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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

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 სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება 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

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, применявшиеся 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
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 compu-ter-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.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.

ავტორთა საყურადღებოდ!

რედაქციაში 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 ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, 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 სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: 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, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის 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, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში 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 რეცენზია, 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. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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SMALL BUT MIGHTY: CHARACTERIZATION OF *VB_SPY_7*, A LYTIC PHAGE TARGETING STREPTOCOCCUS PYOGENES

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Abstract.

The emergence of antibiotic-resistant pathogens necessitates alternative therapies for treating microbial infections, especially in the oral cavity and upper respiratory tract. Our team has developed Phage Pastilles, a controlled-release formulation containing bacteriophages that target common pathogens, including *Streptococcus pyogenes, Streptococcus salivarius, Staphylococcus aureus, Enterococcus faecalis,* and *E. coli*. These pastilles incorporate eucalyptus oil and serve as a reservoir for gradually releasing bacteriophages, enhancing local therapeutic impact. This study focuses on the characterization of a novel lytic bacteriophage, *vB_SPY_7*, specifically targeting *S. pyogenes* which was incorporated into phage pastilles. Phage *vB_SPY_7*, isolated from wastewater, underwent host range analysis, plaque assays, adsorption kinetics, growth curve studies, electron microscopy, and whole-genome sequencing. Genomic analysis revealed that *vB_SPY_7* is a member of the unclassified Caudoviricetes, with a 35,679 bp genome and 46 predicted ORFs, including essential structural and metabolic genes. Sequence analysis and biological characterization have demonstrated that *vB_SPY_7* is a viable candidate for therapeutic use, and it is highly active when combined in a sustained-release format like Phage Pastilles.

Key words. *Streptococcus pyogenes*, bacteriophage, phage pastilles.

Introduction.

Streptococcus pyogenes, a Gram-positive bacterium, is responsible for a wide array of infections ranging from mild cases of pharyngitis to severe systemic infections like necrotizing fasciitis and toxic shock syndrome [1]. The high virulence of *S. pyogenes* is attributed to its ability to produce various toxins and enzymes that aid in immune evasion and tissue invasion, making it a persistent and recurrent pathogen in the human body. In particular, the upper respiratory tract, which includes the pharynx and tonsils, is highly susceptible to *S. pyogenes* colonization, leading to conditions such as strep throat [2]. These infections, while often treatable with antibiotics, can lead to complications like rheumatic fever or glomerulonephritis if untreated or mismanaged, underscoring the need for alternative treatments that can both prevent recurrence and maintain local microbiome balance [3,4].

The rise of antimicrobial resistance (AMR) among pathogenic bacteria has led to a renewed interest in bacteriophages (phages) as alternative or adjunct therapies to traditional antibiotics. Bacteriophages, viruses that specifically infect bacteria, are known for their ability to target and destroy bacterial cells while sparing human cells, making them a promising tool for addressing antibiotic-resistant infections [5,6]. As AMR continues to threaten the effectiveness of antibiotics, particularly in managing infections in clinical settings, phage therapy is increasingly being investigated for its unique potential to combat infections that no longer respond to conventional antibiotics [7]. The delivery method of antimicrobial agents is a critical factor in treatment efficacy, especially in regions like the oral cavity, where constant salivary flow and frequent movements pose challenges for sustained drug retention [8]. Phage-based treatments are particularly well-suited for such localized infections due to their specificity, limited impact on non-target microbes, and the natural tendency of phages to amplify in the presence of their bacterial hosts. This approach not only allows prolonged phage presence in the oral cavity but also reduces the need for repeated administration, which can improve patient compliance. Furthermore, the integration of eucalyptus oil in these pastilles offers potential synergistic effects, as eucalyptus is known for its antimicrobial and anti-inflammatory properties [9,10]. A key focus of our research has been the isolation and characterization of new bacteriophage targeting *Streptococcus pyogenes*, for incorporation into our novel Phage Pastille "Phagovita". To ensure the efficacy and safety of *vB_SPY_7*, we conducted extensive testing, including electron microscopy for morphological characterization, genome sequencing, and bioinformatic analysis. Our analysis revealed that *vB_SPY_7* belongs to the unclassified Caudoviricetes, a phage family known for their specificity and potential therapeutic applications. The phage genome of *vB_SPY_7* contains 46 open reading frames (ORFs), with annotated functions related to structural assembly, host lysis, and DNA/RNA metabolism. Notably, the phage lacks lysogenic cycle genes, suggesting a strictly lytic lifestyle that enhances its safety profile for therapeutic applications by minimizing the risk of horizontal gene transfer. This study contributes to the growing body of research supporting phage therapy as a viable treatment option in the face of escalating antibiotic resistance.

Materials and Methods.

Bacterial Strains:

A total 60 bacterial strains were used from the collection of General Microbiology laboratory of which 10 were *Streptococcus pyogenes*,10 *streptococcus agalactiae*, 10 *Streptococcus sanguinis*, 10 *streptococcus sallivarius*, 10 *Streptococcus mutans* strains, and 10 *Enterococcus faecalis*. All bacterial cultures were maintained in Brain Heart Infusion (BHI) medium (Becton Dickinson and Company, Sparks, MD, USA) at 37°C under aerobic conditions.

Phage Isolation and Purification:

The bacteriophage *vB_SPY_7* was isolated from sewage water collected from the Mtkvari River in Tbilisi, Georgia. The isolation, purification, concentration, and propagation processes followed standard protocols as previously described with small modification [11]. Briefly, 100 mL of wastewater was combined with 10 mL of 10X 1M CaCl2 TSB broth and 1 mL of an exponentially growing bacterial culture (OD600 = 1). After a 24-hour incubation at 37°C, the mixture was centrifuged, and the supernatant was filtered using a sterile 0.22 µm syringe filter. The filtrate was stored at 4°C for further analysis. Phage purification was carried out using the serial dilution and single plaque isolation method. Initially, high-titer phage lysates were diluted in SM buffer to obtain a series of dilutions. Each dilution was mixed with its host bacteria and incorporated into soft agar overlays, which were poured onto TS agar plates. Following incubation at 37°C for 18–24 hours, a single plaque was picked using a sterile pipette tip and transferred into SM buffer to elute the phage particles. This process was repeated at least five times to ensure the purification of the phage. The purified phage stock was then amplified by infecting the host bacterial culture, followed by filtration through a 0.22 µm syringe filter to remove bacterial debris. The purified phage was stored at 4°C for further experiments.

Electron microscopy:

Phage lysate (10^8 pfu/mL) were spotted on carbon-coated grid, dried, stained with 2% phosphotungstic acid, air dried, and analyzed by Transmission electron microscopy (Model Jeol/ JEM 2100 2000X) at 200 kV to study phage morphology. The bar represents a length of 100nm [12].

Host Range:

The host range of the phage was assessed using the spot titer method, where high-titer phage lysates were applied to bacterial lawns from the strain collection. Following an 18-hour incubation at 37°C, the plates were examined for plaque formation. Phage lytic activity was categorized as follows: CL (confluent lysis), SCL (semi-confluent lysis), IP (individual plaques), OL (overgrown lysis), and R (resistant) [13].

Phage Adsorption and One-Step Growth Assay:

Phage adsorption was evaluated by mixing phage and bacterial cultures at a multiplicity of infection (MOI) of 0.1. The mixture was incubated at 37°C, and at intervals of 0, 5, 10, and 12 minutes, 0.1 mL samples were taken and transferred into tubes containing 9.9 mL of BHI broth with 0.4 mL chloroform. After shaking and cooling on ice for 10 minutes, the samples were diluted 100-fold and mixed with semi-solid 1M $CaCl_2$ supplemented TS agar before being poured onto TS agar plates. After a 24-hour incubation at 37°C, the percentage of non-adsorbed phages was calculated using the formula: 100−(PnP0×100)100 - \left(\frac{P_n}{P_0} \times 100\right)100−(P0Pn×100), where PnP_nPn represents non-adsorbed phages, and P0P_0P0 is the initial phage count in the control. Serial dilutions of phages without chloroform served as controls.

For the one-step growth curve, experiments followed established methods to determine the latent period and burst size. Briefly, 0.1 mL of phage suspension (2×10^8 PFU/mL) was added to 0.9 mL of a mid-log-phase bacterial culture (5×10^8 CFU/mL, MOI = 0.4) and incubated at 37°C for 10 minutes to allow adsorption. The mixture was then centrifuged at 6000 × g for 5 minutes, and the pellet was resuspended in 10 mL of BHI broth. Samples (0.1 mL) were collected every 10 minutes over 90 minutes and titrated against the host bacterium. All experiments were performed in triplicate [13].

DNA Extraction, PFGE, Sequencing, and Genome Annotation:

Phage DNA was extracted using the Invitrogen Genomic DNA Mini Kit by Thermo Fisher Scientific (Life Technologies Corp., 5781 Van Allen Way, Carlsbad, CA, USA.). The genome was sequenced using the Illumina Miseq Platform with paired-end (2 ×151-bp) operating mode (Illumina, San Diego, CA, USA) at Macrogen Europe in Amsterdam, The Netherlands. Spades v. 3.15.3 was used for fastq file quality control and SPAdes (version 3.15.3) for de novo assembly with default settings [14,15]. The contig sequence was obtained with a minimum of 100-fold coverage. Open reading frames were predicted by Genemarks v. 4.28 and the genome was annotated via Artemis [16,17]. Function annotation was done by PHROGs v.4. A circular genome map was constructed using Genius [18,19]. As for the genome comparison visualizer, EASYFIG v.3.4 was used. VipTree v.2.2.5. was used for a genome comparison visualizer. For tRNA search, tRNAscan-SE v.1.3.1 software was used [20]. The sequence has been uploaded to GenBank with the accession number OR387861.1.

Results.

Morphological characteristics obtained by transmission electron microscopy together with the genomic information revealed that phage *vB_SPY_7* belongs to the unclassified Caudoviricites (Figure 1A). Phage *vB_SPY_7* contains sdDNA and is 35679 bp in length. A total 46ORFs were predicted, functionally were annotated 27ORFs. Genes with predicted functions were grouped as following: head and packaging module (7 ORFs); tail morphogenesis module (5 ORFs); host cell lysis module (2 ORFs), DNA and RNA metabolism (4 ORFs), 4ORF were annotated as Head to tail connection and 4 ORFs were identified as Auxiliary metabolic gene and host takeover (Figure 1B).

The phage host range was tested on 10 clinical isolates of GAS, 10 clinical isolates of GBS,10 clinical isolates of *Viridans group Streptococci*, and 10 *Enterococcus spp*.; Phage *vB_SPY_7* was able to productively infect only *Streptococcus pyogenes* strains and none of the other *Streptococcus* strains. The approximate time for 89% of phage to attach to host strain was 12 min. Phage *vB_SPY_7* had a 22 min latent period and a burst size of 180 (± 25) progeny per infected host cell (Figure 2A,B).

In an attempt to classify *vB_SPY_7* we compiled the database of similar viruses. Then, we constructed proteomic tree. On the resulting tree *vB_SPY_7* failed to group with phages classified to any known genus. However, the sequence of phage *vB_SPY_7* is having some similarity ~ 59,9% to the genome of Streptococcus phage A25. Phage genome does not contain lysogeny control genes and no tRNAs were identified.

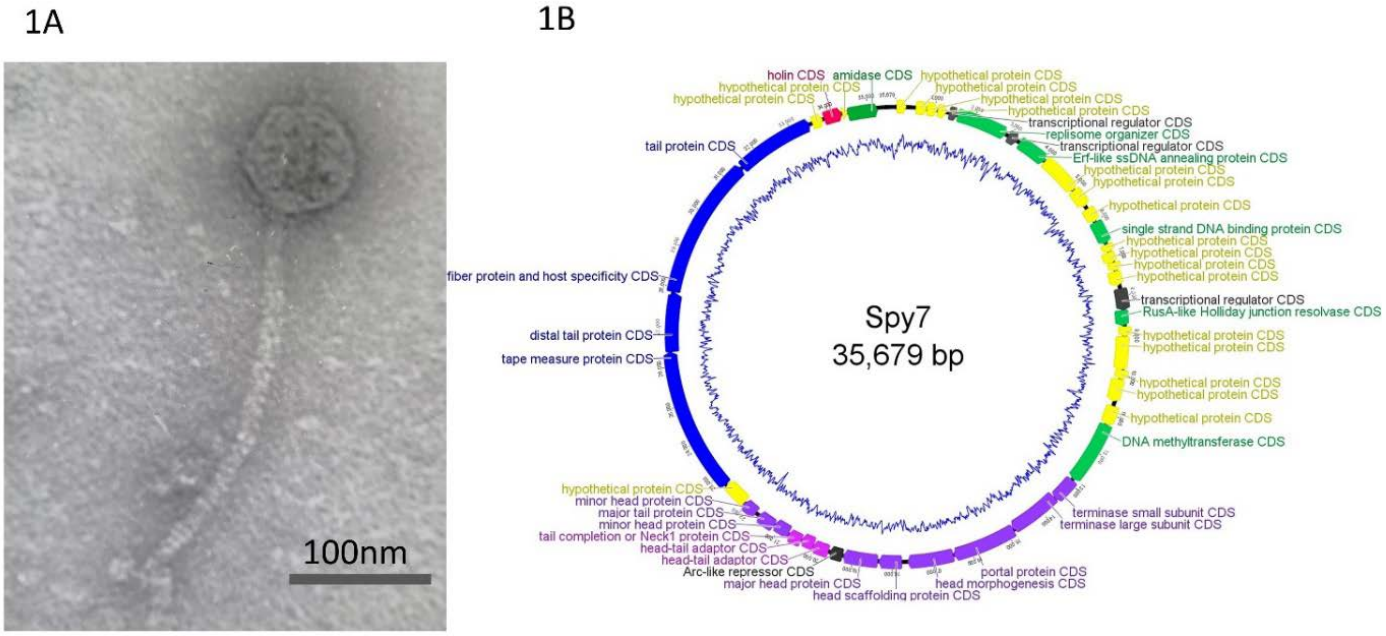

Figure 1A, B. *Transmission electron micrograph and Genome map of phage vB_SPY_7.*

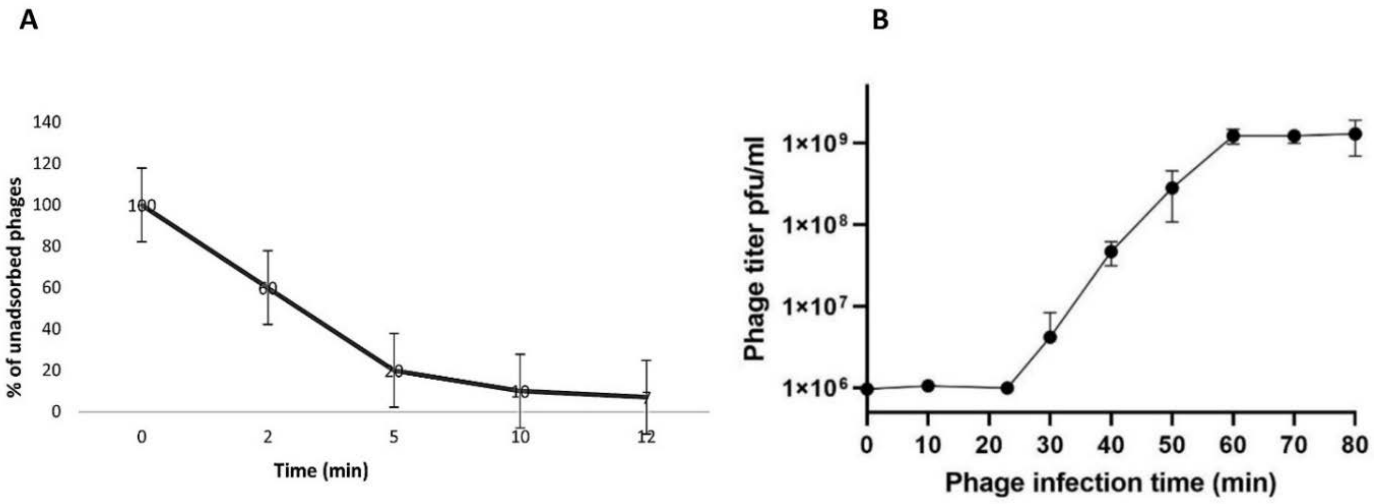

Figure 2A, B. *Phage vB_SPY_7 Adsorption and one step growth curve.*

Discussion.

Our research highlights the potential of bacteriophage *vB_SPY_7* as a novel therapeutic agent against *Streptococcus pyogenes* infections, addressing critical challenges posed by antimicrobial resistance (AMR). The findings demonstrate the phage's specificity for *S. pyogenes*, it's strictly lytic nature, and its promising characteristics for therapeutic applications. The morphological and genomic analyses confirmed that *vB_SPY_7* is an unclassified member of the Caudoviricetes, with a genome devoid of lysogeny-associated genes, minimizing risks such as horizontal gene transfer and lysogenic conversion. These properties establish *vB_SPY_7* as a safe candidate for therapeutic use. Its inability to infect other streptococcal or enterococcal species underscores its host specificity, which is crucial for preserving the microbiome balance during treatment. The observed latent period of 22 minutes and burst size of 180 progeny per infected cell suggest that *vB_SPY_7* can rapidly proliferate in the presence of its bacterial host, enhancing its therapeutic efficacy. Phage-based treatments, such as the novel Phage Pastilles, offer distinct advantages for managing localized infections in the oral cavity and upper respiratory tract. The incorporation of *vB_SPY_7* into this slow-release formulation, combined with the antimicrobial properties of eucalyptus oil, provides a synergistic approach that targets *S. pyogenes* while mitigating the impact on non-target microbial communities. This dual-action strategy addresses both the eradication of pathogens and the prevention of microbiome dysbiosis, a common complication of broad-spectrum antibiotics. The rise of AMR necessitates alternative solutions, and our study adds to the growing evidence supporting phage therapy's viability. By combining *vB_SPY_7* with innovative delivery methods like Phage Pastilles, this research paves the way for more effective

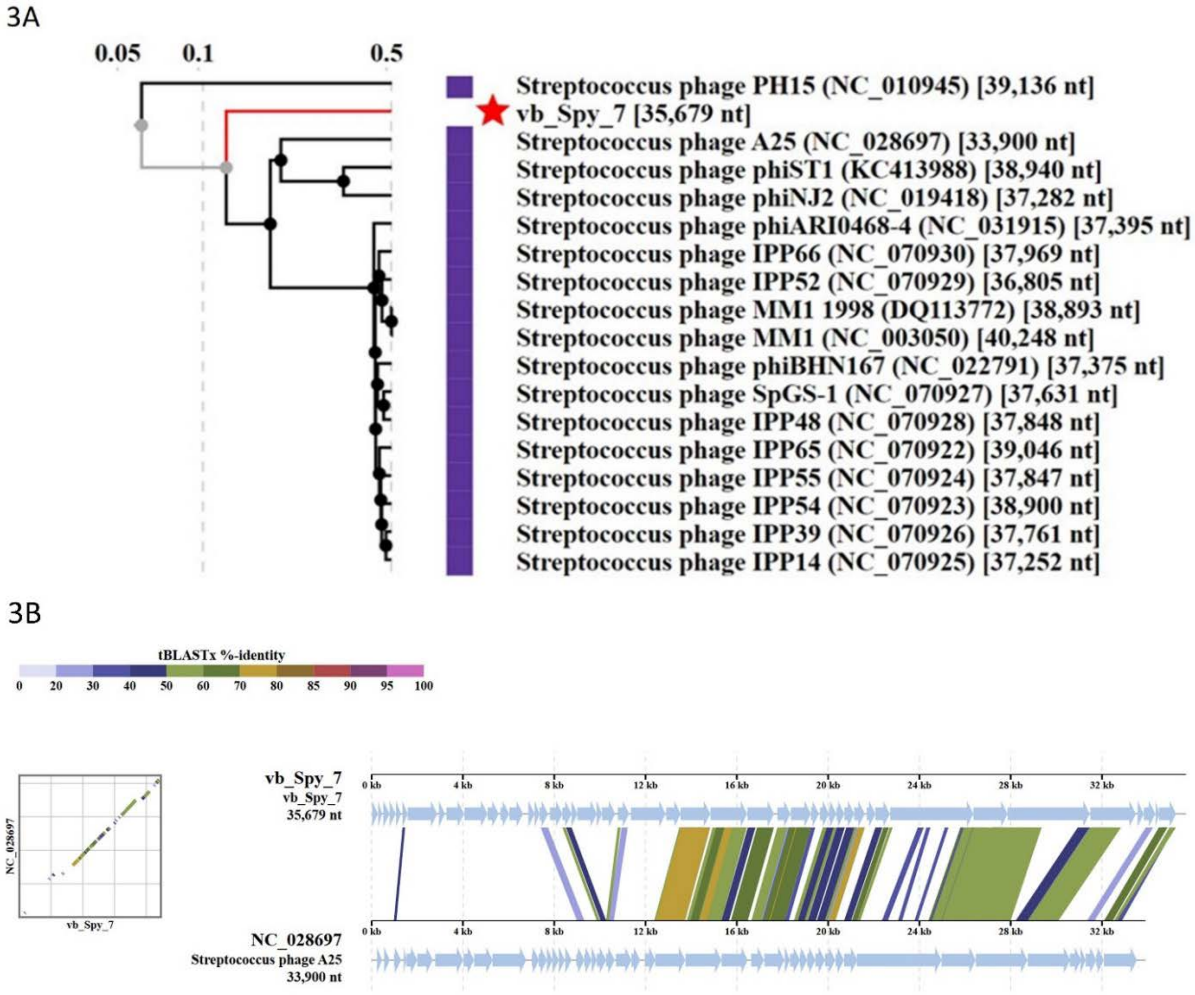

Figure 3A, B. *Proteomic dendrogram(A) and tBLASTx alignment(B) of phage vB_SPY_7 and reference phages.*

and patient-friendly approaches to treating *S. pyogenes*-related infections.

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Declaration of Competing Interests:

All authors declare that they have no relevant financial or non-financial interests to disclose.

Author's contribution:

All authors contributed to the study conception and design. The study was designed, directed and coordinated by S.R., I.K.,

All tasks and methodology were performed by S.R., I.K., N.K., M.G.,

The main text of the paper was written by S.R and improved and revised by S.R., I.K.; visualization and revision were done by S. R. and I.K., N.K

Investigation was performed by S.R., N.K., M.Ch.

Writing-editing was done by S.R., M.G., I.Tch

Sequence analysis and interpretation of data by I.K., N.K.

All authors have read and agreed to the given version the manuscript.

Ethics approval:

This is an observational study. Ethical Approval- not applicable.

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All authors have read and confirm their agreement for the publication of the provided version of the manuscript.

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