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## A century of phage therapy: from novel discoveries about the past to the challenges of the today

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The discovery of bacterial viruses able to kill bacteria, named bacteriophages (phages), more than a century ago, contributed to combating bacterial infections during the first three decades of the 20th century. Vladimir Sertić, a Croatian microbiologist, was one of the pioneers in bacteriophages investigation, who performed significant scientific research on phage strains characterization, isolation, and classification. The important contribution of Vladimir Sertić can be seen from his private archive (from the 1930s) which contains hundreds of valuable archival materials that are today kept at the Department of Pharmacology School of Medicine, Zagreb. The Discovery of this archive was recently described by Lackovic and Toljan. Here we describe a further survey of this archive. In addition to many documents, it contains phage cocktails in a small commercial paper box, with instructions for usage, as clear evidence of their therapeutic application in the pre-antibiotic era. This revelation, not shown in the literature up to now, to the best of our knowledge, motivated us to present it to the wider scientific and professional community with this short article, giving an additional brief overview of the current perspective of phage investigation and therapeutic application.

### 1. Introduction

It is now clear more than ever that microbial infections accompanied by the rapid occurrence of resistance to old as well as new generations of antibacterial drugs represent one of the leading global health problems ([https://amr-review.org/sites/default/files/160525\\_Final%20paper\\_with%20cover.pdf](https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf)). Antibiotic therapy failures were recognized practically as early as they were introduced to cure patients with bacterial infections, and this problem evolved over the decades ([https://www.who.int/medicines/publications/WHO-PPL-Short\\_Summary\\_25Feb-ET\\_NM\\_WHO.pdf](https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf); Magiorakos et al. 2012). The development of novel antibacterial drugs seems not to be a sustainable solution for the continuously evolving resistance problem.

The discovery of bacteriophages (or phages), which can infect, replicate in, and lyse bacterial cells indicated their potential therapeutic application more than a century ago (Salmond and Fineran 2015). Soon after Félix Hubert d'Hérelle, a French-Canadian microbiologist discovered bacteriophages, he started to actively employ them as both prophylactic and therapeutic agents against bacterial infections (d'Hérelle 1917). From that time and until the discovery of antibiotics, phages were used to treat patients with intestinal bacterial infections, mainly dysentery (d'Hérelle, 1917) and cholera (d'Hérelle 1929). However, there are no data on production volume or intake frequency, just as there is no data from which the clinical effectiveness could be assessed.

### 2. Bacteriophages as a tool to fight bacteria – an acknowledgment to Vladimir Sertić

The 2<sup>nd</sup> and 3<sup>rd</sup> decades of the 20<sup>th</sup> century were the most productive in phages research and therapeutic application. In 1928, Vladimir Sertić (Fig. 1), a Croatian microbiologist, joined d'Hérelle's private Laboratoire du Bactériophage in Paris and worked intensively on isolation, characterization, and classification of several thousands of strains of bacteriophages. However, Sertić started some of the

pioneering experiments demonstrating lysins as being specific phage-secreted bacteriolytic enzymes while he was working at the Institute for Pharmacology and Experimental Pathology (Pathophysiology) in Zagreb as a research assistant, several years before he went to Paris (Lacković and Toljan 2020). His intensive research on the elucidation of phage biology greatly contributed to phage



Fig. 1: Dr. Vladimir Sertić during his stay in Paris (VS personal archive, unpublished)

therapeutic applicability. Together with assistant Nikolai Bulgakov he isolated numerous bacteriophage strains and proposed a bacteriophage classification system (Lacković and Toljan 2020). Among the isolated strains,  $\phi$ X174 (the virus that infects *Escherichia coli*, usually wrongly pronounced as *fi ex* 174, instead of as *fi ten* 174) is the most famous as it was the first living organism whose genome was fully sequenced in 1977 by Fred Sanger, a Nobel prize laureate (The Nobel Prize 1980).

Lacković and Toljan, while looking through the content of the Sertić's private archive (entrusted to safekeeping to professor Lacković from a Sertić's family member, now kept at the Department of Pharmacology, University of Zagreb School of Medicine, available from Professor Lacković), revealed archive materials, some of which was described in their recent article dedicated to Sertić (Lacković and Toljan 2020). Among some personal comments, hand-written articles, the archive contained a package (dated 1930) with 520 authentic commercial ampoules containing phages produced in d'Hérelle's laboratory. Additionally, a commercial paper box with 25 glass ampoules, each containing 2 ml of sterile "bouillon" was found (Fig. 2). More carefully exploring the archive, professor Lacković revealed documents (in Croatian, translation into English is given along with Fig. 3) with information about producer, content, indications, and usage, which corresponds to the Patient Information Leaflet that is inserted in every drug package nowadays.

The finding of the commercial phage cocktails, produced in France, along with product description on Croatian, suggests, although without concrete evidence, that this therapy was widespread and applicable in different parts of the European continent.



Fig. 2: From the personal archive of Vladimir Sertić (previously unpublished): **A.** commercial paper box containing therapeutic phage (Bacte-Intesti-Phage<sup>®</sup>), **B.** phage containing sterile "bouillon" in sealed glass vials, **C.** content of the therapeutic box printed on a side of the box (a mixture of different types of phage selected to cause lysis of listed types of bacteria: *Dysentery phage*, *para-dysentery phage*, *Proteus phages*, *Typhi-phages*, *Paratyphi phages*, *Enterococcus phages*, *Pyocyanus phages*, *Coli phages*, *Streptococcus phages*, *Staphy phages*, **D.** expiration date on the side of the box, **E.** warning about the usage (on Croatian) stating that each vial that is opened should be used within three hours, and every cloudy vial should be thrown away.

Translation of Bakte-Intesti-fag<sup>®</sup> document from Croatian:

**Indications:** gastroenteritis, enteritis, colitis, chronic intestinal stasis, entero-renal syndromes, infections of the alimentary tract, infant diarrhea, toxic-infectious bowel disorders of bacterial origin in children and adults. The use of bacteriophages achieves specific treatment of diseases caused by bacteria according to which the used bacteriophage is specific.

**How to use:** to pour out the contents of the ampoule a notch should be made with a file at one end of the ampoule and the tip broken off. A notch should be made at the other end in the same way, but before the tip is broken off, the ampoule should be placed vertically above the dilution water cup so that the open end of the ampoule is facing downwards. Only then should the upper end be broken

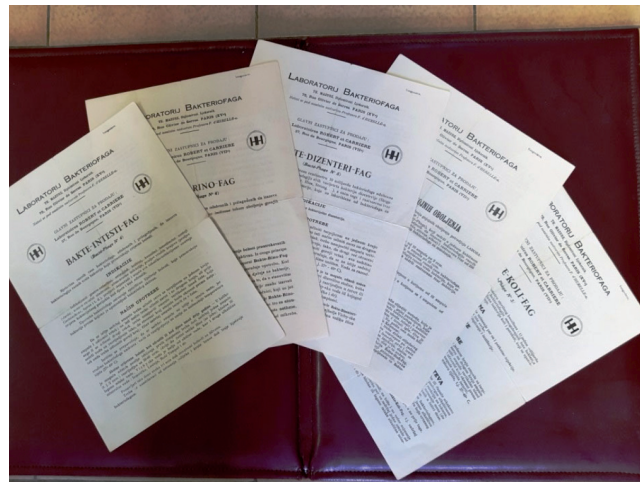


Fig. 3: Description (in Croatian) of phage preparations: BAKTE-INTESTI-FAG, BACTE-RHINO-FAG, BAKTE-DISENTERI-FAG, BAKTE-COLI PHAG (Sertić's private archive: not published before)

off and the entire contents of the ampoule will leak into the water. It is strongly recommended that the contents of the ampoule not be poured into hot water as the heat destroys the bacteriophages. Dilution water must not exceed body temperature (37-40 °C).

Acute intestinal diseases in adults and children older than 2 years - If possible, immediately after the onset of the first symptoms, the contents of one ampoule (2 cm<sup>3</sup>) should be given diluted with 30-60 cm<sup>3</sup> (one to two tablespoons for soup) of cold or slightly lukewarm water. Vichy water is better than ordinary water, as well as other alkaline mineral waters: Jamnička, Lasinja, etc.

Repeat the same dose every three hours until the symptoms of the disease disappear. Milk and solid foods should be refrained from during bacteriophage treatment.

Document translated from Croatian that accompanied commercial box with French description probably reflects wide usage of Bacte-Intesti-Phage<sup>®</sup> in different European countries, and not only in Croatia. As can be seen from the composition of Bacte-Intesti-Phage<sup>®</sup> (Fig. 2), a cocktail of different phage strains was used to treat intestinal disorders. However, what was the rationale for such a cocktail composition, and based on what evidence the respective bacteriophage strains were chosen? As d'Hérelle described in one of his papers: „a therapeutic bacteriophage designed to cause *in vivo* bacterial lysis during a specific disease must be able to attack all the host strains of the bacterial species which causes this disease”, further assuming that no known single bacteriophage meets this requirement (Kuhl and Mazure 2011). Thus, he suggested that only a mixture of carefully selected phage strains is capable of attacking the bacteria species and can treat the respective clinical syndrome. He additionally described in detail a complex process of phage cocktail preparation, which begins with the isolation of pathogenic bacteria from pathological products, followed by isolation and selection of hypervirulent, heat-resistant, and stable bacteriophages that target bacterial species. It was also stressed out that the therapeutic phage cocktails must be constantly improving and changing in composition, based on data from the ongoing research and isolation of new bacteriophage strains, of better characteristics from the older ones (Kuhl and Mazure 2011). This approach, suggested by d'Hérelle witnesses preclinical research which preceded phage cocktails design, even 100 years ago.

The archive of Vladimir Sertić contained documents (corresponding to the Patient Information Leaflet) showing that during the 1920s the Laboratoire du Bacteriophage besides Bacté-Intesti-Phage<sup>®</sup> produced other commercial phage cocktails: Bacté-Coli-Phage<sup>®</sup>, Bacté-Dysentérie-Phage<sup>®</sup>, Bacté-Pyo-Phage<sup>®</sup>, and Bacté-Rhino-Phage<sup>®</sup> (Fig. 3). The composition of phages in those cocktails is not known. These preparations were commercially produced from d'Hérelle's previous company in France until approximately 1978, while during the 1990s, the Pasteur Institutes of Paris and Lyon continued to produce small amounts of bacteriophage preparations on request (Kutter et al. 2010).

### 3. Phage therapy in the post-antibiotic era and current perspective

With the discovery of antibiotics, phage therapy was largely abandoned, but in some countries of the former Soviet Union, such as Georgia and Russian Federation, but also Poland, physicians continued to use phage cocktails and generated practical experience over years. The institute that continuously performed phage research and therapeutic application since its foundation by George Eliava and Felix d'Herelle in 1923 is Eliava Institute in Tbilisi, Georgia (former USSR). Nowadays, at Eliava Institute (Eliava Institute of Bacteriophages, Microbiology and Virology) an individualized phage treatment is provided for various chronic and drug-resistant infections (Kutter et al. 2011; Bacterial Strain and Phage Collection 2021). Some of Eliava's phage preparations, like Intesti Bacteriophage<sup>R</sup>, applied in the prevention and therapy of pyo-inflammatory and enteric infections, are still available in pharmacies without prescription throughout Tbilisi and can be purchased and distributed via Elavia's online pharmacy all over the world Eliava BioPreparations 2021.

Similar to the 100-year-old description of Bacte-Intesti-Phage<sup>R</sup> (Fig. 2, 3), Eliava's commercial product Intesti Bacteriophage<sup>R</sup> contains a mixture of bacterial phage lysates of *Shigella*, *Salmonella*, *Escherichia coli*, *Proteus (vulgaris, mirabilis)*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* (Eliava BioPreparations 2021). According to the information from the producer web page, Intesti Bacteriophage<sup>R</sup> is safe for use in all special patient populations (children, elderly, during pregnancy, and lactation), it is without contraindications and danger of overdosing. Besides Intesti Bacteriophage<sup>R</sup>, Enko Bacteriophage<sup>R</sup>, Fersisi Bacteriophage<sup>R</sup>, SES Bacteriophage<sup>R</sup>, Staphylococcal bacteriophage<sup>R</sup>, Pyo bacteriophage<sup>R</sup> are also available over the counter and distributed by Eliava's online pharmacy (Eliava Pharmacy 2021). These products, based on the *pret-a-porter* approach, are not approved either by the European Medical Agency or US Food and Drug Administration since they do not fulfill the regulatory standards for human application.

The Ludwik Hirsfeld Institute of Immunology and Experimental Therapy in Wroclaw, Poland, has been researching the biological properties and the application of bacteriophages since the 1970s (the only center of phage therapy in Central Europe) (Kutter et al. 2011; Hirsfeld Institute of Immunology and Experimental Therapy 2021). Similarly, as in Georgia, the Phage Therapy Unit treats patients with phage formulations according to patients' needs (based on the *sur-mesure* concept) thus providing personalized treatment, which is in line with the Declaration of Helsinki, Article 37, regulating the usage of Unproven Interventions in Clinical Practice (WMA Declaration of Helsinki 2018).

Ten years ago, the Biological Defence Research Directorate (BDRD) of the USA Navy began an initiative to explore phages as a potential way to deal with biodefense threats associated with multidrug-resistant bacteria. Adaptive Phage Therapeutics (APT) Company got the exclusive right to use the BDRD phage library (PhageBank) commercially. Nowadays, while the world is facing with COVID-19 crisis, APT has made available emergency therapeutic phage treatment to COVID-19 patients with secondary carbapenem-resistant *Acinetobacter baumannii* (Adaptive Phage Therapeutic, Inc. 2021).

In contrast to a large number of preclinical investigations about phages effectiveness on bacterial isolates and animals, evaluation in humans is mostly under-reported, or reported as a description of patient cases, with a lack of well-designed controlled randomized clinical studies.

Although without the approval of regulatory authorities, sporadic phage application continued for critically ill patients. From the pharmaceutical point of view, the preparation of magistral phage cocktails by clinical pharmacists is interesting, and it has been recently introduced in Belgium (Pirnay et al. 2018).

### 4. Conclusion

Hundred years ago, phage therapy was accompanied by controversies and conflicts among scientists. At the time, opinions were grounded on small trials, anecdotal reports, and clinical impressions (Summers 2012). It looks like history has been repeated since some concerns are still ongoing. To provide consistent evidence about efficacy and safety, clinical studies possibly providing necessary answers are currently underway.

Furthermore, more recent basic investigations revealed that phages are not only the natural predators of bacteria but are able to interact directly with the mammalian gut cells and immune system, thus opening new views about phages physiology, potential applicability, or potential danger (Offord 2021).

Nevertheless, the pioneers of phage therapy should be acknowledged since they marked one era in the medicine of the first half of the 20<sup>th</sup> century. One of them is Vladimir Sertić, a Croatian microbiologist, who contributed to phages biology and their therapeutic applicability. The commercial phage cocktails, accompanied with descriptions about their usage, reported here, show the widespread use of phage therapy in the pre-antibiotic era. Nowadays, since contemporary medicine is facing the evolving problem of resistance to antibacterial drugs, but also new views on phage physiology, phage therapy needs evidence-based reassessment of applicability.

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