

# GEORGIAN MEDICAL NEWS

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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии  
საქართველოს სამედიცინო სიახლეები

## GEORGIAN MEDICAL NEWS

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**GMN: Georgian Medical News** is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

**GMN: Медицинские новости Грузии** - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

**GMN: Georgian Medical News** – საქართველოს სამედიცინო ხიახლები – არის უფლებული სამეცნიერო სამედიცინო რევიუზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეცნიელების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რეცენზირდება ინგლისურ ენებზე ქვეყნება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

## WEBSITE

[www.geomednews.com](http://www.geomednews.com)

## К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman (Кириллица)**, для текста на грузинском языке следует использовать **AcadNusx**. Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применяющиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи.** Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - <http://www.spinesurgery.ru/files/publish.pdf> и [http://www.nlm.nih.gov/bsd/uniform\\_requirements.html](http://www.nlm.nih.gov/bsd/uniform_requirements.html) В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

**При нарушении указанных правил статьи не рассматриваются.**

## REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or computer-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman (Cyrillic)**, print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: [http://www.nlm.nih.gov/bsd/uniform\\_requirements.html](http://www.nlm.nih.gov/bsd/uniform_requirements.html)  
[http://www.icmje.org/urm\\_full.pdf](http://www.icmje.org/urm_full.pdf)

In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

**Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.**

## ავტორია საშურალებოდ!

რედაქტორი სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე, დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურნოვან ტექსტებში - **Times New Roman (Кириллицა)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx**. შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სის და რეზიუმების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გამუქდება: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანორმილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოსასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტ-სურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შედეგის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ურნალის დასახელება, გამოცემის ადგილი, წელი, ურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფრჩილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცეზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქტორი იტოვებს უფლებას შეასწოროს სტატიას. ტექსტშე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქტორი ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდიდად წარდგენილი იყო სხვა რედაქტორიაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

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## PHENOTYPIC CHARACTERIZATION OF FIVE PHAGES ACTIVE AGAINST ANTIBIOTIC-RESISTANT *KLEBSIELLA PNEUMONIAE*

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### Abstract.

Widespread antibiotic resistance represents an increasingly significant burden for healthcare systems worldwide. One of the primary contributors to this pressing issue is *Klebsiella pneumoniae*, a pathogen of major clinical concern. Here we describe five distinct phages that infect antibiotic-resistant *K. pneumoniae* strains. A total of 100 clinical bacterial strains were systematically assessed for their antibiotic- and phage susceptibility profiles. Notably, 94 of these strains demonstrated high levels of antibiotic resistance across multiple drug classes. The five described phages, which target nearly all studied strains, were comprehensively characterized regarding their virion morphology, their lytic spectra, their intracellular growth parameters, and their potential to trigger development of phage-resistant bacterial forms. Two Siphoviridae phages exhibited remarkably low rates of phage-resistant form development during the study period. While such rates were comparatively higher for the three Myoviruses tested, bacterial forms that acquired resistance to these particular phages subsequently became sensitive to other phages in the collection. In conclusion, the studied phages effectively targeted 93 out of 100 bacterial strains tested. Such broad coverage of diverse clinical strains by these phages strongly underscores their considerable potential in therapeutic settings, particularly for treating multidrug-resistant infections.

**Key words.** *Klebsiella pneumonia*, bacteriophage, phage therapy, infectious diseases, bacterial infections.

### Introduction.

*Klebsiella* species are a group of Gram-negative bacteria belonging to the *Enterobacteriaceae* family. *K. pneumoniae* as well as other species of the *Klebsiella* genus are responsible for a wide range of infectious diseases in humans [1]. *Klebsiella*-associated diseases include urinary tract infections, pneumonia and bloodstream infections, particularly in immunocompromised patients [2]. *K. pneumoniae* strains are notorious for their ability to acquire antibiotic-resistance against a wide range of antibiotics including beta-lactams, aminoglycosides and carbapenems. This makes *Klebsiella*-associated infectious diseases increasingly difficult to treat [3,4]. As a result, the emergence of multidrug-resistant *Klebsiella* strains have become a major concern in various clinical settings [5]. These pathogens are responsible for community- as well as hospital-acquired infections, with a

fairly high mortality rate, which tends to be particularly high in hospital settings [6-9].

To combat the growing threat from *Klebsiella*-associated infectious diseases, phages have emerged as one of the most promising alternative therapeutic approaches – both independently and in combination with antibiotics [10-15]. Phages represent the largest group of viruses on earth. They can be found in various environments such as air, soil, water and especially wastewater [16]. Phages, as natural predators of bacteria, have demonstrated their therapeutic potential in human healthcare as early as in the beginning of the XX century [17,18]. The golden era of antibiotics overshadowed phage use. However, treatment options for antibiotic-resistant bacteria are now diminishing [19]. Therefore, phages are once again regarded as antibacterial agents of great potential [20,21].

A number of traits highlight great potential and advantages of phages over antibiotics. Phages are widely distributed in nature and their isolation from the environment does not require significant financial resources. Phages are highly specific to their target bacteria, often on species or even on strain level. Such specificity ensures that mammalian cells and healthy microbiota remain untargeted by phage therapy [22-24]. Phages have virtually no side effects; their high specificity to targeted pathogens as well as the fact that due to their widespread presence phages are non-foreign entities for humans, makes phages safe therapeutic agents [25-28]. Presence of target bacterial cells leads to the multiplication of phage particles – increasing their concentration and preventing further spread of the pathogen. Phages can easily penetrate and move within the body, making them successful at reaching sites which are otherwise not so easily accessible – including bacterial biofilms [29,26,30]. While phage-resistance does emerge in clinically relevant bacteria, it is considered less alarming, than the development of antibiotic resistance. Phages are in constant arms race with their hosts, often being successful at overcoming resistance. In phage therapy, one of the strategies for suppressing emergence of phage resistance is combining therapeutic phages in cocktails.

Recent research on *Klebsiella* phages has mainly focused on their isolation, characterization and study of their therapeutic potential. The diversity of *Klebsiella* phages, their genetic plasticity and their ability to evolve quickly to counteract bacterial resistance mechanisms are important factors that make

them a promising therapeutic tool in the fight against bacterial infections [13].

Despite the promising potential of *Klebsiella* phages, there are challenges that need to be addressed before they can be routinely used in clinical settings. These include bacterial potential to develop resistance against phages. Here we characterize five *Klebsiella* phages in terms of their biological properties. We also evaluate the rates at which phage-resistant bacterial forms arise against them and we estimate their potential therapeutic use.

## Materials and Methods.

### Bacterial strains and their cultivation:

100 *Klebsiella pneumoniae* strains of clinical origin were used in this study. All strains had been provided by the Bacteriophage analytical-diagnostic center "Diagnosi 90". The full list of strains and their origin can be found in Table S1.

Bacteria were cultivated at 37 °C for 18-24 hours. Incubation of cultures (both solid and liquid) took place in static conditions. Lysogeny Broth (LB) with and without agar was used as growth medium.

### Antibiotic susceptibility testing of bacterial strains:

Bacterial strains were tested for their antibiotic susceptibility with disk diffusion method according to EUCAST recommendations [31]. Susceptibility evaluation experiments included 23 antibiotics from different groups: Penicillins – 5 antibiotics; Cephalosporins – 6 antibiotics; Carbapenems – 4 antibiotics; Monobactam – 1 antibiotic; Fluoroquinolones – 4 antibiotics; Aminoglycosides – 3 antibiotics. Additionally, the activity of trimethoprim-sulfamethoxazole and chloramphenicol were also tested, bringing the total to 25 antibacterial agents (Table S2).

### PCR detection of $\beta$ -lactamase-encoding genes:

In all bacterial strains of interest, the presence of four  $\beta$ -lactamase encoding genes (Table 1) was detected by conventional PCR. 2  $\mu$ l of heat-extracted DNA was used as a template. All primers are listed in Table 1. DreamTaq Green PCR Master Mix (Thermo Scientific) was used in all reactions and PCR products were analysed on 1 % agarose gel.

### Genotyping of *K. pneumoniae* strains:

*K. pneumoniae* clinical strains of interest were genotyped through pulsed-field gel electrophoresis (PFGE) approach [39]. Agarose-embedded DNA samples were treated with lysis buffer (1 mg/mL lysozyme, 100  $\mu$ g/m proteinase K, 6 mM Tris-HCl (pH 8.0), 1 M NaCl, 100 mM EDTA (pH 8.0), 0.2% deoxycholate, 0.5% N-Lauroylsarcosine). Restriction endonuclease XbaI (NEB) was used for DNA fragmentation. Electrophoresis took place at 14 °C for 18 hours in 1 % agarose and 0.5 x Tris-borate EDTA (TBE) in a pulsed field gel electrophoresis apparatus Gene Navigator™ System (Amersham). Pulse parameters were: 10-50 seconds, 200 volts (6 V/cm). Fragment analysis was conducted using Freetree and TreeView softwares [40].

### Isolation of bacteriophages:

Phages were isolated from environmental water samples by the enrichment method [41]. 90 ml of water sample was enriched with 10 ml of times concentrated LB and 1 ml of bacterial

suspension of strain(s) of interest. After incubating the sample at 37 °C for 18 hours, samples were centrifuged at 9 000 rpm for 20 min. Supernatant was filtered through 0.22  $\mu$ m pore sized filters and was spotted on bacterial lawn.

### Plaque formation and morphology:

The double-layer agar method was used to analyse phage plaque morphology [41]. Ten-fold diluted phage samples were mixed with 0.1 ml of a suspension of the host bacterial strain (10<sup>9</sup> CFU/ml) and 3 ml of semi-solid (0.5 %) LB agar. The mixture was spread on a Petri dish containing solid (1.5 %) LB agar. After incubation at 37 °C for 18-24 hours, the morphology and number of phage colonies were recorded.

### Phage host range and efficiency of plating:

The host range of bacteriophages was studied using the spot-test assay [42]. 100 clinical isolates of *K. pneumoniae* were employed as target strains. 10  $\mu$ l of phage suspensions (with phage titers of 10<sup>7</sup> PFU/ml) were spotted on bacterial soft-agar lawns. The plates were incubated at 37 °C, after which the presence of lysis zones was observed. The following designations were used to assess the degree of antibacterial activity of bacteriophages: CL - the phage causes complete lysis of bacteria; SCL - the phage causes complete lysis, but single bacterial colonies are observed in the lysis zone; OL - the phage does not cause complete lysis of bacteria and there is secondary bacterial growth in the droplet zone; R- bacteria were resistant to phage. The efficiency of plating (EOP) of phages was calculated as average PFU on bacterial strains of interest divided by the average PFU obtained from the original host strain. EOP values from the original host strain were considered a reference - 100%. The EOP was classified into high ( $\geq 0.5$ ), medium (0.1–0.5), or low (0.001–0.1) [43].

### Transmission electron microscopy:

For the visualization of phage particles negative staining Drop-to-Drop method was used [44]. Briefly, 0.05 ml of phage suspension (with a titre of 10<sup>9</sup>-10<sup>10</sup> PFU/ml) was spotted on a formvar and carbon covered copper grid (Electron Microscopy Sciences). After 1 min of incubation extra liquid was blotted with filter paper. Copper grid was then placed on to the 2% phosphotungstic acid (pH 7.2) droplet for 1 min. extra liquid was blotted with filter paper again and the grid was allowed to air dry. Prepared sample was examined using transmission electron microscope JEOL 100- SX (Jeol, Akishima-Shi, Tokyo, Japan).

### One-step growth experiments:

For the one-step growth curve experiment, phages and their host bacterial strains were mixed in LB broth at a multiplicity of infection (MOI) of 0.1. The mixture was incubated at 37 °C in a water bath for 5-15 minutes to allow phage adsorption. To assess the number of unadsorbed phages at 5-minute intervals, 0.1 ml of the sample was transferred into pre-cooled 9.9 ml of LB broth containing chloroform and immediately placed on ice for 10 minutes. The sample was then analyzed using the double-layer agar method. The proportion of adsorbed phages was determined by calculating the percentage of non-adsorbed phage particles, which corresponded to the plaque count. Once the maximum adsorption level was reached, the mixture was diluted 1000 times in fresh LB broth and incubated under the

**Table S1.** List of 100 strains included in the study. Isolation source is indicated next to the strain ID. Strains resistant to all five phages tested in this work are highlighted in yellow. resistance (R) is indicated in purple, full and intermediate sensitivity – in blue (S and I, respectively). Next to each strain, its antibiotic-resistance status is shown: S – sensitive; MDR – multi-drug resistant; XDR – extensively drug resistant; PDR – pan-drug resistant.

#	Strain ID	Isolation source	Phage					
			M66	M222	M143	S884	S706/2	
1	<i>K.p.10</i>	Stool	R	S	R	S	S	MDR
2	<i>K.p.13</i>	Urine	R	S	R	S	S	MDR
3	<i>K.p.16</i>	Urine	R	R	R	S	S	MDR
4	<i>K.p.27</i>	Stool	R	I	R	R	R	MDR
5	<i>K.p.34</i>	Stool	S	I	R	S	S	MDR
6	<i>K.p.37</i>	Throat swab	R	I	R	R	R	XDR
7	<i>K.p.51</i>	Urine	S	S	R	I	S	XDR
8	<i>K.p.54</i>	Sputum	I	I	R	I	I	S
9	<i>K.p.57</i>	Sputum	R	I	R	R	R	MDR
10	<i>K.p.58</i>	Stool	R	R	R	S	R	XDR
11	<i>K.p.63</i>	Urine	R	I	R	I	S	XDR
12	<i>K.p.66</i>	Wound swab	S	I	R	R	R	<b>PDR</b>
13	<i>K.p.72</i>	Stool	R	I	S	R	R	XDR
14	<i>K.p.79</i>	Stool	R	S	R	I	S	XDR
15	<i>K.p.95</i>	Wound swab	R	I	R	R	R	XDR
16	<i>K.p.102</i>	Unknown	R	I	S	S	S	XDR
17	<i>K.p.154</i>	Unknown	R	S	R	R	R	S
18	<i>K.p.155</i>	Wound swab	R	S	R	R	R	XDR
19	<i>K.p.156</i>	Urine	R	S	R	I	S	XDR
20	<i>K.p.158</i>	Urethra	R	R	R	R	S	XDR
21	<i>K.p.159</i>	Stool	R	I	R	R	R	MDR
22	<i>K.p.160</i>	Stool	R	I	R	I	S	XDR
23	<i>K.p.161</i>	Stool	R	S	R	R	R	MDR
24	<i>K.p.162</i>	Stool	R	R	S	R	R	XDR
25	<i>K.p.163</i>	Stool	R	S	R	S	S	XDR
26	<i>K.p.164</i>	Urine	R	S	R	R	I	MDR
27	<i>K.p.165</i>	Urine	R	S	R	R	R	MDR
28	<i>K.p.166</i>	Stool	R	I	R	R	R	XDR
29	<i>K.p.167</i>	Throat swab	R	R	R	R	R	XDR
30	<i>K.p.168</i>	Stool	R	I	R	R	R	MDR
31	<i>K.p.169</i>	Stool	R	R	S	R	R	MDR
32	<i>K.p.170</i>	Stool	R	S	R	R	R	XDR
33	<i>K.p.171</i>	Stool	R	R	R	R	R	MDR
34	<i>K.p.172</i>	Stool	R	I	R	S	I	XDR
35	<i>K.p.173</i>	Stool	R	R	R	I	R	MDR
36	<i>K.p.174</i>	Stool	R	R	R	R	R	MDR
37	<i>K.p.176</i>	Urine	R	S	R	S	S	XDR
38	<i>K.p.177</i>	Sputum	R	S	R	R	R	XDR
39	<i>K.p.178</i>	Wound swab	R	I	R	R	R	S
40	<i>K.p.179</i>	Unknown	S	R	S	R	R	S
41	<i>K.p.180</i>	Urine	R	S	R	I	S	XDR
42	<i>K.p.181</i>	Unknown	R	S	R	I	R	MDR
43	<i>K.p.182</i>	Stool	R	S	R	I	R	MDR
44	<i>K.p.183</i>	Stool	R	R	S	R	R	MDR
45	<i>K.p.184</i>	Throat swab	R	S	R	S	S	MDR
46	<i>K.p.185</i>	Vagina	R	R	R	R	R	MDR
47	<i>K.p.186</i>	Unknown	S	R	R	R	R	S
48	<i>K.p.187</i>	Stool	R	I	R	I	I	S
49	<i>K.p.191</i>	Stool	S	R	R	S	I	XDR
50	<i>K.p.192</i>	Sputum	R	I	R	R	R	XDR
51	<i>K.p.793a</i>	Urine	R	S	R	R	R	XDR
52	<i>K.p.795a</i>	Urine	R	S	R	R	S	XDR

53	<i>K.p.797a</i>	Urine	R	S	R	R	R	XDR
54	<i>K.p.798a</i>	Urine	R	R	R	R	R	XDR
55	<i>K.p.799a</i>	Urine	R	R	R	R	R	XDR
56	<i>K.p.803a</i>	Stool	S	I	R	R	R	XDR
57	<i>K.p.804a</i>	Stool	R	I	R	R	S	XDR
58	<i>K.p.811a</i>	Stool	R	S	R	R	R	XDR
59	<i>K.p.813a</i>	Urine	R	R	R	S	S	<b>PDR</b>
60	<i>K.p.814a</i>	Urine	R	S	R	R	R	XDR
61	<i>K.p.815a</i>	Unknown	R	R	R	R	S	XDR
62	<i>K.p.816a</i>	Unknown	R	S	R	R	R	XDR
63	<i>K.p.820a</i>	Urine	R	I	R	S	S	XDR
64	<i>K.p.821a</i>	Urine	R	S	R	I	R	XDR
65	<i>K.p.823a</i>	Urine	S	R	R	R	R	XDR
66	<i>K.p.828a</i>	Unknown	R	I	R	R	R	XDR
67	<i>K.p.836a</i>	Urethra	R	S	R	R	R	XDR
68	<i>K.p.837a</i>	Sperm	R	S	R	R	R	XDR
69	<i>K.p.842a</i>	Urine	R	S	R	I	I	XDR
70	<i>K.p.853a</i>	Unknown	S	R	R	I	R	XDR
71	<i>K.p.855a</i>	Unknown	R	S	R	S	S	MDR
72	<i>K.p.856a</i>	Wound swab	R	S	R	R	R	XDR
73	<i>K.p.858a</i>	Urine	R	S	R	I	R	XDR
74	<i>K.p.860a</i>	Unknown	R	S	R	R	R	XDR
75	<i>K.p.870a</i>	Unknown	R	R	R	R	R	XDR
76	<i>K.p.872a</i>	Throat swab	R	S	R	R	R	XDR
77	<i>K.p.873a</i>	Unknown	R	S	R	I	R	XDR
78	<i>K.p.879a</i>	Throat swab	R	R	R	R	S	XDR
79	<i>K.p.880a</i>	Unknown	R	R	S	R	R	MDR
80	<i>K.p.884a</i>	Urine	R	R	R	S	R	XDR
81	<i>K.p.887a</i>	Wound swab	R	S	R	S	S	XDR
82	<i>K.p.889a</i>	Wound swab	S	I	S	R	R	XDR
83	<i>K.p.892a</i>	Urine	R	I	S	R	R	XDR
84	<i>K.p.898a</i>	Sputum	S	S	R	I	I	MDR
85	<i>K.p.900a</i>	Sputum	R	S	R	S	S	XDR
86	<i>K.p.904a</i>	Urine	R	I	R	R	R	XDR
87	<i>K.p.910a</i>	Stool	R	S	R	S	S	XDR
88	<i>K.p.912a</i>	Stool	R	R	S	R	R	XDR
89	<i>K.p.918a</i>	Stool	R	I	S	R	R	XDR
90	<i>K.p.924a</i>	Sperm	R	S	R	S	S	XDR
91	<i>K.p.925a</i>	Urine	R	S	R	I	S	XDR
92	<i>K.p.959a</i>	Urine	R	S	R	I	R	XDR
93	<i>K.p.962a</i>	Urine	S	S	R	I	I	XDR
94	<i>K.p.976a</i>	Sperm	R	S	R	I	I	XDR
95	<i>K.p.978a</i>	Prostate	R	I	R	I	I	XDR
96	<i>K.p.983a</i>	Urine	R	S	R	R	R	XDR
97	<i>K.p.984a</i>	Unknown	R	I	R	I	S	XDR
98	<i>K.p.985a</i>	Urine	S	R	R	S	I	XDR
99	<i>K.p.986a</i>	Urine	R	I	R	S	S	MDR
100	<i>K.p.987a</i>	Unknown	R	I	R	R	R	<b>PDR</b>

**Table S2.** List of antibiotics used in this work. Antibiotics are grouped according to their class.

Antibiotic class	Antibiotic
Penicillins	Ticarcillin
	Ampicillin
	Piperacillin
	Amoxicillin-clavulanic acid
	Ampicillin-sulbactam
Cephalosporins	Cefepime
	Cefuroxime
	Ceftriaxone
	Ceftazidime
	Cefotaxime
Carbapenems	Ceftazidime-avibactam
	Doripenem
	Imipenem
	Ertapenem
	Meropenem
Monobactam	Aztreonam
	Oftloxacin
	Moxifloxacin
Fluoroquinolones	Ciprofloxacin
	Levofloxacin
	Tobramycin
Aminoglycosides	Gentamicin
	Amikacin
Other	Trimethoprim-sulfamethoxazole
	Chloramphenicol

same conditions. To track phage propagation, sample aliquots were taken at 5-10 min intervals and were plated using the double-layer agar method.

Phage latent period was defined as the shortest time required for the phages to complete their replication cycle, from adsorption to bacterial lysis and progeny release. The burst size was calculated as the ratio of the phage count before and after lysis, indicating the number of new phages released per infected bacterial cell [41,45].

#### Evaluation of the frequency of the development of phage-resistant bacterial forms:

To estimate the frequency at which phage-resistant forms were created, both solid- and liquid culture approaches were used. In solid culture approach 0.1 ml bacterial culture ( $10^7$ - $10^8$  CFU/ml) was plated onto the LB agar plates pre-treated with phage suspensions ( $10^9$ - $10^{10}$  PFU/ml). After 18 hours of incubation at 37 °C, the number of phage-resistant bacterial colonies formed on the plates was recorded. The frequency of resistance formation was calculated as the CFU of resistant colonies divided by the CFU of colonies from a control experiment (bacteria plated on untreated agar) [41,46]. In liquid culture approach, 96 well microtiter plates were inoculated with 0.1 ml of phage suspension ( $10^9$ - $10^{10}$  PFU) and with 0.05 ml phage host strains ( $10^4$ - $10^5$  CFU). Absorbance was recorded at 590 nm every 20 min for 18 h while shaking at 37 °C.

## Results.

### Characterization of bacterial strains.

#### Antibiotic susceptibility of *K. pneumoniae* strains:

100 *K. pneumoniae* clinical strains, which were studied in this work had been isolated from various clinical samples of both, local and travelling patients. About one third were isolated from urine samples (30 %), followed by faecal isolates (29 %). 16 % of strains have unknown origin. Isolation sites of the remaining 25 strains are as follows: sputum and throat swabs – 11 %, urogenital specimens – 7 % and wound swabs – 7 % (Table S1).

All strains were investigated in terms of their antibiotic resistance. Two most effective antibiotics were chloramphenicol and a combination of ceftazidime with avibactam. Only 17 % and 18 % of strains showed resistance to these two antibacterial agents, respectively (Table 2). In cases of 15 other antibiotics that were tested in this work, percentage of resistant strains always exceeded 50 % and even reached 100 % in case of Ticarcillin. Ticarcillin was followed by ampicillin, doripenem, piperacillin and cefepime, resistance rates to which were 99 %, 95 %, 93 % and 93 %, respectively (Table 2). 86 % of all strains showed resistance to ceftazidime. 78 % and 73 % of studied strains were resistant against ofloxacin and the combination of amoxicillin and clavulanic acid, respectively. Moxifloxacin, aztreonam and ciprofloxacin were ineffective against 64 %, 63 % and 61 % of strains, respectively. Resistance rates to the remaining five antibiotics did not exceed 58 % and were as follows: imipenem - 58 %, cefuroxime - 57 %, meropenem - 56 %, ertapenem - 53 % and ceftriaxone - 51 % (Table 2).

According to these results, most studied strains showed either extensively drug-resistant (XDR) or multidrug-resistant (MDR) phenotypes. Three strains were completely resistant to all tested antibiotics of all classes. From the remaining 97 strains 67 are XDR and 24 are MDR isolates.

#### Evaluation of the presence of selected $\beta$ -lactamase encoding genes:

According to conventional PCR approach, we did not detect any of the four targeted *bla* genes in most of studied strains (70 %). Among the other 30 %, tested *bla* genes occurred either independently or in combination. 11 % of strains harbored *bla*<sub>KPC</sub>. *bla*<sub>CTXM</sub> gene was found in 8 % of strains. These two genes co-occurred in 2 strains. *bla*<sub>NDM</sub> was found in 4 % of studied strains and in all of four cases *bla*<sub>CTXM</sub> was also present. Only five strains were found to harbor *bla*<sub>OXA-48</sub> gene. In one out of these five cases *bla*<sub>OXA-48</sub> was co-detected with *bla*<sub>CTXM</sub>. Since only a small set of genes was selected for PCR detection, PCR-negative strains could still bear antibiotic resistance-encoding genes, which have not been targeted by us. These may include potential alternative mechanisms related to the loss of porins or to the acquisition of different efflux pumps.

#### Pulsed-field gel electrophoresis (PFGE) fingerprinting:

By PFGE fingerprinting we defined phylogenetic relationships between studied *K. pneumoniae* isolates. The strains were

**Table 1.** bla genes targeted by PCR. All primers and PCR setting were selected according to the sources cited in the table.

Targeted genes	Primer sequence	Expected product size	References
<i>bla</i> <sub>KPC</sub>	For: CGTCTAGTTCTGCTGTCTTG Rev: CTTGTCATCCTTGTAGGCG	798 bp	[32-34]
<i>bla</i> <sub>CTXM</sub>	For: AAAAATCACTGCGCCAGTTC Rev: AGCTTATTCACTGCCACGTT	415 bp	[35,36]
<i>bla</i> <sub>OXA-48</sub>	For: CCAAGCATTTTACCCGCATCKACC Rev: GYTTGACCATAACGCTGRCTGCG	389 bp	[37]
<i>bla</i> <sub>NDM</sub>	For: GCAGCTTGTGGCCATGCGGGC Rev: GGTGCGAAGCTGAGCACCGCAT	782 bp	[38]

**Table 2.** Antibiotic susceptibility of clinical isolates of *K. pneumoniae*. Amount of resistant/susceptible strains are presented in percentages. Percent values are calculated from 100 tested strains.

Antibiotic class	Antibiotic	Resistant strains (%)	Sensitive strains (%)
Penicillins	Ticarcillin	100	0
	Ampicillin	99	1
	Piperacillin	93	7
	Amoxicillin-clavulanic acid	73	27
	Ampicillin-sulbactam	50	50
Cephalosporins	Cefepime	93	7
	Cefuroxime	57	43
	Ceftriaxone	51	49
	Ceftazidime	86	14
	Cefotaxime	43	57
Carbapenems	Ceftazidime-avibactam	18	82
	Doripenem	95	5
	Imipenem	58	42
	Ertapenem	58	42
Monobactam	Meropenem	56	44
	Aztreonam	63	37
Fluoroquinolones	Ofloxacin	78	22
	Moxifloxacin	64	36
	Ciprofloxacin	61	39
	Levofloxacin	48	52
Aminoglycosides	Tobramycin	31	69
	Gentamicin	30	70
	Amikacin	30	70
Other	Trimethoprim-sulfamethoxazole	36	64
	Chloramphenicol	17	83

**Table 3.** EOP of five tested phages. EOP of 0.5 or higher corresponds to high efficiency, EOP values between 0.1 and 0.5 is considered medium efficiency, while EOP of 0.1 or less is low efficiency.

Bacteriophage	Number of bacterial strains tested	Quantitative distribution of strains according to the efficiency of each phage		
		EOP $\geq 0.5$	EOP $< 0.5$	EOP = 0
vB_Kp M66	13	7	5	1
vB_Kp M222	29	14	9	6
vB_Kp M143	9	4	0	5
vB_Kp S884	29	13	10	6
vB_Kp S706/2	18	9	6	3

**Table 4.** The rate at which phage resistant forms appear for each tested phage are presented. The rate is calculated by taking into account the initial amount of bacterial CFUs on phage-treated plates and the number of formed colonies.

Bacteriophage	Rate of formation of phage-resistant forms
vB_Kp M66	$8 \times 10^{-4}$
vB_Kp M222	$6 \times 10^{-3}$
vB_Kp M143	$3 \times 10^{-4}$
vB_Kp S884	$1.5 \times 10^{-3}$
vB_Kp S706/2	$1 \times 10^{-8}$

grouped according to their origin: 50 strains from local patients in one group and 50 strains from non-Georgian residents – in another group. Strains were compared with other strains from their assigned group. *Xba*I digestion resulted in detectable and reproducible fingerprints in 96 cases, while DNA of 4 (Three local patients' strains and one strain from a patient from abroad) strains was repeatedly degraded by restriction digest. Obtained fingerprints were used for constructing dendograms, where multiple clusters can be observed (Figure 1). Tight clusters represent strains with higher level of genetic relatedness. No big clusters or large groups of highly similar strains were detected – highlighting high genetic diversity among studied strains. No correlation was found between the clustering of strains and their antibiotic susceptibility patterns.

### Characterization of phages.

#### Phage host range and efficiency of plating (EOP):

From an initial set of 31 candidate phages five were selected for subsequent experiments. Main selection criterion for these phages was their lytic spectra. Phage M222 showed activity against 74 (out of 100) isolates, phages S884 and S706/2 were active against 44 strains and 40 strains, respectively. Phages M66 and M143 were not distinguished because of their wide lytic activity, however, they showed activity against strains, which were otherwise resistant to the three other phages. This way we ended up with a set of five phages, which, collectively act against 93 out of 100 target bacterial strains (Table S1).

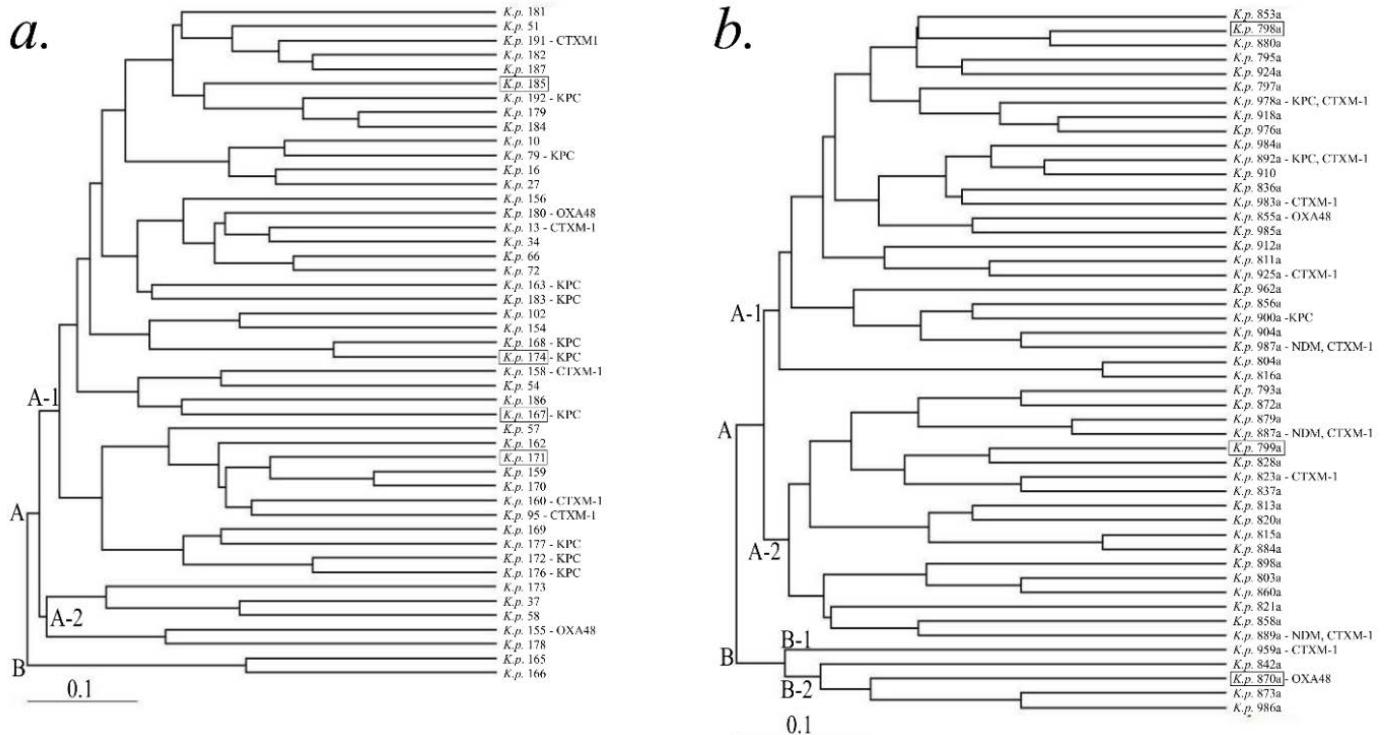
While some strains showed intermediate sensitivity (e.g. OL) towards phages of interest, only fully sensitive strains were

selected for the evaluation of the efficiency of plating (EOP) experiments. All five phages showed high EOP ( $\geq 0.5$ ) on roughly half of tested strains (Table 3). Phage M143, which was characterized by the narrowest host range, indeed showed plaque formation only on 4 out of 9 tested strains. Even in these four cases plaques were not always fully transparent. The other four phages showed plaque formation on most tested strains. Phage M66 formed plaques on 12 out of 13 strains (with high efficiency on 7 strains), phage M222 and phage S884 both formed plaques on 23 out of 29 and 26 strains respectively (with high efficiency on 14 and 13 strains, respectively) and phage S706/2 formed plaques on 15 out of 18 strains (with high efficiency on 9 strains) (Table 3).

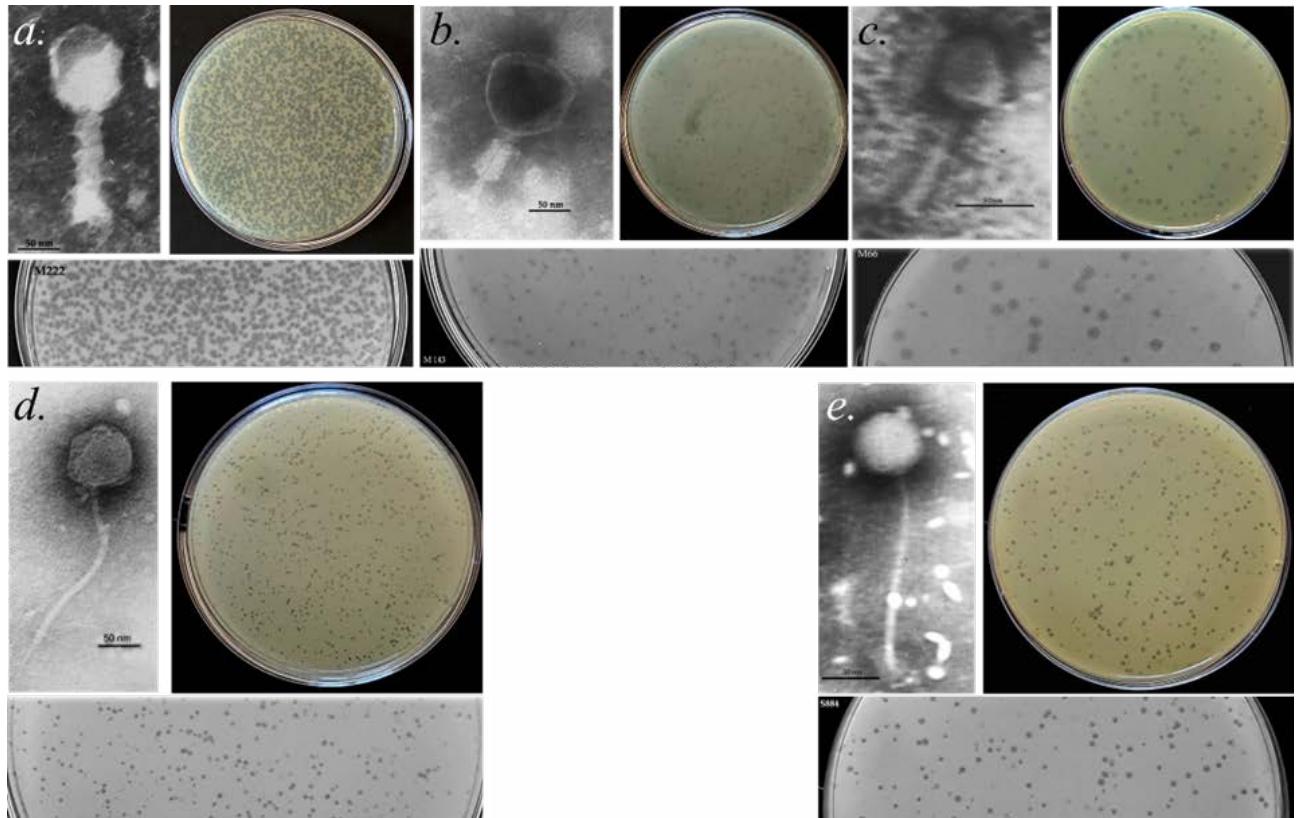
#### Phage virion and plaque morphology:

Phage virion morphology was studied via transmission electron microscopy (TEM). TEM investigation revealed two different phage morphologies. Phages M66, M222 and M143 showed morphology characteristic for the Myoviridae morpho group – all three phages having hexagonal heads and contractile tails (Figure 2 a, b, c – top left panels). On the other hand, phages S884 and S706/2 showed smaller heads and non-contractile tails, putting them in the Siphoviridae morphological group (Figure 2 d, e – top left panels).

Plaque formation varied among the studied five phages. Phage plaques on bacterial lawns differed in terms of their size and in terms of the presence or absence of halos around plaque centers. While phages M222, S884 and S706/2 formed small, clear plaques, in cases of phages M143 and M66 halo formation was also observed (Figure 2 b, c, bottom and right panels).



**Figure 1.** Dendograms of *Xba*I digest-based PFGE fingerprinted strains of *K. pneumoniae*. Strains of local patients (a) and of patients travelling from abroad (b) are presented in two separate trees. Clusters A and B (and corresponding sub-clusters) are indicated for each dendrogram. Seven strains, which showed resistance to all five phages are boxed. Detected presence of  $\beta$ -lactamase encoding genes is indicated next to relevant strains.



**Figure 2. Morphology of phage particles and plaques.** Phage particles were visualized by transmission electron microscopy, at x320 000 magnification. Scale bar – 50 nm. Plaque morphology was assessed on double layered agar plated. For each phage top-left panel depicts its virion morphology, top-right panel shows a full plate with phage plaques and the bottom panel depicts magnified picture of plaque-containing plate in greyscale. a. Phage M222 – Myoviridae, approximate head and tail sizes: 108 x 87 nm and 135 35 nm, respectively. b. Phage M 143 – Myoviridae, approximate head and tail sizes: 105 x 106 nm and 102 x 30 nm, respectively. c. Phage M66 – Myoviridae, approximate head and tail sizes: 43.7 x 43.7 nm and 75 x 9.5 nm, respectively. d. Phage S706/2 – Siphoviridae, approximate head and tail sizes: 78 x 78 nm and 218 x 8 nm, respectively. e. Phage S884 – Siphoviridae, approximate head and tail sizes: 65 x 65 nm and 188 x 6 nm, respectively.

#### Phage one-step growth experiments:

In terms of their growth characteristics, all five phages showed different properties. Phages M222 and M143 proved to be the fastest absorbers, 95 % and 92 % of phage particles of M222 and M143, respectively successfully absorbing to host cells in 5 minutes. In all other aspects these two phages differed drastically. With 130 min, phage M143 has the longest (among the studied phages) latent period. Its burst size fluctuates between 90 and 100 PFUs per infected cell. These properties are quite similar to what we saw in case of phage M66, which is also characterized by long latent period (100-120 min) and a burst size of 100-120 PFUs per infected cell. It is noteworthy, that maximum adsorption by this phage takes place at 10 minutes (with 90 % of phage particles adsorbing to the host). Fast-adsorbing phage M222 has a much shorter latent period of 15-20 min and its burst size is 40 PFUs per infected cell. Adsorption of phage particles of S706/2 and S884 peaks at 10 minutes in both cases (with 95 % and 90 % of corresponding phage particles adsorbing at 10 min time point), however their latent period and burst size are significantly different. Phage S706/2 has a 50 min-long latent period concluded by the release of 90-100 PFUs from each infected cell, while phage S884 produces 30 PFUs per infected cell after a latent period of only 20-25 min.

#### Determination of the rate of formation of phage-resistant forms:

We studied the rate at which phage-resistant forms arise in target bacterial strains. For phages S884 and S706/2, the rate was very low ( $1-1.5 \times 10^{-8}$ ). This number was higher for the three other phages (Table 4). Importantly, in case of phage M222 (with a rate of  $6 \times 10^{-3}$ ), resistant forms were found to be unstable – already after the first round of cultivation, phage resistance was no longer detected. It is interesting to note, that phage-resistant forms, which developed against phage M222 as well as phages M143 (at the rate of  $3 \times 10^{-4}$ ) and M66 (at the rate of  $8 \times 10^{-4}$ ) acquired sensitivity to other phages, including phage M198 [47] (data not shown).

In accordance with the results obtained by plate-based assays (described above), we found that phage resistance against phages S884 and S706/2, but also against phage M222 did not occur in liquid culture experiments. Bacterial forms resistant to phages M143 and M66 only arose after 8-12 hours of cultivation and even then, cell density remained low.

#### Discussion.

In this work we evaluated therapeutic potential of five phages targeting clinical strains of *K. pneumoniae*. *K. pneumoniae* is

an important pathogen, with more and more cases of infections, where conventional antibiotics have failed [48-52]. Since the number of successful clinical cases, where *K. pneumoniae* has been effectively targeted by personalized phages, is on the rise, it is obvious, that *Klebsiella* phages represent a promising alternative to antibiotics [12,13,53-59]. The fact that there are no ready-made *Klebsiella*-targeting phage cocktails available on the pharmaceutical market, creates obstacles for rapid, effective and accessible phage therapy options for *Klebsiella*-related infections. We therefore believe, that characterization of phages for potential therapeutic application (for personalized use as well as for potential cocktail development) is an important step in this direction.

While the need for well characterized *Klebsiella* phages is apparent, we understand the limitations of current study. Phenotypically characterized phages, which are described in this work have not yet been studied in terms of their genetic characteristics. This is an important next step, which would allow us to confidently declare therapeutic potential of these phages – as we have recently done for another *Klebsiella* phage – Phage M198 [47].

Phenotypic tests suggest, that all five phages described in this work are most likely lytic phages, which effectively target clinical isolates of *K. pneumoniae*. Also, the fact that these five phages vary by their intracellular growth characteristics (most importantly by their adsorption times and latent periods), makes them good candidates for phage combinations. Relatively low rates of resistance formation is also an important aspect, which needs to be noted. While presented data does not allow to pinpoint any mechanisms behind emergence or loss of phage-resistance, these are long known, but not fully understood occurrences. Since we did not study genetic backgrounds of different generations of the bacterial host and neither did we assess heterogeneity of phage-treated populations, possible pseudolysogeny cannot be fully excluded. It is also plausible to assume, that what we observed are not true resistant mutants, but rather cells in a carrier state [60,61]. It would therefore be interesting to find out whether the cells in different generations respond to phage infection through receptor-involving mechanisms such as by adapting LPS synthesis pathways or through reversible phase-variation [62,63]. These aspects of the emergence and loss of phage resistance towards described five phages remains to be understood.

Importantly, characterized phages lyse strains, which proved to be extensively drug-resistant – highlighting their therapeutic potential. Also, phenotypic antibiotic resistance profiles or presence of resistance genes tested by us does not seem to correlate with phage susceptibility of studied strains.

It is interesting to note, that the distribution of detected beta-lactamase encoding genes was uneven between the two groups of strains tested here. KPC, which is responsible for resistance to carbapenems and which is often found on mobile genetic elements, was more frequently encountered in strains originating from Georgia. Since we have not studied how alternative processes could contribute to observed antibiotic resistance, we are unable to definitively identify the specific mechanisms responsible for phenotypically observed resistance to a wide range of antibiotics. However, previous studies suggest that

loss of porins or acquisition of efflux pumps may contribute to resistance in strains, which, according to our limited PCR experiments, did not harbor beta-lactamase encoding genes. It has long been known, that outer membrane permeability is an important factor in drug resistance in *Klebsiella* [64]. Loss of outer membrane porins, such as OmpK35 and OmpK36 has been established as one of the factors contributing to resistance to beta-lactams, such as imipenem and ertapenem [64-66]. While efflux pumps are rarely the sole determinants of high-level antibiotic resistance, they are playing an important role in the development of multidrug resistance in *Enterobacteriaceae* [67]. It is therefore highly likely, that PCR-negative strains from this work possess mechanisms, not detectable by highly specific PCR used by us.

Our observation that these phages have covered 93 % of randomly selected drug-resistant, genetically diverse (according to PFGE fingerprinting) strains from different sources, suggests that they could be effective against a large variety of clinical isolates. However, we understand that the variety of targeted *K. pneumoniae* strains, being notorious for its capsule type diversity, needs to be confirmed by strain capsular typing. While such typing has not been a part of this project, we are planning to address this shortcoming in future work involving these phages.

Despite its shortcomings, we view this work as an important step towards ensuring that a large and diverse set of therapeutic *Klebsiella* phages are available. As a foundation for future experiments, this work would be followed by experiments, which would aim at phage cocktail design by more detailed characterization of phages as well as of their target strains.

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## Authorship contribution statement

L.L.: writing – original draft (lead); writing - review and editing (equal); Methodology (lead); Formal analysis (equal); Investigation(equal); Projectadministration(lead); Visualization (lead). D.B.: Investigation (equal). L.A.: Investigation (equal). M.Ch.: Investigation (equal). M.Kh.: Investigation (equal). G.Ts.: Investigation (equal). N.B.: Conceptualization (lead); Funding acquisition (lead); Project administration (supporting). L.K.: Conceptualization (supporting); Formal analysis (equal), Project administration (supporting); Visualization (supporting); Supervision (lead). E.Z.: writing – original draft (supporting); writing - review and editing (equal); Investigation (equal), Project administration (supporting); Visualization (supporting).

## REFERENCES

1. Clegg S, Murphy C.N. Epidemiology and Virulence of *Klebsiella Pneumoniae*. In Urinary Tract Infections; Mulvey, M.A., Klumpp, D.J., Stapleton, A.E., Eds.; ASM Press: Washington, DC, USA. 2016:435-457.
2. Podschun R, Ullmann U. *Klebsiella Spp.* as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors. Clin Microbiol Rev. 1998;11:589-603.

3. Holt K.E, Wertheim H, Zadoks R.N, et al. Genomic Analysis of Diversity, Population Structure, Virulence, and Antimicrobial Resistance in *Klebsiella Pneumoniae*, an Urgent Threat to Public Health. *Proc. Natl. Acad. Sci. U.S.A.* 2015;112.

4. Dong N, Yang X, Chan E.W.-C, et al. Klebsiella Species: Taxonomy, Hypervirulence and Multidrug Resistance. *EBioMedicine*. 2022;79:103998.

5. Ko W.-C. Community-Acquired *Klebsiella pneumoniae* Bacteremia: Global Differences in Clinical Patterns. *Emerg. Infect. Dis.* 2002;8:160-166.

6. Borer A, Saidel-Odes L, Riesenber K, et al. Attributable Mortality Rate for Carbapenem-Resistant *Klebsiella pneumoniae* Bacteremia. *Infect. Control Hosp. Epidemiol.* 2009;30:972-976.

7. Ben-David D, Kordevani R, Keller N, et al. Outcome of Carbapenem Resistant *Klebsiella pneumoniae* Bloodstream Infections. *Clinical Microbiology and Infection*. 2012;18:54-60.

8. Chetcuti Zammit S, Azzopardi N, Sant J. Mortality Risk Score for *Klebsiella pneumoniae* Bacteraemia. *European Journal of Internal Medicine*. 2014;25:571-576.

9. Li D, Huang X, Rao H, et al. *Klebsiella pneumoniae* Bacteremia Mortality: A Systematic Review and Meta-Analysis. *Front. Cell. Infect. Microbiol.* 2023;13:1157010.

10. Corbellino M, Kieffer N, Kutateladze M, et al. Eradication of a Multidrug-Resistant, Carbapenemase-Producing *Klebsiella pneumoniae* Isolate Following Oral and Intra-Rectal Therapy with a Custom Made, Lytic Bacteriophage Preparation. *Clinical Infectious Diseases*. 2020;70:1998-2001.

11. Nobrega F.L, Costa A.R, Kluskens L.D, et al. Revisiting Phage Therapy: New Applications for Old Resources. *Trends in Microbiology*. 2015;23:185-191.

12. Corbellino M, Kieffer N, Kutateladze M, et al. Eradication of a Multidrug-Resistant, Carbapenemase-Producing *Klebsiella pneumoniae* Isolate Following Oral and Intra-Rectal Therapy with a Custom Made, Lytic Bacteriophage Preparation. *Clinical Infectious Diseases*. 2020;70:1998-2001.

13. Herridge W.P, Shibu P, O'Shea J, et al. Bacteriophages of *Klebsiella* Spp., Their Diversity and Potential Therapeutic Uses. *J Med Microbiol.* 2020;69:176-194.

14. Eskenazi A, Lood C, Wubbolts J, et al. Combination of Pre-Adapted Bacteriophage Therapy and Antibiotics for Treatment of Fracture-Related Infection Due to Pandrug-Resistant *Klebsiella pneumoniae*. *Nat Commun.* 2022;13:302.

15. Weber-Dąbrowska B, Źaczek M, Łobocka M, et al. Characteristics of Environmental *Klebsiella pneumoniae* and *Klebsiella Oxytoca* Bacteriophages and Their Therapeutic Applications. *Pharmaceutics*. 2023;15:434.

16. Weinbauer M.G. Ecology of Prokaryotic Viruses. *FEMS Microbiol Rev.* 2004;28:127-181.

17. Chanishvili N. Phage Therapy—History from Twort and D'herelle through Soviet Experience to Current Approaches. In *Advances in Virus Research*; Elsevier: Cambridge, MA, US. 2012;83:3-40.

18. Salmond G.P.C, Fineran P.C. A Century of the Phage: Past, Present and Future. *Nat Rev Microbiol.* 2015;13:777-786.

19. Antimicrobial Resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report 2020; European Centre for Disease Prevention and Control: Stockholm. 2022.

20. El Haddad L, Harb C.P, Gebara M.A, et al. A Systematic and Critical Review of Bacteriophage Therapy Against Multidrug-Resistant ESKAPE Organisms in Humans. *Clinical Infectious Diseases*. 2019;69:167-178.

21. Gordillo Altamirano F.L, Barr J.J. Phage Therapy in the Postantibiotic Era. *Clin Microbiol Reviews*. 2019;32:e00066-18.

22. Vandenheuvel D, Lavigne R, Brüssow H. Bacteriophage Therapy: Advances in Formulation Strategies and Human Clinical Trials. *Annu. Rev. Virol.* 2015;2:599-618.

23. Langdon A, Crook N, Dantas G. The Effects of Antibiotics on the Microbiome throughout Development and Alternative Approaches for Therapeutic Modulation. *Genome Med.* 2016;8:39.

24. Palma M, Qi B. Advancing Phage Therapy: A Comprehensive Review of the Safety, Efficacy, and Future Prospects for the Targeted Treatment of Bacterial Infections. *Infect Dis Rep.* 2024;16:1127-1181.

25. Loc-Carrillo C, Abedon S.T. Pros and Cons of Phage Therapy. *Bacteriophage*. 2011;1:111-114.

26. Barr J.J. A Bacteriophages Journey through the Human Body. *Immunological Reviews*. 2017;279:106-122.

27. Principi N, Silvestri E, Esposito S. Advantages and Limitations of Bacteriophages for the Treatment of Bacterial Infections. *Front. Pharmacol.* 2019;10:513.

28. Łusiak-Szelachowska M, Weber-Dąbrowska B, Źaczek M, et al. The Presence of Bacteriophages in the Human Body: Good, Bad or Neutral? *Microorganisms*. 2020;8:2012.

29. Tait K, Skillman L.C, Sutherland I.W. The Efficacy of Bacteriophage as a Method of Biofilm Eradication. *Biofouling*. 2002;18:305-311.

30. Fong S.A, Drilling A, Morales S, et al. Activity of Bacteriophages in Removing Biofilms of *Pseudomonas aeruginosa* Isolates from Chronic Rhinosinusitis Patients. *Front. Cell. Infect. Microbiol.* 2017;7:418.

31. The European Committee on Antimicrobial Susceptibility Testing Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 12.0. 2022.

32. Poirel L, Walsh T.R, Cuvillier V, et al. Multiplex PCR for Detection of Acquired Carbapenemase Genes. *Diagnostic Microbiology and Infectious Disease*. 2011;70:119-123.

33. Robledo I.E, Aquino E.E, Vázquez G.J. Detection of the KPC Gene in *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* during a PCR-Based Nosocomial Surveillance Study in Puerto Rico. *Antimicrob. Agents Chemother.* 2011;55:2968-2970.

34. Tzouvelekis L.S, Markogiannakis A, Psichogiou M, et al. Carbapenemases in *Klebsiella pneumoniae* and Other Enterobacteriaceae: An Evolving Crisis of Global Dimensions. *Clinical Microbiology Reviews*. 2012;25:682-707.

35. Shams E, Firoozeh F, Moniri R, et al. Prevalence of Plasmid-Mediated Quinolone Resistance Genes among Extended-Spectrum  $\beta$ -Lactamase-Producing *Klebsiella pneumoniae* Human Isolates in Iran. *Journal of Pathogens*. 2015;2015:1-7.

36. Calbo E, Freixas N, Xercavins M, et al. Foodborne Nosocomial Outbreak of SHV1 and CTX-M-15-Producing *Klebsiella pneumoniae*: Epidemiology and Control. *Clinical Infectious Diseases*. 2011;52:743-749.

37. Voets G.M, Fluit A.C, Scharringa J, et al. A Set of Multiplex PCRs for Genotypic Detection of Extended-Spectrum  $\beta$ -Lactamases, Carbapenemases, Plasmid-Mediated AmpC  $\beta$ -Lactamases and OXA  $\beta$ -Lactamases. International Journal of Antimicrobial Agents. 2011;37:356-359.

38. Doyle D, Peirano G, Lascols C, et al. Laboratory Detection of Enterobacteriaceae That Produce Carbapenemases. J Clin Microbiol. 2012;50:3877-3880.

39. Durmaz R, Otu B, Koksal F, et al. The Optimization of a Rapid Pulsed-Field Gel Electrophoresis Protocol for the Typing of *Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella* spp. Jpn J Infect Dis. 2009;62:372-377.

40. Hampl V, Pavlicek A, Flegr J. Construction and Bootstrap Analysis of DNA Fingerprinting-Based Phylogenetic Trees with the Freeware Program FreeTree: Application to Trichomonad Parasites. International Journal of Systematic and Evolutionary Microbiology. 2001;51:731-735.

41. Adams M.H. Bacteriophages. Appendix: Methods of Study of Bacterial Viruses; An Interscience reprint; Interscience Publishers. 1959.

42. Clokie M.R.J, Kropinski A.M, Eds.; Bacteriophages. Methods in Molecular Biology; Humana Press: Totowa, NJ. 2009;501.

43. Khan Mirzaei M, Nilsson A.S. Isolation of Phages for Phage Therapy: A Comparison of Spot Tests and Efficiency of Plating Analyses for Determination of Host Range and Efficacy. PLoS ONE. 2015;10:e0118557.

44. U.S. Centers for Disease Control and Prevention Negative Staining Electron Microscope Protocol for Rash Illness. 2024.

45. Middelboe M, Amy M, Chan N.P, et al. One-Step Growth Experiments (Bacteriophages). 2015;1.

46. Чанишвили Т, Капанадзе Ж. Определение Скорости Развития Фагоустойчивости Различных Видов. 1967:91-98.

47. Leshkasheli L, Kusradze I, Bolkvadze D, et al. *Klebsiella pneumoniae* Phage M198 and Its Therapeutic Potential. Viruses. 2025;17:115.

48. Livermore D.M. Has the Era of Untreatable Infections Arrived? Journal of Antimicrobial Chemotherapy. 2009;64:i29-i36.

49. Karaiskos I, Giannarellou H. Multidrug-Resistant and Extensively Drug-Resistant Gram-Negative Pathogens: Current and Emerging Therapeutic Approaches. Expert Opinion on Pharmacotherapy. 2014;15:1351-1370.

50. Holt K.E, Wertheim H, Zadoks R.N, et al. Genomic Analysis of Diversity, Population Structure, Virulence, and Antimicrobial Resistance in *Klebsiella pneumoniae*, an Urgent Threat to Public Health. Proc. Natl. Acad. Sci. U.S.A. 2015;112.

51. Miftode I.-L, Nastase E, Miftode R.-Ştefan, et al. Insights into Multidrug-resistant *K. pneumoniae* Urinary Tract Infections: From Susceptibility to Mortality. Exp Ther Med. 2021;22:1086.

52. Gomes M.Z.R, De Lima E.M, Martins Aires C.A, et al. Outbreak Report of Polymyxin-Carbapenem-Resistant *Klebsiella pneumoniae* Causing Untreatable Infections Evidenced by Synergy Tests and Bacterial Genomes. Sci Rep. 2023;13:6238.

53. Cano E.J, Caflisch K.M, Bollyky P.L, et al. Phage Therapy for Limb-Threatening Prosthetic Knee *Klebsiella pneumoniae* Infection: Case Report and in Vitro Characterization of Anti-Biofilm Activity. Clin Infect Dis. 2020;73:e144-e151.

54. Townsend E.M, Kelly L, Gannon L, et al. Isolation and Characterization of *Klebsiella* Phages for Phage Therapy. PHAGE. 2021;2:26-42.

55. Doub J.B, Shishido A, Srikanth U, et al. Salphage: Salvage Bacteriophage Therapy for a Recalcitrant *Klebsiella pneumoniae* Prosthetic Shoulder Infection — a Case Report. ActaO. 2022;93:756-759.

56. Onallah H, Hazan R, Nir-Paz R, et al. Compassionate Use of Bacteriophages for Failed Persistent Infections During the First 5 Years of the Israeli Phage Therapy Center. Open Forum Infectious Diseases. 2023;10:ofad221.

57. Pirnay J.-P, Djebara S, Steurs G, et al. Personalized Bacteriophage Therapy Outcomes for 100 Consecutive Cases: A Multicentre, Multinational, Retrospective Observational Study. Nat Microbiol. 2024;9:1434-1453.

58. Gholizadeh O, Ghaleh H.E.G, Tat M, et al. The Potential Use of Bacteriophages as Antibacterial Agents against *Klebsiella pneumoniae*. Virol J. 2024;21:191.

59. Abbas S, Kanwar R, Ullah K, et al. Bacteriophage Therapy: A Possible Alternative Therapy against Antibiotic-Resistant Strains of *Klebsiella pneumoniae*. Front. Microbiol. 2025;16:1443430.

60. Mäntynen S, Laanto E, Oksanen H.M, et al. Black Box of Phage–Bacterium Interactions: Exploring Alternative Phage Infection Strategies. Open Biol. 2021;11:210188.

61. Abedon S.T. Disambiguating Bacteriophage Pseudolysogeny: An Historical Analysis of Lysogeny, Pseudolysogeny, and the Phage Carrier State. In Contemporary trends in bacteriophage research; Nova Science Publishers, Incorporated: New York, NY. 2009:285-307.

62. Debray R, De Luna N, Koskella B. Historical Contingency Drives Compensatory Evolution and Rare Reversal of Phage Resistance. Molecular Biology and Evolution. 2022;39:msac182.

63. Turkington C.J.R, Morozov A, Clokie M.R.J, et al. Phage-Resistant Phase-Variant Sub-Populations Mediate Herd Immunity Against Bacteriophage Invasion of Bacterial Meta-Populations. Front. Microbiol. 2019;10:1473.

64. Jacoby G.A, Mills D.M, Chow N. Role of  $\beta$ -Lactamases and Porins in Resistance to Ertapenem and Other  $\beta$ -Lactams in *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2004;48:3203-3206.

65. Tsai Y.-K, Fung C.-P, Lin J.-C, et al. *Klebsiella pneumoniae* Outer Membrane Porins OmpK35 and OmpK36 Play Roles in Both Antimicrobial Resistance and Virulence. Antimicrob Agents Chemother. 2011;55:1485-1493.

66. Wassef M, Abdelhaleim M, AbdulRahman E, et al. The Role of OmpK35, OmpK36 Porins, and Production of  $\beta$ -Lactamases on Imipenem Susceptibility in *Klebsiella pneumoniae* Clinical Isolates, Cairo, Egypt. Microbial Drug Resistance. 2015;21:577-580.

67. Rovier F, Brunel J.-M, Pagès J.-M, et al. Efflux-Mediated Resistance in Enterobacteriaceae: Recent Advances and Ongoing Challenges to Inhibit Bacterial Efflux Pumps. Antibiotics. 2025;14:778.