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ACTIVITY OF BACTERIOPHAGES TO MULTIPLY RESISTANT STRAINS OF SALMONELLA AND THEIR VARIOUS SEROTYPES

Phage therapy is one of the promising "new" methods of treatment, which attracts more attention abroad. Despite the fact that phage therapy is traditionally used in former Soviet countries, its importance in Western countries began to be assessed with the advent of a multitude of antibiotic-resistant forms of bacteria that are no longer being treated. In this regard, the number of deaths among patients exposed to antibiotic-resistant infections increases every year. Phage terapy can be used as an alternative method of treatment. Numerous literature sources indicate that resistance to antibiotics and bacteriophages does not match. However, little is known about how specific the bacteriophages are to various bacterial serotypes, which are simultaneously characterized by multiple drug resistance (MDR).

Key words: *bacteriophage (phage), salmonella, multiple drug resistance (MDR), serotype*

Introduction

Phage therapy is one of the promising "new" methods for treatment of infectious diseases of bacterial origin, which nowadays attracts increased attention abroad [1]. Phage therapy has been traditionally used in the former Soviet countries and ex-Soviet countries, however its importance began to be appreciated in Western countries with the advent of a multiply drug-resistant (MDR)

untreatable forms of bacteria [2]. In this regard, the number of deaths among patients exposed to antibiotic-resistant infections increases year by year [3].

Salmonella is one of the four main causes of diarrheal diseases worldwide. Most cases of salmonellosis are mild; however, sometimes salmonellosis can be life threatening. The severity of the disease depends on the factors associated with the host, and on the serotype of *Salmonella.* Recently, a certain number of resistant serotypes appeared in the food chain, which can often lead to fatal consequences. For example, in 2010, more than 30 Kaliningraders were poisoned with salads purchased in a supermarket, 27 injured were hospitalized. The infection was caused by a salmonella strain of serotype *S*. Typhimurium [4]. Only in 2017 in the United States were recorded several outbreaks caused by different serotypes of *Salmonella: Salmonella* Urbana. *Salmonella* Newport, *Salmonella* Infantis, *Salmonella* Anatum, *Salmonella* Thompson, *Salmonella* Kiambu, *Salmonella* Agona, and *Salmonella* Gaminara. [5]. Food poisoning of 8 people through raw sprouts was recorded in January 2018 in two US states, Illinois and Wisconsin, in the Jimmy John's chain of restaurants [6]. Severe poisoning of 16 people by chicken burgers infected with *S.* Typhimurium and *S*.Agona serotypes was registered in February 2018 in Georgia [7]. Most serotypes isolated from patients are characterized by a high degree of antibiotic resistance. In 2017, a case of typhoid fever in a six-year old boy in the Democratic Republic of the Congo (DRC) caused by a *S.* Typhi isolate producing CTX-M-15 extended spectrum β-lactamase (ESBL) and showing decreased ciprofloxacin susceptibility, has been reported [8].

Numerous literature sources indicate that resistance to antibiotics and bacteriophages does not match. However, little is known about how specific bacteriophages are to various bacterial serotypes, which are also characterized by multiple antibiotic resistance.

The aim of our work was to elucidate the activity of phages against antibiotic-resistant strains and their specificity to various serotypes of bacteria belonging to the genus *Salmonella.*

Materials and methods

The work used 226 strains of *Salmonella* obtained from different countries: Georgia, Armenia, Ireland, Germany and Congo. Strains were isolated from diarrhea patients (mainly from feces and in some cases from blood); from animals (chickens, ducks, pigs, cows, fish), food (sandwiches, cheese, vegetables, etc.).

Biochemical identification was performed using standard biochemical tests.

MALDI-TOF identification of strains at the *Salmonella* species level was carried out using mass spectrometry (Bruker microflex ™). The output was provided with the flexControl software version 3.0 (8).

Serological typing of isolates was performed according to White-Kauffmann-Le Minor's scheme using commercially available polyvalent antiserum for flagellate (H) and lipopolysaccharide (O) antigens [9].

Molecular serotyping of the *Salmonella* strains was carried out using a semi-automatic Rep-PCR system (DiversiLab®System, bioMérieux, Marcy l'Etoile, France).

The antibiotic resistance profile of the strains was determined in accordance with the regulations adopted by the National Committee for Clinical Laboratory Standards (NCCLS) [10,11]. The following antibiotic disks were used (Liofilchem® srl Italy) with antibiotic concentrations: ampicillin (A, 10 μg), amoxicillin + clavulanic acid (Au, 20 μg / 10 μg), azithromycin (Az, 15 μg), ceftriaxone (Cx, 30 μg), chloramphenicol (C, 30 μg), ciprofloxacin (Cip, 5 μg), nalidixic acid (N, 30 μg), streptomycin (Sm, 10 μg), tetracycline (Tc, 30 μg), trimethoprimsulfamethoxazole (Tm / Su, 1.25 μg / 23.75 μg), sulfamethoxazole Cy, 50 μg). The results of sensitivity testing were evaluated based on the criteria proposed in [10].

All strains demonstrating resistance to ampicillin and amoxicillin / clavulanate or to ampicillin and ceftriaxone have been tested for the presence of an ESBL phenotype using E-test strips (ESBL CT / CTL, BIOMÉRIEUX S.A., France, REF 532208) according to the manufacturer's instructions [11].

Isolation of phages: Fourteen phage clones, isolated during the period of 2013-2017 from the waters of the river Mtkvari (Kura) in Tbilisi, the Black Sea water samples and from the sewerage system in Tbilisi (Table 1). The isolation was performed according to the procedure described in [12]. Besides that 5 series of the commercial preparation INTESTI-bacteriophage (manufactured by Eliava BioPreparations, Ltd) were used in this work, which includes the salmonella component.

Morphological analysis of the phages was carried out using transmission electron microscopy (TEM) confirmed their main classification (Table 1). The samples were purified and contrasted in accordance with Hans-W. Ackerman [13], the preparations were examined using JEOL-JEM-1400 TEM. Phages belonged to the families Siphoviridae (n = 6), Myoviridae (n = 6) and Podoviridae (n = 2). The genome-based grouping allowed us to further assign these phage clones to individual phage / genera types, as indicated in Table 1.

Genetic analysis of the phages. To assert the strictly lytic nature of these phages, high resolution genome maps of 12 of the 14 individual phages were obtained using nanopore sequencing. A pooled library consisting of barcoded genomic DNA of the phages was prepared using native barcodes and the 1D ligation kit from Oxford Nanopore Technology (ONT). The result was then sequenced on a MinION device, equipped with a R9.4 flowcell. For the data analysis, Albacore v2.1 (ONT) was used for base-calling the reads, followed by porechop v0.2.1 in order to remove barcode sequences. Genome map assembly was performed with Canu v1.6 [14]. All the assembled genomes were subsequently processed with Racon v0.5 for better consensus sequences [15], and nanopolish v0.8.3 for higher accuracy of base-called nucleotides in the sequences. Considering the intrinsic properties of nanopore sequencing, together with the run coverage (30X to 60X), we define these assemblies as high resolution phage maps, rather than fully accurate genome sequences. A BLASTn search revealed close homology to known phages, commonly used in phage therapy settings and allows their classification (Table 1).

The bacterial susceptibility to the phages was performed according to [16].

Results and discussion

The activity of phages was determined to 226 strains of *Salmonella,* of which 102 were of clinical, 105 - veterinary and 19 - of unknown origin. Isolates were obtained from Georgia (33), Armenia (44), Germany (7) and Ireland (141) and Congo (1). These isolates belonged to the following serotypes: *S*. Typhimurium (83), *S*. Enteritidis (38), *S.* Dublin (22), *S*. Anatum (11), *S.* Infantis (8), *S*. Derbey (8), *S* Newport (8), *S*.Agona (7), *S.* Bredney (5), *S*. Branderburg (3), *S*.Paratyphi B (3), *S*. Germinara (2), *S.* Uganda (2), *S* Kentucky (2), *S* Reading (2), *S*. Senftenberg (1), *S.* Java (1), *S*. Bareilly (1), *S*. Virchow (1), *S*. Goldcost (1), *S.* Kottbus (1), *S*. Poona (1), *S*.Typhi (1), and unknown serotypes (14). Thus, 23 serotypes of *Salmonella* were included into the work. Distribution of serotypes according to clinical and veterinary strains is given in graph No. 1. The clinical serotypes cover only 11 variants, among which: *S*. Typhimurium and *S*. Enteritidis predominated; while among veterinary isolates a greater variety of serotypes was observed, among which *S*. Typhimurium and *S*. Dublin prevail, followed by *S*. Anatum, *S*. Enteritidis, *S.* Infantis, etc.

Table 1. Characterization of 14 bacteriophages active to various serotypes of *S. enterica*

No	Name	Source	Date	Host strain	Morphology group	Relatedness of phages	Activity of phages to clinical strains of *S. enterica*, % (n=101)	Activity of phages to the strains of veterinary strains and unknown origin *S. enterica*, % (n =125)
1	ΦGE_vB_S.E_N3	r.Kura	2013	*S. enter. 3*	*Siphoviridae*	ND	**87.8**	**60.5**
2	ΦGE_vB_S.E_N5	r.Kura	2013	*S. enter. 3*	*Siphoviridae*	*E. coli T5 strain ATCC 11303-B5*	**57.6**	**46.5**
3	ΦGE_vB_S.E_N8	r.Kura	2013	*S. enter. 3*	*Siphoviridae*	*phage SPC35*	**74.6**	**52.6**
4	ΦGE_vB_S.E_MG	Sewage water	2013	*S. enter. 3*	*Myoviridae*	*S. phage PVP-SE1*	**48.3**	**35.0**
5	ΦGE_vB_S.T_BS	Black Sea	2013	*S. typh. 4*	*Myoviridae*	*S SPT-1, partial genome*	**89.7**	**73.7**
6	ΦGE_vB_S.T_B1	r.Kura	2013	*S. typh. 6*	*Myoviridae*	*S. phage Mushroom*	**89.6**	**76.3**
7	ΦGE_vB_S.T_B3	r.Kura	2013	*S. typh. 6*	*Myoviridae*	*S. phage Mushroom*	**88.7**	**72.8**
8	ΦGE_vB_S.T_NS7	Raw milk	2015	*S. typh. 6*	*Myoviridae*	*S. phage Mushroom*	**82.3**	**66.7**
9	ΦGE_vB_S.e_M4	Black Sea	2016	*S. enter. 232*	*Siphoviridae*	*S. phage vB_SenS-Ent3*	**34.8**	**12.1**
10	ΦGE_vB_S.e_M5	Black Sea	2016	*S. enter. 407*	*Siphoviridae*	*S. phage vB_SenS-Ent3*	**42.5**	**32.4**
11	ΦGE_vB_S.e_Tr	r.Kura	2017	*S. typh. 641*	*Podoviridae*	*S. phage BTP1*	**74.1**	**32.5**
12	ΦGE_vB_S.e_Hi	r.Kura	2017	*S. enter. 765*	*Siphoviridae*	*S. phage vB_SenS-Ent3*	**77.8**	**52.6**
13	ΦGE_vB_S.e_7A	r.Kura	2017	*S. typh. 1328*	*Myoviridae*	*S. phage BPS15Q2*	**63.0**	**29.8**
14	ΦGE_vB_S.e_M1	Black Sea	2016	*S. enter. 104*	*Podoviridae*	ND	**13.7%**	**ND**

ND. – no data/ нет сведений.

Notably, most of the strains showed resistance from four to eight classes of antibiotics, with the highest levels of resistance to: nalidixic acid (synthetic quinolone antibiotics) - 67.86%, sulfonamide (sulfonamides) - 65.48%, ampicillin (penicillins) - 57.14%, amoxicillin + clavulanic acid - 51, 19% and ceftriaxone (cephalosporins) - 45.24%, ciprofloxacin (fluoroquinolones) - 15.48%. Most clinical strains related to *S.* Typhimurium (31), *S.* Enteritidis (8), *S.* Derby (3), *S.* Anatum (1), and *S.* Newport (1) isolated in Armenia and *S.*Typhi from Congo demonstrated ESBL phenotype.

The investigated phage clones showed lytic activity for almost all *Salmonella* serotypes, but individual clones lyzed from 13.7% to ~ 90% of clinical, and from 12.1% to 76.3% of veterinary strains and of the unknown origins (Table 1). Phages that were the most active both in clinical and veterinary serotypes are: ΦGE_vB_S.T_BS (Myoviridae, 89.7% and 73.7%, respectively), ΦGE_vB_S.T_B1 (Myoviridae, 89.6%, 76.3%), ΦGE_vB_S.T_B3 (Myoviridae, 88.7%, 72.8%), ΦGE_vB_S.E_N3 (Siphoviridae, 82.3%, 60.5%), and ΦGE_vB_S.T_NS7 (Myoviridae, 88.8%, 66.7%). The fact that the phages are most active to clinical strains is due to a smaller variety of serotypes among them among which the serotypes of *S.* Typhimurium and *S.* Enteritidis dominate.

From the available literature [17] it is known that the phages are adsorbed on the receptors located in the lipopolysaccharide (LPS) layer. LPS is a complex that consists of three parts: lipid A, a basic polysaccharide and O-polysaccharide. Lipid A, as a rule, consists of fatty acids attached to disaccharides of glucosamine phosphate. The key polysaccharide is connected to the lipid A through a ketodeoxicotonate linker. Cells that contain all the three LPS components are designated as smooth (S) type, and those that do not have the O-polysaccharide part are distinguished as coarse (R) type. In general, the saccharides that make up the O-antigen vary greatly, and the saccharides of the main polysaccharide layer remain conservative among the species. Because of this, phages specific for S-type strains tend to focus on O-polysaccharide and thus tend to have a narrower range of hosts compared to those that are capable of adsorbing R-type cells [17].

According to Silva *et al.* [18] in the case of *Salmonella* phages, the cellular receptors allowing phage adsorption on the surface of the host cell are similar to the *E. coli* receptors: of the 19 characterized *Salmonella* phages, 11 used proteins, 7 adsorbed on sugar residues, and 1 for one phage, both types of receptors However, it is known that *Salmonella* phages can also be adsorbed on the bacterial flagella, pili and capsules, for example, on the acetyl group of the Vi-exopolysaccharide capsule (α-1,4-linked N-acetylgalactoaminuronate polymer). It is also known that the phages of Gram negative bacteria belonging to *Siphoviridae* class typically adsorb on proteins, at the same time phages related to *Myoviridae* class prefer sugars, although they can also attach to protein and combined receptors, while Podoviridae phages can only be adsorbed on sugars. Based on the above knowledge, we can conclude that the phages from our collection belonging to the class *Myoviridae* apparently may have a large range of surface receptors, which explains their relatively broad host range, and, accordingly, high activity for various serotypes of *Salmonella.*

It is known that the mechanisms of antibiotic resistance are associated with: changes of the cell wall structure, damage of its synthesis, bacterial DNA and RNA modifications and disruption of protein synthesis [19]. At the same time, the mechanism of resistance to phages is mainly determined by a change in the structure of adsorption receptors that prevent adsorption and further multiplication of phages in to the host cells [20]. To investigate the presence of any connection between the antibiotic resistance profiles and the sensitivity to phages, the Pearson correlation coefficient was calculated using a two-sided significance test. Based on the obtained data, it was approved that there was no significant relationship between antibiotic resistance profiles and phage susceptibility. In other words, antibiotic-resistant strains, including those demonstrating ESBL phenotype, show sensitivity (are lysed) to specific Salmonella phages. In addition, it should be noted that the phages

used in this study appeared to be active in virtually all salmonella serotypes, including the ESBL strain of S. Typhi from the Democratic Republic of the Congo [20].

Conclusions

Our research showed that the phages under investigation do not have a certain predisposition to any specific *Salmonella* strains. They are equally active to multiply resistant strains, as well as to various serotypes of *S. enterica*.

The studied phages revealed a high activity both to clinical (~ 90%) and veterinary strains (›70%), which suggests that they may be used not only in human medicine, but also in veterinary as a preventive measure, and also an disinfection agent for treatment of surfaces, premises, etc. This will significantly reduce the frequency of the spread of MDR strains and pathogenic serotypes of *Salmonella* through the food chain, improve the quality of veterinary products, which ultimately will have a positive impact on human health. In many cases, phage-based preparations can become an effective alternative to antibiotics. However, before phages are included in such preparations, they must be well characterized at the molecular and biological levels. In addition, to extend the efficiency and host range of the phage-based preparations it is recommended to include into these combinations the phages belonging to different morphological groups targeting broad spectrum of cellular receptors.

REFERENCE

1. Watts G. (2017) Phage therapy: revival of the bygone antimicrobial. *Lancet,* 390, 2539-2540.

2. Chanishvili N. (2016) Bacteriophages as Therapeutic and Prophylactic Means: Summary of the Soviet and Post Soviet Experiences. *Curr Drug Deliv*, 13: 309-323.

3. Nagel TE, Chan BK, De Vos D, El-Shibiny A, Kang'ethe EK, Makumi A, et al. (2016) The Developing World Urgently Needs Phages to Combat Pathogenic Bacteria. *Front Microbiol*, 7: 882.

4. RIA News, *Retrieved from* https://ria.ru/spravka/20100408/219600988.html)/

5. Reports of *Salmonella* Outbreak Investigations from **2017.** *Retrieved from* https://www.cdc.gov/salmonella/outbreaks-2017.html

6. Multistate Outbreak of *Salmonella* Montevideo Infections Linked to Raw Sprouts, **(2018)** *Retrieved from* https://www.cdc.gov/salmonella/montevideo-01-18/index.html.

7. 16 people were poisoned after receiving a chicken sandwich made in "Carrefour", (2018) *Palitra News*, *Retrieved from* https://www.allnews.ge/sazogadoeba/158532

8. Phoba MF, Barbé B, Lunguya O, Masendu L, Lulengwa D, Dougan G, et al. (2017) *Salmonella enterica* serovar Typhi Producing CTX-M-15 Extended Spectrum β-Lactamase in the Democratic Republic of the Congo. *Clin Infect Dis,* 65: 1229-1231.

9. Grimont P. A.D., Weill F.-X. (2007), *Antigenic formulae of the salmonella serovars, Institut Pasteur WHO Collaborating Centre for Reference and Research on Salmonella* .

10. NCCLS. (1997) *Performance Standards for Antimicrobial Disk Susceptibility Tests*— Sixth Edition; Approved Standard. NCCLS Document M2-A6. Wayne, Pennsylvania: NCCLS.

11. NCCLS (1997). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Fourth Edition; Approved Standard. NCCLS Document M7-A4. Wayne, Pennsylvania .

12. Kutter, E. and Sulakvelidze, A. (Eds.) (2005) Bacteriophages—Biology and Application. FL.:CRC Press. [in English]

13. Ackermann HW. (2009) Basic phage electron microscopy. *In: Clokie MRJ and Kropinski A. Bacteriophages: Methods and protocols*. Vol 1. New York, NY: Humana Press; 113-126 [in English].

14. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. (2017) Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome research*, 27: 722-736 [in English].

15. Vaser R, Sović I, Nagarajan N, Šikić M. (2017) Fast and accurate de novo genome assembly from long uncorrected reads. *Genome research*, 27: 737-746 [in English].

16. Kutter E. (2009) Phage host range and efficiency of plating. *In: Clokie MRJ and Kropinski A. Bacteriophages: Methods and protocols.* Vol 1. New York, NY: Humana Press, 141-149 [in English].

17. Rakhuba DV, Kolomiets EI, Dey ES & Novik GI (2010), Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. *Pol J Microbiol.,* 59(3):145-55 [in English].

18. Silva J.B., Storms Z., Sauvageau D. (2016), Host receptors for bacteriophage adsorption. FEMS Microbiollogy Letters, 363(4), 1 February 2016, fnw002, *Retrieved from* https://doi.org/10.1093/femsle/fnw002 [in English]

19. Blair J.M.A., Webber M.A., Baylay A.J., Ogbolu D.O., & Piddoc L.J.V. (2015), Molecular mechanisms of antibiotic resistance, *Nature Reviews Microbiology,* 13:42–51; doi:10.1038nrmicro2315 [in English]

20. Labrie S.J., Samson J.E., and Moineau S. (2010), Bacteriophage resistance mechanisms**,** *Nature Reviews Microbiology,* **2010;** 8:317-327; doi: 10.1038 nrmicro2315 [in English]

АКТИВНОСТЬ БАКТЕРИОФАГОВ К МНОЖЕСТВЕННО РЕЗИСТЕНТНЫМ ШТАММАМ САЛЬМОНЕЛЛ И ИХ РАЗЛИЧНЫМ СЕРОТИПАМ */ Макалатия Хатуна, Какабадзе Елена, Бакурадзе Ната, Грдзелишвили Нино, Наторшвили Гульнара, Кусрадзе Ия, Годердзишвили Марина, Седракян Анаит, Аракелова Карине, Мкртчян Мхитар, Мачарашвили Нино, Папиашвили Екатерина, Лавин Роб, Ли Дэвид, Коффи Айден, Де Вос Даниэл, Пирней Жан-Поль, Чанишвили Нина*

Фаготерапия является одним из перспективных «новых» методов лечения, который привлекает все больше внимания за рубежом. Несмотря на то, что фаготерапия традиционно применяется в бывших советских странах, ее значение в западных странах стало оцениваться с появлением множественно антибиотико-резистентных форм бактерий, которые больше не подвергаются лечению. В связи с этим с каждым годом возрастает количество летальных случаев среди пациентов, подверженных антибиотико-резистентным инфекциям. Фаготерапия может быть использована как альтернативный метод лечения. Многочисленные литературные источники указывают, что резистентность к антибиотикам и бактериофагам не совпадает. Однако, мало известно о том насколько специфичны бактериофаги к различным бактериальным серотипам, одновременно характеризующихся множественной антибиотико- резистентностью.

Ключевые слова*: бактериофаги (фаги), сальмонелла, множественная резистентность к препаратам, серотипы*

АКТИВНІСТЬ БАКТЕРІОФАГІВ ДО МНОЖИННОСТІ РЕЗИСТЕНТНИХ ШТАМІВ САЛЬМОНЕЛИ ТА ЇХ РІЗНИМИ СЕРОТИПАМИ / Макалатії Хатуна, Какабадзе Олена, Бакурадзе Ната, Грдзелішвілі Ніно, Наторшвілі Гульнара, Кусрадзе Ія, Годердзішвілі Марина, Седракян Анаід, Аракелова Карині, Мкртчян Мхітар, Мачарашвілі Ніно, Папіашвілі Катерина, Лавін Роб , Лі Девід, Коффі Айден, Де Вос Даніел, Пірні Жан-Поль, * Чанішвілі Ніна

Вступ. Фаготерапія є одним з перспективних «нових» методів лікування, який привертає все більше уваги за кордоном. Бактеріофагами (коротко - фагами) є природними вірусами бактерій, які вражають людину, тварин і рослин. Численні літературні джерела вказують, що резистентність до антибіотиків і бактеріофагів не збігається. Однак, мало відомо про те наскільки специфічні бактеріофаги до різних бактеріальних серотипам, одночасно характеризуються множинною антибіотикорезистентністю.

Метою нашої роботи було з'ясування питання про активність фагів щодо антибіотикорезистентних штамів і їх специфічності до різних серотипам бактерій, що відносяться до роду Salmonella.

Матеріали та методи досліджень. У роботі було використано 226 штамів сальмонел, отриманих з різних країн: Грузії, Вірменії, Ірландії, Німеччини та Конго. Штами були виділені від хворих на діарею (в основному з фекалії і в деяких випадках з крові); від тварин (курей, качок, свиней, корів, риб), продуктів харчування (бургери, сир, овочі і т.д). Біохімічна ідентифікація проводилася за допомогою стандартних біохімічних тестів. У роботі також були використана масс-спектрометрія, методом матрично активованої лазерної десорбціонной іонізації; серологічне типування ізолятів, молекулярне серотипування штамів сальмонел за допомогою напівавтоматичної системи Rep-PCR; антибіотикорезистентність штамів визначали методом дисків і серійних розведень. У роботі були використані чотирнадцять фагових клонів, Морфологічний аналіз фагів проводили з використанням трансмісійної електронної мікроскопії (ТЕМ) фагових клонів. Для підтвердження строго літичного характеру фагів відібраних для тестування були отримані карти геному з високою роздільною здатністю 12 з 14 окремих фагів з використанням нанопористого секвенування. Активність бактерій до фагів визначали за допомогою т.зв. точкового тесту.

Результати досліджень та їх обговорення. Активність вищевказаних фагів була визначена до 226 штамів сальмонел, з яких 102 були клінічного, 105 - ветеринарного і 19 невідомого походження, усього диференціювали 23 серотипи сальмонел. Більшість штамів, включених в наше дослідження, виявляли резистентність від чотирьох до восьми класів антибіотиків. Тільки дев'ять штамів з Грузії і п'ять з Вірменії виявилися повністю чутливими до всіх антибіотиків. Жоден штам не був стійким до всіх антибіотиків, які використовуються в цьому дослідженні. Досліджені клони фагів були виділені з водних джерел, використовуючи штами *S.* Typhimurium і *S.* Enteritidis як штамів-господарів. Незважаючи на це вони проявляли літичну активність практично до усіх серотипів сальмонел, проте окремі клони проявили різну активність до тестованих штамів. На підставі отриманих даних не було виявлено суттєвої взаємозв'язку між профілями резистентності до антибіотиків та чутливістю до фагів. Іншими словами, антибіотикорезистентні штами, включаючи демонструють ESBL фенотип, виявляють чутливість (лизируются) специфічними сальмонельозний фагами. Крім того, слід зазначити, що використані фаги були активні практично до всіх серотипам сальмонел, включаючи штам ESBL S. Typhi з ДРК.

Висновки та перспективи подальших досліджень

1. Наші дослідження показали, що досліджувані фаги з точки зору їх лізуючого активності однаково активні як щодо множинне резистентних штамів, так і до різних серотипам, що відносяться до виду S. enterica.

2. В той же час дані фаги мають високу активність як до клінічних (~ 90%), так і ветеринарним штамів (> 70%), що дозволяє запропонувати їх використання не тільки в медицині, але і ветеринарії як засіб лікувального, профілактичного засобу, а також для санітарної обробки поверхонь.

3 У багатьох випадках фагів препарати можуть стати ефективною альтернативою антибіотиків. Однак, перш ніж включати фаги до складу таких препаратів, вони повинні бути добре охарактеризовані на молекулярному і біологічному рівні. Крім того, посилення спектра дії фагів препаратів, рекомендовано включати до їх складу фаги з різною морфологією, націлені на широке коло бактеріальних рецепторів.

Ключові слова: *бактеріофаги (фаги), сальмонела, множинна резистентність до препаратів, серотипи*

СПИСОК ЛІТЕРАТУРИ

1. Watts G. Phage therapy: revival of the bygone antimicrobial. Lancet, **2017**; 390: 2539-2540.

2. Chanishvili N. Bacteriophages as Therapeutic and Prophylactic Means: Summary of the Soviet and Post Soviet Experiences. Curr Drug Deliv, **2016**; 13: 309-323.

3. Nagel TE, Chan BK, De Vos D, El-Shibiny A, Kang'ethe EK, Makumi A, Pirnay JP. The Developing World Urgently Needs Phages to Combat Pathogenic Bacteria. Front Microbiol, **2016**; 7: 882.

4. RIA News, https://ria.ru/spravka/20100408/219600988.html)/

5. Reports of *Salmonella* Outbreak Investigations from **2017**; https://www.cdc.gov/salmonella/outbreaks-2017.html

6. Multistate Outbreak of *Salmonella* Montevideo Infections Linked to Raw Sprouts, **2018**; https://www.cdc.gov/salmonella/montevideo-01-18/index.html.

7. 16 people were poisoned after receiving a chicken sandwich made in "Carrefour", Palitra News, January 25, **2018** ; https://www.allnews.ge/sazogadoeba/158532

8. Phoba MF, Barbé B, Lunguya O, Masendu L, Lulengwa D, Dougan G, Wong VK, Bertrand S, Ceyssens PJ, Jacobs J, Van Puyvelde S, Deborggraeve S. *Salmonella enterica* serovar Typhi Producing CTX-M-15 Extended Spectrum β-Lactamase in the Democratic Republic of the Congo. Clin Infect Dis, **2017**; 65: 1229-1231.

9. Grimont P. A.D., Weill F.-X. , Antigenic formulae of the salmonella serovars, Institut Pasteur WHO Collaborating Centre for Reference and Research on Salmonella, **2007**.

10. NCCLS. Performance Standards for Antimicrobial Disk Susceptibility Tests— Sixth Edition; Approved Standard. NCCLS Document M2-A6. Wayne, Pennsylvania: NCCLS;**1997**.

11. NCCLS. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Fourth Edition; Approved Standard. NCCLS Document M7-A4. Wayne, Pennsylvania: NCCLS;**1997**.

12. Kutter, E. and Sulakvelidze, A. (Eds.) Bacteriophages—Biology and Application. FL: CRC Press. – 2005– 485 p.

13. Ackermann HW. Basic phage electron microscopy. In: Clokie MRJ and Kropinski A. Bacteriophages: Methods and protocols. Vol 1. New York, NY: Humana Press; **2009**: 113-126.

14. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome research, **2017**; 27: 722-736.

15. Vaser R, Sović I, Nagarajan N, Šikić M. Fast and accurate de novo genome assembly from long uncorrected reads. Genome research, **2017**; 27: 737-746.

16. Kutter E. Phage host range and efficiency of plating. In: Clokie MRJ and Kropinski A. Bacteriophages: Methods and protocols. Vol 1. New York, NY: Humana Press; **2009**: 141-149.

17. Rakhuba DV, Kolomiets EI, Dey ES, Novik GI, Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. Pol J Microbiol. **2010**;59(3):145-55.

18. Silva J.B., Storms Z., Sauvageau D., Host receptors for bacteriophage adsorption. FEMS Microbiollogy Letters, **2016**; 363(4), 1 February 2016, fnw002, https://doi.org/10.1093/femsle/fnw002.

19. Blair J.M.A., Webber M.A., Baylay A.J., Ogbolu D.O., and Piddoc L.J.V., Molecular mechanisms of antibiotic resistance, *Nature Reviews Microbiology,* **2015**; 13:42–51; doi:10.1038nrmicro2315

20. Labrie S.J., Samson J.E., and Moineau S., Bacteriophage resistance mechanisms**,** *Nature Reviews Microbiology,* **2010;** 8:317-327; doi: 10.1038 nrmicro2315