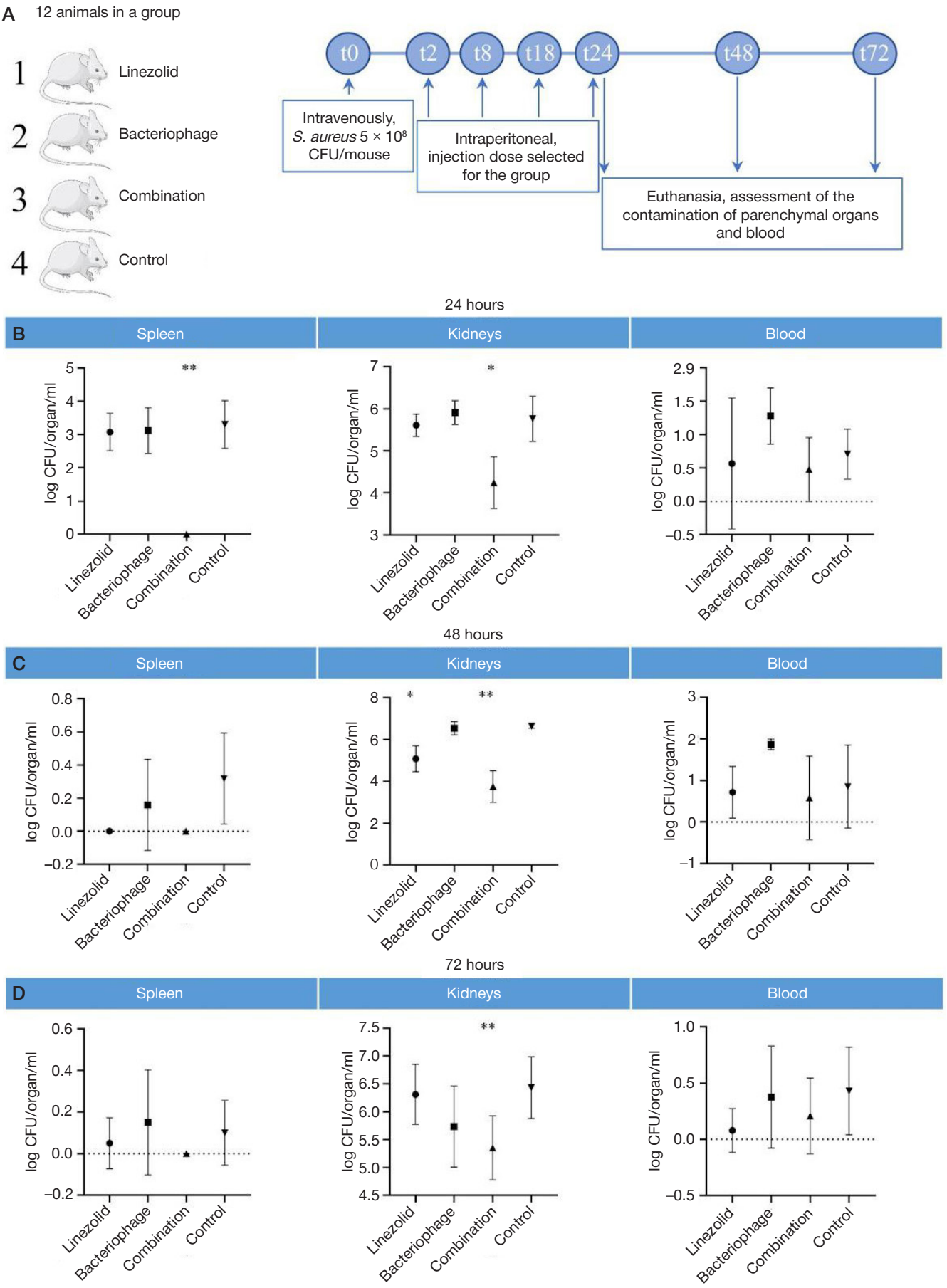

Fig. 2. Evaluation of the effectiveness of the combined effect of linezolid and bacteriophage: experiment design and results. **A**. Experiment design. **B–D**. Contamination of parenchymal organs and blood 24, 48, and 72 hours after infection, respectively. * — $p < 0.05$, ** — $p < 0.001$.

to compare the means between the groups. The differences were considered significant for $p < 0.05$.

Statement of compliance with ethical standards

All experiments with laboratory animals were approved by the Bioethics Commission of the State Research Center for Applied Microbiology and Biotechnology and conducted in accordance with the Guide for the Care and Use of Laboratory Animals [23].

RESULTS

Staphylococcal infection model

To build an adequate model of staphylococcal infection in laboratory animals, we conducted preliminary studies to select and infecting dose that ensures contamination of parenchymal organs (spleen, kidneys) and blood with the *S. aureus* strain SA413 on the third day after infection while avoiding animal mortality.

According to the results of those studies, intraperitoneal administration of bacteria at a dose of 5×10^8 CFU/mouse yielded death of all animals on the first day after infection, which is presumably due to the high concentration of the investigated strain in the area of administration and the subsequent toxic shock. Intraperitoneal administration of the bacterial culture at concentrations of 5×10^6 and 5×10^7 CFU/mouse, same as intravenous administration at any of the concentrations studied, left the mice alive three days after the infection. Only the mice that received a dose of 5×10^8 CFU/mouse intravenously exhibited downed motor activity and drowsiness, tousled hair and eyelid hyperemia, which indicate the development of an infectious process.

According to the autopsy, on the third day after intravenous injection of 5×10^8 CFU/mouse the animals had staphylococci in the kidneys (8.9×10^5 – 3.6×10^6 CFU/organ/ml) and a small amount of the pathogen in the spleens (37–52 CFU/organ/ml) and blood (3.4×10^2 – 1.3×10^3 CFU/ml). Smaller doses produced either isolated bacterial colonies or none at all, regardless of the method of infection.

Identification of the minimum inhibitory and therapeutic doses of linezolid and bacteriophage against staphylococcal infection

The minimum inhibitory and therapeutic doses of antimicrobial agents were evaluated for two concentrations of linezolid and three variants of bacteriophage doses. Eighteen mice were used for the purpose (Fig. 1).

Visual examination showed physical depression, tousled hair, and eyelid hyperemia in mice in the control group and four of the five experimental groups (linezolid 10 mg/kg of animal weight, and all doses of bacteriophage). No animals died through the entire experiment. Animals that received linezolid in the dose of 40 mg/kg of weight did not have the above symptoms.

An autopsy on the third day revealed low spleen contamination (0–25 CFU/organ/ml) in all groups and no bacteria in the blood, with the exception of the control group (0–3 CFU/ml) and the group that received the bacteriophage in the dose of 2×10^5 PFU/mouse. Kidney contamination was the most illustrative indicator. A dose of linezolid 40 mg/kg of animal weight ensured the pathogen was eliminated from the kidneys, indicating this was the therapeutic dose. A dose of 10 mg/kg of animal weight slowed formation of the bacterial colonies by one order of magnitude, made it a minimum inhibitory dose. The doses of bacteriophage 2×10^5 and 2×10^6 PFU/mouse did not deliver results significantly different from those registered in the control group, and were considered ineffective. The dose of bacteriophage 2×10^7 PFU/mouse reduced kidney contamination by one order of magnitude, and was recognized as the minimum inhibitory dose. An amount that could constitute a therapeutic dose was not found.

We detected phage particles only in the kidneys of mice that received a dose of 2×10^7 PFU/mouse (30–70 PFU/organ). There were no bacteriophages found in the blood and spleens of the animals.

Evaluation of the effectiveness of the combined effect of linezolid and bacteriophage

We used 48 mice to assess the combined effect of antimicrobial agents in minimum inhibitory concentrations (linezolid: 10 mg/kg animal weight; bacteriophage: 2×10^7 PFU/mouse) (Fig. 2).

By visual indicators, animals in all groups had the infectious process developing, and their condition was depressed, as described in the previous experiment.

On the first day, we detected no differences in the contamination of parenchymal organs between the monotherapy groups and the control group (Fig. 2B). In the combined therapy group, the spleen contamination dropped to almost zero values ($p = 0.0014$), and that of the kidneys was by one to two orders of magnitude lower than in the control group ($p = 0.0318$), while blood contamination remained comparable to that in the control group.

The results registered on the second day are shown in Fig. 2B. There were insignificant amounts of staphylococci in the spleens of animals of all groups, except those receiving linezolid, where the spleens were clean of the bacteria. Kidney contamination in the bacteriophage group remained at the level of the control group, and in the antibiotic group it significantly decreased by less than an order of magnitude ($p = 0.0127$); combined therapy pushed the value of this indicator down by two to three orders of magnitude compared to the control group ($p = 0.0028$). Blood contamination in all groups remained at the level of up to 10^2 CFU/ml.

On the third day after infection, we registered insignificant amounts of staphylococci in platings from spleen homogenates sampled in all the groups, which points to this organ's ability to independently eliminate the pathogen (Fig. 2D). Kidney contamination in mice treated with linezolid returned to the level peculiar to the control group, while in mice treated with bacteriophage it remained at that level throughout. In the combined therapy group, the contamination rate was an order of magnitude lower than the control values ($p = 0.0079$). Blood contamination in all groups remained at the control level.

As for the bacteriophage, its content was insignificant during the entire experiment (20–250 PFU/organ/ml) in the kidneys of the animals that received 2×10^7 PFU/mouse thereof as monotherapy, and in the combined use scenario. We registered no significant differences between the groups. No bacteriophage was detected in the spleens and blood.

DISCUSSION

Combined bacteriophages and antibiotics therapy is, presumably, one of the most promising approaches to the treatment of MDR pathogens. Numerous in vitro studies show promising results, demonstrating the synergistic effect of these agents. However, it is important to conduct *in vivo* experiments

to confirm their effectiveness and practical potential. Animal model studies allow assessing the possibilities and limitations of such therapy in conditions close to those of real-life clinical practice, and enable identification of the aspects that require further study before a full-fledged adoption.

To evaluate the effectiveness of the combined use of the bacteriophage vB_SauM-515A1 and linezolid, we chose a model of systemic infection in BALB/c mice, which aligns with the approaches practiced in similar studies [24, 25]. In the preliminary experiments, special attention was paid to the choice of the method of administration, selection of the infecting dose, and establishment of the minimum inhibitory concentrations of active agents. Previously published studies have shown that the infecting dose of *S. aureus* varies depending on the strain in the range from 10^6 to 10^8 CFU/mouse [24, 26, 27]. For example, a dose of 10^6 CFU/mouse was selected for the USA300 strain, known for its high virulence; in this case, the observation period was limited to 24 hours [28]. At the same time, a dose of 10^8 CFU/mouse used in the studies dedicated to the MDR strains partially killed the animals within 10–24 hours and, in some cases, by the third day [24, 27]. We focused on the sequence type 8 SA413 strain, one of the most common and associated with hospital infections worldwide, and found the optimal dose to be 5×10^8 CFU/mouse, administered intravenously. The dose ensured stable organ contamination after three days, thus creating adequate conditions for registration of the effects of therapy. Consistent with the findings reported by other authors, we have established that the results are most reliably reproducible when the injections are intravenous [27, 29].

The identified minimum inhibitory concentration of linezolid that does not cause pathogen elimination in monotherapy regimens and, consequently, should be investigated further, is 10 mg/kg of animal weight. This concentration of the antibiotic reduced bacterial contamination minimally, which is also consistent with the results reported by other researchers [10]. The concentration of 40 mg/kg of animal weight completely eliminated bacteria from the kidneys by the third day of the experiment, which is also similar to the data registered by other authors [29]. It should be noted that linezolid and bacteriophage were administered intraperitoneally to avoid vascular damage and the risk of hemorrhages associated with repeated injections. In particular, the effectiveness of this way was demonstrated in staphylococcal infection mice models that have thus received K-like phage ϕSA039 [30].

According to the published data on therapeutic use of bacteriophages in mouse models, the amount of antimicrobial agent varies from 10^6 to 10^{10} PFU/mouse [27, 31]. In our study, the minimum inhibitory dose of the bacteriophage was 2×10^7 PFU/mouse. This dose only partially decreased the level of bacterial contamination of kidneys, which underscores the need to use high concentrations of bacteriophages in monotherapy regimens. Moreover, lack of phages in blood and spleen indicates that there probably are some limitations to the system-wide spread of bacteriophages, which once again points to the need for an integrated approach in therapy.

Compared to monotherapy, combined use of linezolid and bacteriophage in minimum inhibitory doses had a more pronounced effect: within the first 24 hours, kidney contamination level decreased by two to three orders of magnitude versus the control values, a fact that backs the synergistic potential of antimicrobial agents. However, by the third day, bacterial contamination damping effect produced by the combination was not as strong as initially, which may indicate the need for a longer course of treatment to achieve the full therapeutic effect.

The evidence of the greater effectiveness of combination therapy compared with monotherapy are consistent with a number of reports covering animal model studies that investigated the effectiveness of the combined use of linezolid and *Herelleviridae* bacteriophages against other types of infections caused by *S. aureus*. Previously, it was demonstrated in a mouse model of a diabetic foot staphylococcal infection that a single injection of a Herelleviridae family phage delivers results comparable to those produced by linezolid, and combination therapy was much more effective in stopping the entire infectious process (bacterial load, number of lesions, foot myeloperoxidase activity, and histopathology), as well as accelerating the general tissue healing process [16]. A study that assessed the effectiveness of a linezolid and bacteriophage MR-5 (family *Herelleviridae*) combination against a skin infection modeled in mice has shown its potency: the agents, taken together, have significantly decreased the bacterial load and, consequently, boosted recovery [17]. There is also a report describing a combined therapy success in a modeled S. aureus infection case after arthroplasty. Mice were implanted with a wire coated with phage (10^9 PFU/ml) and/or linezolid into the intramedullary canal of the femur, and then inoculated with MRSA. In the group that received wire with a combination of agents, bacterial adhesion was reduced, and the limb's motor functions restored faster [18].

CONCLUSIONS

This study confirmed the promise held by the combined therapy with linezolid and bacteriophage vB_SauM-515A1 for treatment of systemic infections caused by *S. aureus*. Minimum inhibitory doses of the antibiotic and the bacteriophage were established to significantly decrease the level of bacterial contamination of parenchymal organs, which indicates a synergistic effect. The results of this study demonstrate that combination therapy is more effective than monotherapy, especially at the early stages, and can help reduce the dosage of the antibiotic, thus minimizing the possible side effects.

References

1. Cheung GYC, Bae JS; Otto M. Pathogenicity and Virulence of Staphylococcus Aureus. Virulence 2021; 12: 547–69, DOI: 10.1080/21505594.2021.1878688.
2. Guo Y, Song G, Sun M, Wang J, Wang Y. Prevalence and Therapies of Antibiotic-Resistance in Staphylococcus Aureus. Front Cell Infect Microbiol. 2020; 10: 107, DOI: 10.3389/fcimb.2020.00107.
3. Ikuta KS, Swetschinski LR, Robles Aguilar G, Sharara F, Mestrovic T, et al. Global Mortality Associated with 33 Bacterial Pathogens in 2019: A Systematic Analysis for the Global Burden of Disease Study 2019. The Lancet. 2022; 400: 2221–48, DOI: 10.1016/S0140-6736(22)02185-7.
4. Łusiak-Szelachowska M, Międzybrodzki R, Drulis-Kawa Z, Cater K Knežević P, Winogradow C, Amaro K, et al. Bacteriophages and Antibiotic Interactions in Clinical Practice: What We Have Learned so Far. J Biomed Sci. 2022; 29: 23, DOI: 10.1186/s12929-022-00806-1.
5. Dickey J, Perrot V. Adjunct Phage Treatment Enhances the Effectiveness of Low Antibiotic Concentration against Staphylococcus Aureus Biofilms in Vitro. PLoS ONE. 2019; 14:

e0209390, DOI: 10.1371/journal.pone.0209390.
6. Kumaran D, Taha M, Yi Q, Ramirez-Arcos S, Diallo J-S, Carli A, et al. Does Treatment Order Matter? Investigating the Ability of Bacteriophage to Augment Antibiotic Activity against Staphylococcus Aureus Biofilms. Front Microbiol. 2018; 9: 127, DOI: 10.3389/fmicb.2018.00127.
7. Kornienko M, Kuptsov N, Gorodnichev R, Bespiatykh D, Guliaev A, Letarova M, et al. Contribution of Podoviridae and Myoviridae Bacteriophages to the Effectiveness of Anti-Staphylococcal Therapeutic Cocktails. Sci Rep. 2020; 10: 18612, DOI: 10.1038/s41598-020-75637-x.
8. Leskinen K, Tuomala H, Wicklund A, Horsma-Heikkinen J, Kuusela P, Skurnik M, et al. Characterization of vB_SauM-fRuSau02, a Twort-Like Bacteriophage Isolated from a Therapeutic Phage Cocktail. Viruses. 2017; 9: 258, DOI: 10.3390/v9090258.
9. Abatángelo V, Peressutti Bacci N, Boncompain CA, Amadio AF, Carrasco S, Suárez CA, et al. Correction: Broad-Range Lytic Bacteriophages That Kill Staphylococcus Aureus Local Field Strains. PLoS ONE. 2017; 12: e0187387, DOI: 10.1371/journal.pone.0187387.
10. Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, et al. Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant Staphylococcus aureus infections in adults and children: executive summary. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2011; 52 (3): 285–92. Available from: https://doi.org/10.1093/cid/cir034.
11. Bozdogan B, Appelbaum PC. Oxazolidinones: Activity, Mode of Action, and Mechanism of Resistance. International Journal of Antimicrobial Agents. 2004; 23: 113–9. DOI: 10.1016/j.ijantimicag.2003.11.003.
12. Hui L-A, Bodolea C, Vlase L, Hiriscau EI, Popa A. Linezolid Administration to Critically Ill Patients: Intermittent or Continuous Infusion? A Systematic Literature Search and Review. Antibiotics. 2022; 11: 436. DOI: 10.3390/antibiotics11040436.
13. Abdraimova NK, Kornienko MA, Bespiatykh DA, Kuptsov NS, Gorodnichev RB, Shitikov EA. Combined effects of bacteriophage vB_SauM-515A1 and antibiotics on the Staphylococcus aureus clinical isolates. Bulletin of RSMU. 2022; DOI: 10.24075/brsmu.2022.052.
14. Kaur S, Harjai K, Chhibber S. Bacteriophage Mediated Killing of Staphylococcus Aureus In Vitro on Orthopaedic K Wires in Presence of Linezolid Prevents Implant Colonization. PLoS ONE. 2014; 9: e90411. DOI:10.1371/journal.pone.0090411.
15. Wang B, Xu Y, Zhao H, Wang X, Rao L, Guo Y, et al. Methicillin-Resistant Staphylococcus Aureus in China: A Multicentre Longitudinal Study and Whole-Genome Sequencing. Emerging Microbes & Infections. 2022; 11: 532–42. DOI: 10.1080/22221751.2022.2032373.
16. Chhibber S, Kaur T. Sandeep Kaur Co-Therapy Using Lytic Bacteriophage and Linezolid: Effective Treatment in Eliminating Methicillin Resistant Staphylococcus Aureus (MRSA) from Diabetic Foot Infections. PLoS ONE. 2013; 8: e56022. DOI: 10.1371/journal.pone.0056022.
17. Kaur S, Chhibber S. A Mouse Air Pouch Model for Evaluating the Anti-Bacterial Efficacy of Phage MR-5 in Resolving Skin and Soft Tissue Infection Induced by Methicillin-Resistant Staphylococcus Aureus. Folia Microbiol. 2021; 66: 959–72, DOI: 10.1007/s12223-021-00895-9.
18. Kaur S, Harjai K, Chhibber S. In Vivo Assessment of Phage and Linezolid Based Implant Coatings for Treatment of Methicillin Resistant S. Aureus (MRSA) Mediated Orthopaedic Device Related Infections. PLoS ONE. 2016; 11: e0157626, DOI: 10.1371/journal.pone.0157626.
19. Berryhill BA, Huseby DL, McCall IC, Hughes D, Levin BR. Evaluating the Potential Efficacy and Limitations of a Phage for Joint Antibiotic and Phage Therapy of Staphylococcus Aureus Infections. Proc Natl Acad Sci USA. 2021; 118, e2008007118. DOI: 10.1073/pnas.2008007118.
20. Kumaran D, Taha M, Yi Q, Ramirez-Arcos S, Diallo J-S, Carli A, Abdelbary H. Does Treatment Order Matter? Investigating the Ability of Bacteriophage to Augment Antibiotic Activity against Staphylococcus Aureus Biofilms. Front Microbiol. 2018; 9: 127. DOI: 10.3389/fmicb.2018.00127.
21. Kornienko M, Fisunov G, Bespiatykh D, Kuptsov N, Gorodnichev R, Klimina K, et al. Transcriptional Landscape of Staphylococcus Aureus Kayvirus Bacteriophage vB_SauM-515A1. Viruses. 2020; 12: 1320. DOI: 10.3390/v12111320.
22. Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of Bacteriophages Using the Small Drop Plaque Assay System. In: Clokie MRJ, Kropinski AM, editors. Bacteriophages. Methods in Molecular Biology. Humana Press: Totowa, NJ. 2009; p. 81–85.
23. Guide for the Care and Use of Laboratory Animals. National Academies Press: Washington, D.C., 2011.
24. García P, Moscoso M, Fernández MC, Fuentes-Valverde V, Pérez A, Bou G. Comparison of the in Vivo Efficacy of Ceftaroline Fosamil, Vancomycin and Daptomycin in a Murine Model of Methicillin-Resistant Staphylococcus Aureus Bacteraemia. International Journal of Antimicrobial Agents. 2023; 62: 106836. DOI: 10.1016/j.ijantimicag.2023.106836.
25. Suligoy CM, Díaz RE, Gehrke A-K, Ring N, Yebra G, Alves J, et al. Acapsular Staphylococcus Aureus with a Non-Functional Agr Regains Capsule Expression after Passage through the Bloodstream in a Bacteremia Mouse Model. Sci Rep. 2020; 10: 14108 DOI: 10.1038/s41598-020-70671-1.
26. Kim HK, Missiakas D, Schneewind O. Mouse Models for Infectious Diseases Caused by Staphylococcus Aureus. Journal of Immunological Methods. 2014; 410: 88–99, DOI: 10.1016/j.jim.2014.04.007.
27. Oduor JMO, Onkoba N, Maloba F, Arodi WO, Nyachieo A. Efficacy of Lytic Staphylococcus Aureus Bacteriophage against Multidrug-Resistant Staphylococcus Aureus in Mice. J Infect Dev Ctries. 2016; 10: 1208–13. DOI: 10.3855/jidc.7931.
28. Sharma-Kuinkel BK, Zhang Y, Yan Q, Ahn SH, Fowler VG. Host Gene Expression Profiling and In Vivo Cytokine Studies to Characterize the Role of Linezolid and Vancomycin in Methicillin-Resistant Staphylococcus Aureus (MRSA) Murine Sepsis Model. PLoS ONE. 2013; 8: e60463. DOI: 10.1371/journal.pone.0060463.
29. Gordon O, Dikeman DA, Ortines RV, Wang Y, Youn C, Mumtaz M, et al. The Novel Oxazolidinone TBI-223 Is Effective in Three Preclinical Mouse Models of Methicillin-Resistant Staphylococcus Aureus Infection. Microbiol Spect. 2022; 10: e02451-21, DOI: 10.1128/spectrum.02451-21.
30. Fujiki J, Nakamura T, Nakamura K, Nishida K, Amano Y, Watanabe Y, et al. Biological properties of Staphylococcus virus –SA012 for phage therapy. Scientific reports. 2022; 12 (1): 21297. Available from: https://doi.org/10.1038/s41598-022-25352-6.
31. Plumet L, Ahmad-Mansour N, Dunyach-Remy C, Kissa K, Sotto A, Lavigne J-P, et al. Bacteriophage Therapy for Staphylococcus Aureus Infections: A Review of Animal Models, Treatments, and Clinical Trials. Front Cell Infect Microbiol. 2022; 12: 907314. DOI: 10.3389/fcimb.2022.907314.

Литература

1. Cheung GYC, Bae JS; Otto M. Pathogenicity and Virulence of Staphylococcus Aureus. Virulence 2021; 12: 547–69, DOI: 10.1080/21505594.2021.1878688.
2. Guo Y, Song G, Sun M, Wang J, Wang Y. Prevalence and Therapies of Antibiotic-Resistance in Staphylococcus Aureus. Front Cell Infect Microbiol. 2020; 10: 107, DOI: 10.3389/fcimb.2020.00107.
3. Ikuta KS, Swetschinski LR, Robles Aguilar G, Sharara F, Mestrovic T, et al. Global Mortality Associated with 33 Bacterial Pathogens in 2019: A Systematic Analysis for the Global Burden of Disease Study 2019. The Lancet. 2022; 400: 2221–48, DOI: 10.1016/S0140-6736(22)02185-7.
4. Łusiak-Szelachowska M, Międzybrodzki R, Drulis-Kawa Z, Cater K Knežević P, Winogradow C, Amaro K, et al. Bacteriophages and Antibiotic Interactions in Clinical Practice: What We Have Learned so Far. J Biomed Sci. 2022; 29: 23, DOI: 10.1186/s12929-022-00806-1.
5. Dickey J, Perrot V. Adjunct Phage Treatment Enhances the Effectiveness of Low Antibiotic Concentration against Staphylococcus Aureus Biofilms in Vitro. PLoS ONE. 2019; 14: e0209390, DOI: 10.1371/journal.pone.0209390.
6. Kumaran D, Taha M, Yi Q, Ramirez-Arcos S, Diallo J-S, Carli A, et

al. Does Treatment Order Matter? Investigating the Ability of Bacteriophage to Augment Antibiotic Activity against Staphylococcus Aureus Biofilms. Front Microbiol. 2018; 9: 127, DOI: 10.3389/fmicb.2018.00127.
7. Kornienko M, Kuptsov N, Gorodnichev R, Bespiatykh D, Guliaev A, Letarova M, et al. Contribution of Podoviridae and Myoviridae Bacteriophages to the Effectiveness of Anti-Staphylococcal Therapeutic Cocktails. Sci Rep. 2020; 10: 18612, DOI: 10.1038/s41598-020-75637-x.
8. Leskinen K, Tuomala H, Wicklund A, Horsma-Heikkinen J, Kuusela P, Skurnik M, et al. Characterization of vB_SauM-fRuSau02, a Twort-Like Bacteriophage Isolated from a Therapeutic Phage Cocktail. Viruses. 2017; 9: 258, DOI: 10.3390/v9090258.
9. Abatángelo V, Peressutti Bacci N, Boncompain CA, Amadio AF, Carrasco S, Suárez CA, et al. Correction: Broad-Range Lytic Bacteriophages That Kill Staphylococcus Aureus Local Field Strains. PLoS ONE. 2017; 12: e0187387, DOI: 10.1371/journal.pone.0187387.
10. Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, et al. Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant Staphylococcus aureus infections in adults and children: executive summary. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2011; 52 (3): 285–92. Available from: https://doi.org/10.1093/cid/cir034.
11. Bozdogan B, Appelbaum PC. Oxazolidinones: Activity, Mode of Action, and Mechanism of Resistance. International Journal of Antimicrobial Agents. 2004; 23: 113–9. DOI: 10.1016/j.ijantimicag.2003.11.003.
12. Hui L-A, Bodolea C, Vlase L, Hiriscau EI, Popa A. Linezolid Administration to Critically Ill Patients: Intermittent or Continuous Infusion? A Systematic Literature Search and Review. Antibiotics. 2022; 11: 436. DOI: 10.3390/antibiotics11040436.
13. Абдраймова Н. К., Корниенко М. А. Беспятых Д. А., Купцов Н. С., Городничев Р. Б., Шитиков Е. А. Комбинированное воздействие бактериофага VB_SAUM-515A1 и антибиотиков на клинические изоляты Staphylococcus aureus. Вестник РГМУ. 2022; DOI: 10.24075/vrgmu.2022.052.
14. Kaur S, Harjai K, Chhibber S. Bacteriophage Mediated Killing of Staphylococcus Aureus In Vitro on Orthopaedic K Wires in Presence of Linezolid Prevents Implant Colonization. PLoS ONE. 2014; 9: e90411. DOI:10.1371/journal.pone.0090411.
15. Wang B, Xu Y, Zhao H, Wang X, Rao L, Guo Y, et al. Methicillin-Resistant Staphylococcus Aureus in China: A Multicentre Longitudinal Study and Whole-Genome Sequencing. Emerging Microbes & Infections. 2022; 11: 532–42. DOI: 10.1080/22221751.2022.2032373.
16. Chhibber S, Kaur T. Sandeep Kaur Co-Therapy Using Lytic Bacteriophage and Linezolid: Effective Treatment in Eliminating Methicillin Resistant Staphylococcus Aureus (MRSA) from Diabetic Foot Infections. PLoS ONE. 2013; 8: e56022. DOI: 10.1371/journal.pone.0056022.
17. Kaur S, Chhibber S. A Mouse Air Pouch Model for Evaluating the Anti-Bacterial Efficacy of Phage MR-5 in Resolving Skin and Soft Tissue Infection Induced by Methicillin-Resistant Staphylococcus Aureus. Folia Microbiol. 2021; 66: 959–72, DOI: 10.1007/s12223-021-00895-9.
18. Kaur S, Harjai K, Chhibber S. In Vivo Assessment of Phage and Linezolid Based Implant Coatings for Treatment of Methicillin Resistant S. Aureus (MRSA) Mediated Orthopaedic Device Related Infections. PLoS ONE. 2016; 11: e0157626, DOI: 10.1371/journal.pone.0157626.
19. Berryhill BA, Huseby DL, McCall IC, Hughes D, Levin BR. Evaluating the Potential Efficacy and Limitations of a Phage for Joint Antibiotic and Phage Therapy of Staphylococcus Aureus Infections. Proc Natl Acad Sci USA. 2021; 118, e2008007118. DOI: 10.1073/pnas.2008007118.
20. Kumaran D, Taha M, Yi Q, Ramirez-Arcos S, Diallo J-S, Carli A, Abdelbary H. Does Treatment Order Matter? Investigating the Ability of Bacteriophage to Augment Antibiotic Activity against Staphylococcus Aureus Biofilms. Front Microbiol. 2018; 9: 127. DOI: 10.3389/fmicb.2018.00127.
21. Kornienko M, Fisunov G, Bespiatykh D, Kuptsov N, Gorodnichev R, Klimina K, et al. Transcriptional Landscape of Staphylococcus Aureus Kayvirus Bacteriophage vB_SauM-515A1. Viruses. 2020; 12: 1320. DOI: 10.3390/v12111320.
22. Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of Bacteriophages Using the Small Drop Plaque Assay System. In: Clokie MRJ, Kropinski AM, editors. Bacteriophages. Methods in Molecular Biology. Humana Press: Totowa, NJ. 2009; p. 81–85.
23. Guide for the Care and Use of Laboratory Animals. National Academies Press: Washington, D.C., 2011.
24. García P, Moscoso M, Fernández MC, Fuentes-Valverde V, Pérez A, Bou G. Comparison of the in Vivo Efficacy of Ceftaroline Fosamil, Vancomycin and Daptomycin in a Murine Model of Methicillin-Resistant Staphylococcus Aureus Bacteraemia. International Journal of Antimicrobial Agents. 2023; 62: 106836. DOI: 10.1016/j.ijantimicag.2023.106836.
25. Suligoy CM, Díaz RE, Gehrke A-K, Ring N, Yebra G, Alves J, et al. Acapsular Staphylococcus Aureus with a Non-Functional Agr Regains Capsule Expression after Passage through the Bloodstream in a Bacteremia Mouse Model. Sci Rep. 2020; 10: 14108 DOI: 10.1038/s41598-020-70671-1.
26. Kim HK, Missiakas D, Schneewind O. Mouse Models for Infectious Diseases Caused by Staphylococcus Aureus. Journal of Immunological Methods. 2014; 410: 88–99, DOI: 10.1016/j.jim.2014.04.007.
27. Oduor JMO, Onkoba N, Maloba F, Arodi WO, Nyachieo A. Efficacy of Lytic Staphylococcus Aureus Bacteriophage against Multidrug-Resistant Staphylococcus Aureus in Mice. J Infect Dev Ctries. 2016; 10: 1208–13. DOI: 10.3855/jidc.7931.
28. Sharma-Kuinkel BK, Zhang Y, Yan Q, Ahn SH, Fowler VG. Host Gene Expression Profiling and In Vivo Cytokine Studies to Characterize the Role of Linezolid and Vancomycin in Methicillin-Resistant Staphylococcus Aureus (MRSA) Murine Sepsis Model. PLoS ONE. 2013; 8: e60463. DOI: 10.1371/journal.pone.0060463.
29. Gordon O, Dikeman DA, Ortines RV, Wang Y, Youn C, Mumtaz M, et al. The Novel Oxazolidinone TBI-223 Is Effective in Three Preclinical Mouse Models of Methicillin-Resistant Staphylococcus Aureus Infection. Microbiol Spect. 2022; 10: e02451-21, DOI: 10.1128/spectrum.02451-21.
30. Fujiki J, Nakamura T, Nakamura K, Nishida K, Amano Y, Watanabe Y, et al. Biological properties of Staphylococcus virus –SA012 for phage therapy. Scientific reports. 2022; 12 (1): 21297. Available from: https://doi.org/10.1038/s41598-022-25352-6.
31. Plumet L, Ahmad-Mansour N, Dunyach-Remy C, Kissa K, Sotto A, Lavigne J-P, et al. Bacteriophage Therapy for Staphylococcus Aureus Infections: A Review of Animal Models, Treatments, and Clinical Trials. Front Cell Infect Microbiol. 2022; 12: 907314. DOI: 10.3389/fcimb.2022.907314.