Title: The forgotten tale of Brazilian phage therapy

Authors: Gabriel Magno de Freitas Almeida, PhD. Lotta-Riina Sundberg, Associate Professor.

Affiliation: Department of Biological and Environmental Science, Nanoscience Centre, University of Jyvaskyla, Jyvaskyla, Finland

Corresponding author details: Gabriel Magno de Freitas Almeida. Survontie 9C, FI-40014, Jyvaskyla, Finland. Phone number: +358469333383. Email addresses: gabriel.m.almeida@jyu.fi ; gabriel.magno@gmail.com

Summary (179/200 words)

The use of bacteriophages to treat bacterial infections (phage therapy) is considered a possible solution to the antimicrobial resistance crisis. However, phage therapy is not a new concept. The discovery of phages in the early twentieth century was closely tied to clinical practice, and phage therapy quickly spread around the world. The use of phage therapy in twentieth century South America is still shrouded in mystery and has been mentioned only briefly in recent scientific literature. Research on Brazilian reference collections of medical texts revealed that Brazil was an important, but so far little-known, player of phage therapy, uncovers interesting priority claims and missing pieces of phage therapy history. Of note, there is the widespread use of phages against bacillary dysentery and staphylococcal infections, with Dr José da Costa Cruz from the Institute Oswaldo Cruz as Brazil's leading expert and pioneer. This review about historical phage use in a South American country fills gaps in our knowledge about the "golden years" of phage therapy, providing information about successful experiences that can be useful against dangerous pathogens in our time.

Key points

- Brazil had an important role in the golden years of phage therapy, which lasted until the mid- twentieth century.
- Dr José da Costa Cruz from the Institute Oswaldo Cruz in Rio de Janeiro was the leading expert.
- The first phage therapy cases in Brazil took place in 1921 during an outbreak of dysentery in Barbacena.
- The mass testing of the Institute Oswaldo Cruz phage product during the Paulista revolution of 1924 preceded Dr d'Herelle's tests in India and the Soviet Union's tests on military troops.
- Successful phage use in Brazil was against bacillary dysentery and staphylococcal infections.
- The available information about phage therapy clinical cases and phage products was retrieved from the literature investigated and presented in this publication.

Research in context

Evidence before this study

Reports summarising the use of phage therapy in Brazil were scarce before this review. Only three recent publications mentions, briefly and without details, that Brazilian doctors were involved with phage therapy in the 1920s. Sixteen sources of Brazilian medical publications were checked for phage-related information, looking at the period from 1915 (when Dr Frederik Twort published his phage discovery) to 1952. Any phage or phage therapy related words in titles and abstracts were used as inclusion criteria. The risk of bias associated with data inclusion is minimal, because the objective was to have all published material concerning phages. However, since routine phage therapy cases were not always published, the data presented here are probably only a fraction of the actual phage use at the time.

Added value of this study

With all the information at hand, it was possible to reconstruct phage therapy practices in Brazil during the first half of the twentieth century, covering the beginning of Brazilian phage therapy to its demise. Interesting claims of priority, acknowledgement of important researchers and Institutes, and successful phage use against bacillary dysentery and staphylococcal infections are described. This review fills missing gaps in phage therapy history, describes practices and potential international collaborations of the past and constitutes the most complete description of phage therapy use in the history of a South American country to date.

Implications of all the available evidence

From a historical point of view, the recollection in this review has importance for the history of medical science and consolidates the important, yet forgotten, role of Brazil in phage therapy. The current article may serve as inspiration for similar publications about the history of other nations, filling gaps and improving our current knowledge with experiences from the past. From practical and applied perspectives, this review provides clinical data and medical practices that can help against important bacterial pathogens from our time. Phage therapy in Brazil was focused against bacillary dysenteries and staphylococcal infections. Both still cause problems for humans, the first being the second major cause of diarrheal-associated deaths in the world and the second being an important infection with many clinical manifestations that is becoming increasingly more dangerous as resistance to antibiotics spreads. The experience and clinical data described in this review can be used to shape modern phage use and clinical trials.

Search strategy and selection criteria

Sixteen sources of Brazilian medical publications were checked for phage-related information. The name of these journals, and the time periods verified, were: *Archivos Brasileiros de Medicina* (1920-1940, 1942-1945), *Arquivos de Higiene* (1927-1945), *O Bisturi* (1930, 1933-1945), *Boletim da academia nacional de medicina* (1920-1937, 1939-1940), *Brasil-Medico* (1915-1952), *Brasil Medico Cirurgico* (1941-1942, 1945), *Folha medica* (1920-1932, 1934-1945), *Gazeta Clinica* (1920-1931, 1933-1943, 1945), *O Hospital* (1924, 1931-1945), *Revista Brasileira de Medicina* (1944-1950), *Revista Medica Brasileira* (1939-1945), *Revista Medico Cirurgica do Brasil* (1920-1921, 1923-1945), *Revista de Medicina e Higiene Militar* (1921-1927, 1929-1930, 1939-1945), *Memorias do Instituto Oswaldo Cruz* (1915-1952), *Tribuna Medica* (1920-1930) and *Vida Medica* (1931-1947). Temporal gaps are due to missing editions in the archives. None of these journals were focused on microbiology alone, so space was used for all facets of medical practice at the time. Whole volumes were checked for any phage-related publication, page by page whenever possible or through its summaries. Search criteria was focused on phage therapy related words, searched in the summaries and publication titles of the available journals. Examples of keywords used were “fago, bacteriofago, fagoterapia, principio litico de d’Herelle” and their variations. Although a few cases might have slipped unnoticed, most if not all phage work published at these journals at the verified timeframes were found and are mentioned here. Since most of these publications have no other source besides the physical collections in which they are kept, this review presents a rich and important retelling of Brazilian phage therapy to the modern world.

Main text (4462/4500 words)

Since the 1980s, the use of bacteriophages to treat bacterial infections (phage therapy) has been considered a viable solution for the antimicrobial resistance crisis[1]. Many research groups[2] and modern clinical trials[3-5] have focused on this subject of growing importance, with Belgium recently pioneering with alternative regulatory pathways for the clinical use of phages[6]. However, phage therapy is not a new concept. The discovery of phages in the early twentieth century was closely tied to clinical practice and, in the early 1920s, phage therapy spread from France to the rest of Europe and the Americas[7-8]. Heralded as a significant advance in medical care at the time, phage therapy was used as a routine treatment in several countries until, in the 1940s, a shift to the use of antibiotics occurred, which made it disappear from most of the world. Although the Georgian, Russian and Polish experiences with phage therapy are well documented, much of what has been achieved in other countries remains under-reported.

One interesting missing piece of phage therapy history lies within South America. Mentioned only three times in recent scientific literature, the role of Brazil in using phages as a treatment is still shrouded in mystery. First, "*the epic of phage therapy*" (text based on a presentation given by Dr Alain Dublanchet in Oxford) mentions that in the 1920s, bacteriologists from Germany, USA and Brazil tried to verify Dr Felix d'Herelle's phage therapy results, but failed[8]. Second, a report published in Portuguese by the Paraense[A] Academy of Sciences[9] highly praises Dr José da Costa Cruz[B] career. He is said to have used "*bacteriophagetherapy*" to treat diarrhoea in children and created a phage-based product for clinical use. The third mention appears in a chapter focusing on the history of phage therapy, written by Dr Nina Chanishvili from the Eliava Institute[10]. It states that the Institute Oswaldo Cruz in Rio de Janeiro started to produce anti-dysentery bacteriophages to be used in Latin America in 1924. From these publications, it is clear that Brazilian researchers were involved with phage therapy, moving from failure in a clinical trial to mass production of phages within only a few years.

Additional information could be obtained from Dr Cruz's obituary published in 1941[11], which also shows a picture of him (**Figure 1A**) The text confirms Dr Cruz's interest in "*bacteriophagy*" in his studies of child diarrhoea at the Institute Oswaldo Cruz. He is said to have had spectacular success in treating dysentery cases, and in a short time the Institute started to produce a commercial phage preparation. Its large scale production and application, as well as the rational therapeutic use of phages to treat a number of infectious diseases at the time, were attributed to him. From the obituary, it was possible to track his publications, and the name of the journals in which he published. These were investigated, along with other sources of medical-related information from Dr Cruz's time.

From failed clinical cases to mass production of phages (1921 to 1924)

By the 1920s Brazil was a republic with Rio de Janeiro as its capital, which is where the Institute Oswaldo Cruz was located. The Institute was founded in 1900 and became important for sanitary campaigns, the production of biological products and medical research. The first publication mentioning phages in the Brazilian literature was a study about their nature, co-authored by Dr Cruz in 1921[12]. For more information on this publication and other historical Brazilian phage research papers, see the *Supplementary Material*. The first mention of the clinical use of phages in Brazil appeared in a publication in 1923 describing the results of two sets of clinical tests against dysentery[13]. In this publication, Dr Cruz affirms that anyone who sees the *in vitro* activity of a phage is "*filled with hope that a new therapeutic against infectious diseases will immediately appear*", and states that he was oblivious to Dr d'Herelle's clinical observations at the time. The first phage therapy cases took place at the Mantiqueira Mountains[C], near the city of Barbacena. Despite Shiga bacillus being isolated from two patients, phage treatment was not successful in this early attempt. The second testing of phage therapy took place in 1923, when cases of dysentery started to appear around the Institute Oswaldo Cruz in Rio de Janeiro, and phages were tested in "*better working conditions*". Twenty-four clinical cases were reported, with most patients reporting improvement of their symptoms four to five hours after the start of therapy. It was concluded that phages were recommendable for dysenteries for being easy to administer, innocuous for the patients and efficient in cases in which other treatments have failed.

More information about Dr Cruz's early success with phage therapy was published in French in 1924[14] and in a review written by himself in 1940[15]. After one year of experience in applying oral phage therapy against dysentery, probably as a follow-up to the 1923 trial in Rio de Janeiro, the Institute Oswaldo Cruz prepared

10.000 phage vials and distributed them to numerous doctors in Brazil. Positive results against acute and chronic cases were reported from the states of Pará, Maranhão, Pernambuco, Paraná and São Paulo. It was concluded that phage therapy was the best treatment for dysentery, surpassing any other treatment by quickly supressing symptoms in a few hours and resulting in cure after one or two days. The positive feedback led the Institute Oswaldo Cruz to recommend phage use, and it started to provide phages by request, under the name *Bacteriofagina disenterica*. This product was noted to be the first therapeutic phage preparation offered to the public based on thorough observation. It reached national and international reputation because of the product's quality and the "*choosing of the cases sent for observation*". By this affirmation, Dr Cruz meant that the success of the product could be attributed to cases in which the phage preparation was given in a timeframe close to the beginning of the disease, hence improving its success rate. The *Bacteriofagina disenterica* was largely employed during the 1924 revolution[D] in São Paulo among government troops, but no specific details besides its successful use were reported. This was probably the first large scale human trial in history, preceding the ones made by Dr d'Herelle in India[16] and the ones by the Soviet Union[10].

The earlier years (1923 to 1929)

Dr Cruz's knowledge of phage therapy is clear in his 1923 publication, in which he discusses international research and issues concerning successful phage use[13]. Broad knowledge of phage therapy was probably widespread in the Brazilian medical community because three clinical cases against staphylococcal infections are described by Dr Nelson Barbosa on the same year[17]. The Brasil-Medico journal published comments on two Italian papers in 1924[18-19]. In one of these comments, the lytic principle of d'Herelle is acknowledged for slowly being established as a therapeutic agent in Brazil, with contributions by Dr Cruz and Dr Barbosa, confirming their roles as leading phage therapists of the time.

In 1924, phage therapy work from Brazil was presented at the Society of Medicine and Surgery of Rio de Janeiro. The first presentation was about "*bacteriophagy*" and immunity, discussing whether phages were alive or not and cited Dr Cruz's work[20]**.** The second presentation concentrated on the therapeutic use of "*bacteriophagy*"[21]. After an introduction on Dr d'Herelle's work and a recollection of clinical phage use in other countries, the presenter cited Dr Cruz's results and praised the phage product provided by the Institute Oswaldo Cruz, affirming that he had used it to treat almost fifty dysentery cases with success. The third presenter discussed immune responses to injected phages, the choice of phages and isolation of bacteria, and presented a recollection of his laboratorial and clinical cases[22]. Although no details about the cases are given by the conference summaries, they demonstrate wide use of phage therapy.

During the *IV South American Conference of Hygiene, Microbiology and Pathology* held in 1929, Dr Cruz summarised the therapeutic use of phages worldwide and presented more details about the Brazilian phage therapy experience[23]. He mentioned his own failed attempts to treat bacteraemia in cases of typhus and paratyphus and that his plans to test phage therapy against cholera in 1924 ended because he was not able to isolate specific phages. At the same conference Dr Oscar Pereira, a microbiology professor at the Medicine School of Porto Alegre, shared his own phage therapy clinical experience[24]. He was using phages against bacillary dysentery since 1924, resulting in a complete cure for the patients. According to him, phage therapy was also important for abolishing carrier states, because after phage use the patient's stool would not contain any infectious agent. He stated that the mortality related to dysentery in two hospitals from Porto Alegre city decreased considerably after phage therapy started to be used. He also presented one successful clinical case of phage therapy against a coli bacillus infection on the urinary tract and mentioned nine cases of pyodermitis and thirty-two of furunculosis treated with anti-staphylococcal phages.

The widespread knowledge on phage therapy is also supported by the published syllabus of a course offered by the Institute Oswaldo Cruz, in which one of the topics covered is "*bacteriophagy*"[25]. This shows that health workers in Brazil were being trained in phage therapy as part of their studies at the time. A picture of students approved in the 1928 course can be seen in **Figure 1B**.

From novelty to routine (1930 to 1944)

In 1930, the Brasil-Medico journal published a translated version of a German phage paper, with a note stating: "*among us the anti-dysenteric bacteriophagina is largely employed. As for its efficacy, a lot is discussed*"[26]. A few years later, in 1934, Dr Cruz was one of the authors of a communication presented to the Brazilian National Academy of Medicine[27]. Arguing that publications concerning the use of phage therapy against staphylococcal infections were rare and still did not attracted the deserved attention, it describes the clinical case of a young patient suffering from staphylococcal septicaemia preceded by a furuncle. After a month with no results using other treatments, a phage from a pre-existing collection was adapted to the bacterial strain infecting the patient and used. As a result, the clinical status of the patient progressively improved, and he was cured after six phage injections. Phages were also used topically with success to treat an abscess that appeared near the original furuncle.

Brazilian phage therapy cases continued to appear in the literature, with four therapeutic notes presented in 1935. The first describes a case of staphylococcal furunculosis in a child treated with *Estaphylofagina* ampoules and cured[28]. In the second note, the patient was an undernourished child with pyelitis presenting diarrhoea, urine incontinence and fever. She was treated with *Colifagina from Dr Raul Leite Laboratory*. The fever disappeared in three days, and she recovered completely[29]. The third case was also a child with long-term pyelitis, again successfully treated with *Colifagina*[30]. This note ends with a suggestion that this case was "*enough reason to always recommend*" the phage product. The fourth note describes the cure of a patient with osteomyelitis by using injections of the *Estafilofagina* phage preparation[31]. The names of the phage preparations used as treatments on these cases are of commercial phage products. They were advertised at the medical literature of the time (advertisements of phage-based products in Brazil are shown in **Figure 2**), and prepared by the Raul Leite Laboratory. For more details see the *Supplementary Material*.

In 1938, seventeen years after his first phage publication, Dr Cruz published a review about thirty-three cases of septicaemia that he was involved with during treatment[32]. The cases were divided into gonococcal (one), streptococcal (fourteen), staphylococcal (twelve) and caused by a coli bacillus (six). Phage therapy was used only for seven of the staphylococcal cases and attempted but not continued in one[E]. Considering the staphylococcal cases, he concludes that the best treatments were blood transfusions and phage therapy, suggesting that injected phage preparations could be a first order therapeutic agent for staphylococcal septicaemia, curing even those cases that transfusion could not. Mortality was lower than predicted among phage-treated patients, and all who reached the third phage dose had sterile haemocultures. Although phage therapy cleared bacteraemia in all patients, the appearance of phage-resistant localised bacterial foci led to death or to the need for surgery in some cases. Of the four patients treated with phages who passed away, one had an acute infection and did not reach the second phage dose, one died because of surgery complications and two had localised focal infections. Dr Cruz's review also gives an interesting overview of the phage therapy practices in Brazil. First, not all bacterial diseases were targeted by phage therapy, and even in cases in which phages could be used, other treatments were also applied. For example: of the twelve staphylococcal cases, five were treated with phage-therapy only, two were treated with phage therapy after other approaches failed, two had phage preparations made but not used because the patients recovered before, and three did not use phages at all. Second, consent for using phages was also important for deciding its use. Dr Cruz mentions that no other phage preparations were used in the case in which the patient had a strong reaction to the lysate because the patient's family was against the use of phages since the beginning, and only reluctantly consented on the first dose.

Dr Genesio Pacheco, a co-worker of Dr Cruz from the Institute Oswaldo Cruz and author of his obituary, published a review on "*bacteriophagetherapy*" in 1939[33]. Written in a personal and critical tone it denounces the high number of pharmaceutical products in Brazil and associated their mass scale production and abusive advertising to the search for high profit. The problem with most commercial phage preparations was attributed to the fact that the technical requirements for a perfect product made them unattractive monetarily, and that profit was often made from technical imperfections that affected product quality. Then the association of different medications was said to be responsible for "*a multitude of products cluttering pharmacy shelves and the pockets of pharmaceuticals and producers*". A product combining phages to lactic ferments approved by public health officials was cited as an example. An advertisement of this product from 1934 can be seen in **Figure 2C**. As a conclusion, Dr Pacheco classifies phage production as an activity that should not be subjected to commercial ends, being as important as justice, culture, education and health-related issues such as preparation of medicine. Although his point of view is similar to that of Dr d'Herelle regarding commercial phage products, his connection to phage manufacturers in Brazil (for more details, see the Supplementary *Material*) might present an undisclosed conflict of interest.

In his 1940 review, Dr Cruz mentions his involvement in two Brazilian phage therapy clinical trials against typhoid fever: one in 1924 in São Paulo and the other "*recently*" at the Hospital São Sebastião, both with negative outcomes[15]. His experience with phage therapy against pyogenic infections, from dermal infections to staphylococcal septicaemias, was positive as described previously. He considered phage therapy in these infections extremely successful and the only solution to cases in which other treatments have failed, but he notes that using phages adapted to the patient's clinical isolates, as opposed to growing the phages in reference bacterial strains, was critical for success. This was the last paper published by Dr Cruz, who passed away in the same year.

In 1939, notes about international papers describing negative results for phage therapy appeared in the Brazilian literature. These describe phages failing to protect rabbits that were experimentally infected with *Staphylococcus*[34]. In 1944, a review on phage therapy against staphylococcal infections in Brazil was published[35]. It mentions that phage treatments in these infections were rarely used, and usually done when other treatments failed. The author reports good results using the oral route for treating furunculosis and acne, with 60% of furuncle cases being cured in eight days, and with reincidive cases not cured by other means being cured by phage therapy. Phages were considered to be a "*heroic therapeutic*", and cases in which the bacteria became resistant to the phage are noted to be rare. This publication was discussed on a questions and answers section of the same journal, by a reader who only used phages against dysentery but became interested in phage therapy against staphylococcal infections[36].

Slowly fading away (1944 onwards)

The first half of the 1940s was marked by an increase in publications about other antibacterial substances. For example, a short note discussing an international publication on phage therapy use against staphylococcal meningitis appeared in 1944[37]. Although mentioning that the exclusive use of phages could cure patients, the authors' recommend using sulphonamides in association with phages and anti-toxic sera during the first week of treatment. Then phage use could be continued for a longer time, to avoid complications such as cerebral abscesses. A comment on publication by Jern and Meneley mentions that sulphonamides were not as efficient against *Staphylococcus* as they were for *Streptococcus* infections, with the former being treated more efficiently by phage therapy[38]. Then an experiment using chicken eggs injected with *Staphylococcus* and many antibacterial agents, including antibiotics and phages, was presented. This comparative approach revealed that penicillin was the most efficient treatment. Another comment on an international paper by Boyd and Portnoy was published in 1945, describing the negative results of human trials of phage therapy on war prisioners[39].

Phages are mentioned again in the questions and answers section in 1948[40]. Even though noting that sulphonamides were the current preference for treating enteric infections, a reader asks about the concomitant use of phages and lactic ferments. This highlights that there was still some interest in phages, but that sulphonamides/antibiotics were becoming the first choice of treatment and questionable phage products were still on the market. A question about the use of phages to treat abdominal typhus also appears in the same year[41]. In 1950, a doctor from the University of São Paulo published a review about the treatment of amoebal dysentery[42]. Here, phages are indicated as auxiliary medication to treat cases in which secondary infections might be present, together with antibiotics.

From these publications and comments, it is clear that there was still interest in phage therapy in the 1940s. The associated use of phages and antibacterial medicine also became clear during this decade, as did the divulgation of the international literature concerning the efficacy of antibiotics and failed phage clinical trials. Some examples of advertisements for sulphonamides and penicillin found in the Brazilian medical literature are shown in **Figure 3**.

Why has phage therapy vanished?

There is no clear delimitation for when phage therapy ended in Brazil. The literature used as a source for this review does not contain any publications recommending that phages were not to be used, nor any mention to laws or regulations on this matter. This was confirmed by a consultation made with the Brazilian Health Regulatory Agency (contact protocol 2019084054), which determined that there are no current regulations

concerning the use of phages in Brazil. Dr Cruz's obituary, published in 1941, highlights his efforts in phage therapy, with no mention of problems or any intentions to interrupting it[11]. However, around the same time, publications about phages and clinical phage therapy cases became rarer in the Brazilian literature. Coincidentally, publications on antibiotic use increased in the early 1940s, culminating with a large focus on penicillin in 1944. In this year, the Brasil-Medico journal published a translated version of a Dr Alexander Flemming's paper, a translated transcript of a Winston Churchill speech praising the use of penicillin, and several clinical cases of antibiotic use. In 1945 the *Memorias do Instituto Oswaldo Cruz* journal published a series of penicillin trials, and a proposition about lowering penicillin taxes was being discussed by the government in 1946. Also in 1946, Dr Flemming travelled to Rio de Janeiro to attend a medical conference and visited the Institute Oswaldo Cruz. Furthermore, the 1940s marked the end of the generation trained during phage discovery, with many health workers probably retiring or passing away during this decade. This, along with the new and promising antibiotics, might have made phages an outdated therapy in the eyes of the new medical generation. Health professionals might have seen antibiotics as an easier and more efficient novelty to treat bacterial infections, including those not targeted by phage therapy. The flooding of the Brazilian market by suboptimal commercial phage preparations, as implied by Dr Pacheco in 1939[33], could also have had a negative impact on the reliability of phage therapy compared with antibiotics. Thus, the transition from phage therapy to antibiotics appears to have been gradual, with the success of antibiotic use replacing the use of phages during the 1940s. Years later, when bacterial resistance to antibiotics became a serious threat, much of the phage collections and phage therapy expertise were already lost beyond repair and the search for new antibiotics became more attractive than reviving phage treatments.

Conclusions

The Brazilian medical literature from the first half of the twentieth century reveals that Brazilian doctors and researchers were active practitioners of phage therapy. Dr Cruz, from the Institute Oswaldo Cruz in Rio de Janeiro, was the leading expert on the subject. He coordinated the first human cases in Brazil in 1921 and 1923, resulting in a phage product distributed around the country for many years. Dr Cruz had some interesting claims of priority. He mentioned that his 1921 tests were made before being aware of Dr d'Herelle's results with human patients; claimed that his phage product was the first backed by strong trial data; and its mass testing on soldiers during the 1924 revolution in São Paulo preceded Dr d'Herelle's mass test of phages in Calcutta[16] and the Russian tests on troops[10] by a few years. A timeline showing an overview of major phage therapy milestones, from Brazil and the rest of the world, is presented in **Figure 4**.

No evidence has been found to support a direct interaction between Brazilian researchers and Dr George Eliava or Dr d'Herelle. The origin of the anti-dysentery phages used in Brazil remains a mystery, while at least part of the anti-staphylococcal phages were obtained from the Pasteur Institute in Belgium. Clinical cases found often lacked specific details, such as phage dosages, and all information available in the original references are compiled in **Table 1**. No records of the destiny of Brazilian phage collections or large-scale phage production and purification protocols could be retrieved. For more details about the origin of phages used in Brazil, their production, administration protocols, and international collaborations of Brazilian phage researchers, see the *Supplementary Material*.

Phage preparations were made by the Institute Oswaldo Cruz and distributed throughout the country, while commercial phage products were also made and sold by private companies. Dr Cruz and Dr Pacheco believed that phages were important enough to be made by state owned quality labs. However, both researchers were also involved with a private laboratory. There is no clear milestone pointing to the stop of phage usage in Brazil. What could be perceived is that the interest in phages decreased as the use of antibiotics increased, and this serves as an indication that the same trend might have happened in other Western countries.

The use of phages in Brazil was mostly against bacillary dysentery and staphylococcal infections, with successful outcomes reported for both. Phages were administered orally for dysenteries and injected for staphylococcal infections, but local and oral use were also recorded for the latter. Details of past Brazilian experiences in successfully treating these diseases are priceless to modern researchers and clinicians. In 2010 there were 188 million cases of bacillary dysentery caused by *Shigella* around the world, and these infections are the second leading cause of diarrhoeal related deaths worldwide[48]. Staphylococcal infections are still an important threat with many clinical manifestations, and they are becoming increasingly more dangerous because

of the spread of antibiotic resistance[49-50]. The safe and efficient use of phages against these targets, as suggested by the Brazilian experience shown in this review, can be taken as a guideline to develop modern phage therapy trials and shape phage use in our time.

This review is also important for showing that Brazil has a strong past with phage use, bringing to light original publications and clinical cases that were hidden from the international community by language barriers and for being part of physical collections only. It fills some gaps in the past of phage therapy, with importance to the history of medical science and to the phage research community. Despite its rich past with phage therapy, the use of phages is not currently applied in modern Brazilian medical practice. The revival of phage therapy would in theory fit well within the current Brazilian unified health system[51], which with the support from Institutes and Universities for phage collections and research, could provide efficient and cost-effective phage treatments for the population. However, regulatory and political issues allied to increasing austerity measures would make it hard to be achieved. For a country that after a brief respite is undergoing successive cuts in research funds[52-55], going through a loss of historical memory[56] and suffering from anthropogenic environmental catastrophes[57-58], this review might serve as an inspiration to look into a successful past and aim for a better future.

Footnotes

[A] Paraense is an adjective used to designate someone born at or something originated from the Pará state, northern Brazil.

[B] It is important not to mistake Dr Oswaldo Cruz (1872-1917), namesake of the Institute Oswaldo Cruz with Dr José da Costa Cruz (1894-1940), the author responsible for many publications cited in this text. Dr José da Costa Cruz worked at Institute Oswaldo Cruz and was responsible for much of the phage work in Brazil.

[C] Serra da Mantiqueira (Mantiqueira Mountains) is a mountain range in South-eastern Brazil, extending over the states of São Paulo, Minas Gerais and Rio de Janeiro. The location of the outbreak was mentioned to be around the city of Barbacena, Minas Gerais.

[D] The 1924 revolution in São Paulo mentioned is probably the Paulista Revolution, part of the Tenentist movement. Army dissidents attempted to depose president Artur Bernardes, leading to a 23 days long occupation of the city that ended after the city was bombarded by the Federal Government troops. The estimative are of 1000 dead and 4000 injured.

[E] In this particular case the treatment was discontinued because a strong reaction to the phage started after only a few drops of the medication. A footnote mentions that the reaction was probably not due to the phage itself, explaining that although the phage preparation used was sterile, it was opaque and not transparent as it should be. The opaqueness was attributed to a faulty candle used during the phage preparation, which released kaolin to the filtrate.

Contributors

Gabriel MF Almeida made the literature search, collected the data, and prepared the first version of the manuscript. Both authors contributed in writing the manuscript.

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Conflicts of interest

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Figure legends

Figure1: Images related to Brazilian phage therapy.

A) Dr José da Costa Cruz, phage therapy pioneer in Brazil. Picture from his obituary[11]. B) Students approved on an applied course offered by the Institute Oswaldo Cruz in 1928, in which "*bacteriophagy*" was included in the syllabus[25]. These images are in public domain.

Figure 2: Advertisements for phage therapy products in Brazil.

A) Advertisement for the *Bacteriophagina dysenterica* phage preparation made by the Institute Oswaldo Cruz, printed at the Folha de São Paulo newspaper in 1928. B) Advertisement for the *Lisogenina-coli* product sold by the Raul Leite Laboratory, printed in the Brasil-Medico journal in 1935 (n.14, p. 328). C) Advertisement for the *Lactozym Alfa* product, which combined a polyvalent phage to a lactic probiotic, 1934. D) Advertisement of

many phage preparations made by the Raul Leite Laboratory, 1936. C and D were printed on the “O Bisturi” periodic, which contained general and medical related information for medicine students (1934, n.8, p.6 and 1936, n.15, p.4 respectively). These images are in public domain.

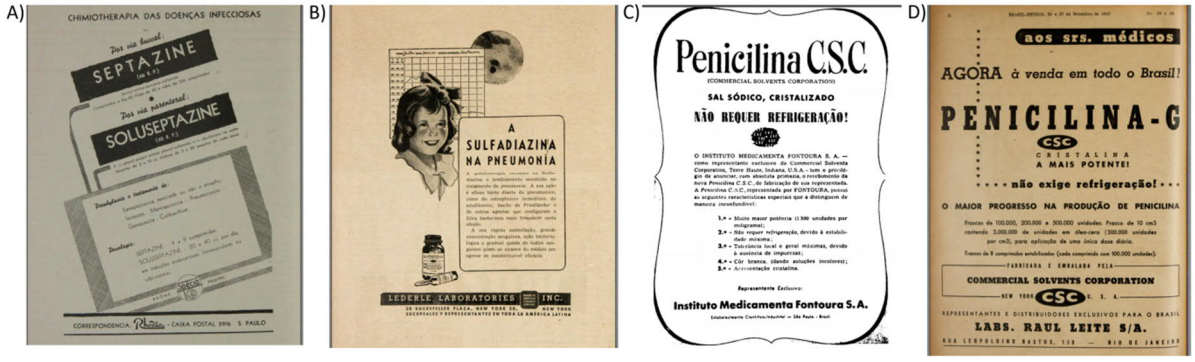

Figure 3: Advertisements for sulphonamides and penicillin in the Brazilian medical literature (1938-1947).

A) One of the earliest advertisements for sulphonamides found (Brasil-Medico, n.18, 1938). B) Advertisement from an American sulphonamide preparation (Brasil-Medico, n. 16-17, p.26, 1945). C) Penicillin advertisement from a company in São Paulo (Brasil-Medico, n.42-43 , p.27, 1946). D) Penicillin advertisement from the Raul Leite Laboratory (Brasil-Medico, n.38-39, p.2, 1947). These products were imported from the United States of America and sold by Brazilian representatives. The images are in public domain.

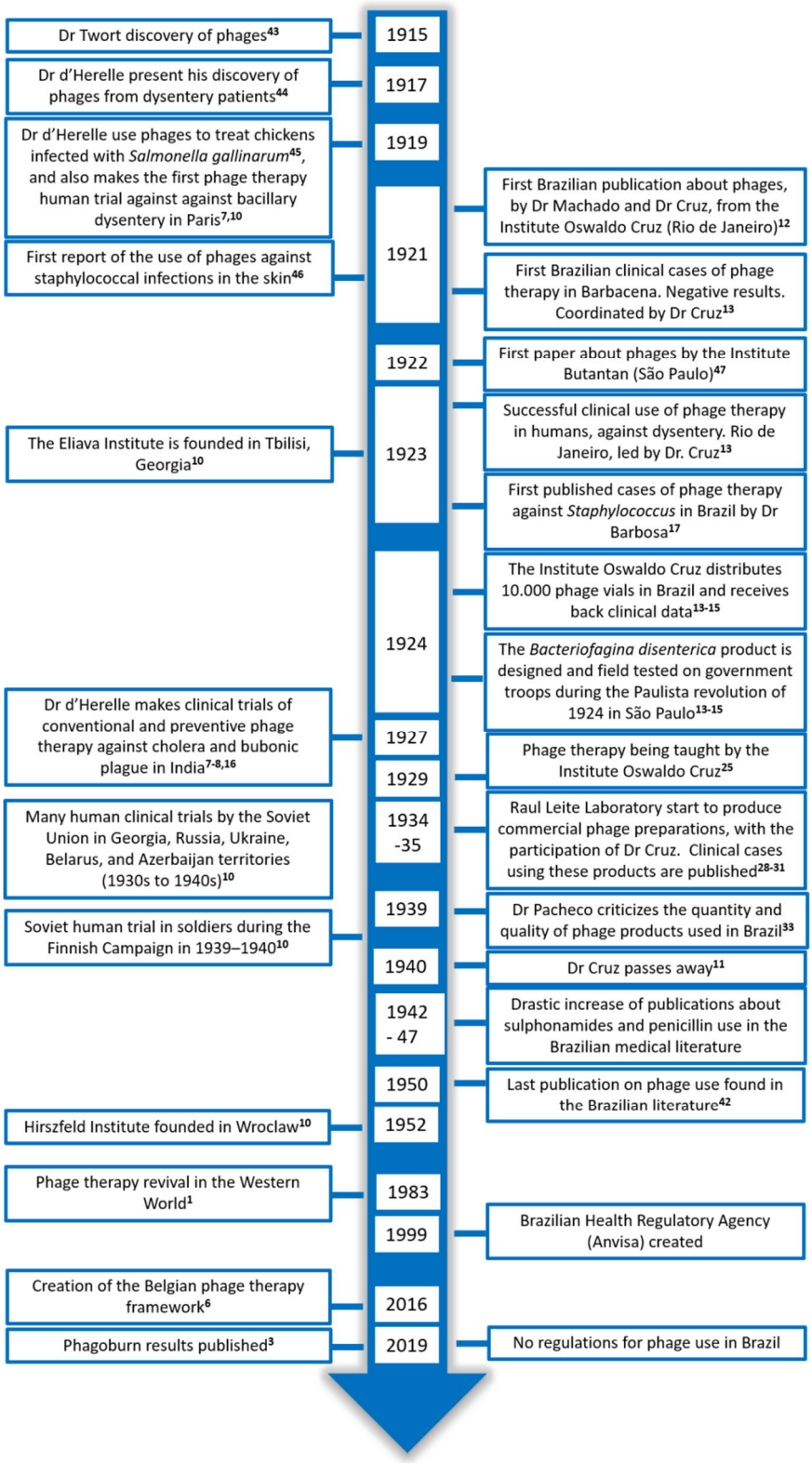

Figure 4: Timeline showing major phage therapy milestones.

On the left there are the well-documented international events, and on the right the events specific for Brazilian phage therapy. These were hidden in old publications and became known through this review. The starting time point is the original publication by Dr Twort describing phages in 1915, and the last time point is the publication of the results from the PhagoBurn European phage therapy clinical trial in 2019.

547 **Table 1:** Compilation of phage therapy clinical cases published in the Brazilian literature in the first half of the
548 twentieth century. An empty entry means that the information was not mentioned on the case description.

Bacterial agent	Case description	Age	Sex	Survived	Number of phage doses	Details	Phage administration routine	Other treatments	Reference and year of publication
Hiss Bacillus	6 days of stomach pain and diarrhoea	25	Male	Yes	2	Better few hours after dose 1, cured on next day	20 cc. , one dose per day		13 ; 1923
Hiss Bacillus	4 days of vomiting, fever, headchaches and body pain followed by bloody diarrhoea	16	Male	Yes	2	Better few hours after dose 1, cured on next day	20 cc. , one dose per day		13 ; 1923
Hiss Bacillus	2 days of stomach pain and fever followed by bloody diarrhoea	17	Female	Yes	1	Cured after the first dose			13 ; 1923
Hiss Bacillus	3 days of bloody diarrhoea	11	Female	Yes	3	Better 2 hours after dose 1, cured 4 days later	One dose per day		13 ; 1923
Flexner Bacillus	Fever, headaches, diarrhoea	32	Male	Yes	3	First dose cured diarrhoea, two more to cure stomach pain	Two days interval between doses 1 and 2		13 ; 1923
Flexner Bacillus	Chronic dysentery for years with monthly crisis	36	Male	Not cured	at least one	The patient bacterial strain was shown to be phage resistant *in vitro*			13 ; 1923
Hiss Bacillus	Diarrhoea	16	Female	Yes	3	Cured 48 hours after dose 1	12 hours interval between doses		13 ; 1923
Hiss Bacillus	3 days of diarrhoea	40	Female	Yes	3	Cured	One dose per day		13 ; 1923
No bacillus isolated	Diarrhoea	42	Male	Yes	1	Felt cured 48 hours later			13 ; 1923
Flexner Bacillus	Diarrhoea	Widow#	Female	Yes	2	Cured after 2 days			13 ; 1923
Flexner Bacillus	Diarrhoea	23	Male	Yes	3	Cured after 2 days			13 ; 1923
Shiga Bacillus	2 months of diarrhoea	19	Male	Yes	many	Better after dose 1, cured after a few days	12 hours interval between doses	Many other treatments including emetine injections	13 ; 1923
Fecal exams not made	2 days of bloody diarrhoea	Married#	Male	Yes	3	Better after dose 1, cured after dose 3	12 hours interval between doses		13 ; 1923
Fecal exams not made	Bloody diarrhoea, stomach pain, fever	42	Female	Yes	at least one	Better after dose 1, cured after 2 days			13 ; 1923
Fecal exams not made	Diarrhoea and fever	6	Male	Yes	at least one	Cured in 24h			13 ; 1923
Fecal exams not made	Fever	4	Male	Yes	at least one	Cured in 24h			13 ; 1923
Fecal exams not made	5 days of dysentery	39	Female	Yes	2	Better after dose 1, cured after dose 2			13 ; 1923
Fecal exams not made	2 days of diarrhoea, fever, vomiting and stomach pain	5	Male	Yes	2	Better after dose 1, cured after dose 2			13 ; 1923
Fecal exams not made	6 days of diarrhoea, vomiting and stomach pain	3	Male	Yes	3	No recovery after dose 1, better after 2, cured after 3			13 ; 1923
Fecal exams not made	2 months of intestinal problems, with recent fever and diarrhoea	2	Female	Yes	1	Immediate recovery			13 ; 1923

Fecal exams not made	Diarrhoea, fever and stomach pain	4	Male	Yes	1	Immediate recovery			13 ; 1923
Fecal exams not made	Dysentery		Female	Yes	2	Cured after dose 2		Emetine	13 ; 1923
Fecal exams not made	Fever, stomach pain and bloody diarrhoea for 4 days		Male	Yes	2	Better after dose 1, cured after dose 2			13 ; 1923
Fecal exams not made	6 months of dysentery	16	Male	Yes	6	Better after dose 2, stomach pains cured after four more doses		Other medications (serum, emetine, others not specified)	13 ; 1923
Staphylococcal	Tibia osteomielitys followed by pyelonephritis	Around 40	Female	Yes	1	Urine culture sterile 2 days after phage treatment	Injection of 1 cc.	23 days of "various therapeutic resources" before phages	17 ; 1923
Staphylococcal	Kidney pain and painful urination	Madam[#]	Female	Yes	1	Urine culture clearer after 24h later and sterile after 3 days	Injection of 2 cc.	Rotropine	17 ; 1923
Staphylococcal	Infected grenade wound during the "recent revolution"	Lady[#]	Female	Yes	Several	Pus almost gone after first dose	Injection of 40 cc. on the wound (daily at first, then every 2 days)	Removal of necrotic tissue, 15 days of common antiseptics	17 ; 1923
	Dysentery (around 50 cases)			Yes		Cure. Phage preparation provided by the Institute Oswaldo Cruz.			21 ; 1924
Coli bacillus	Urinary tract infection resistant to conventional treatments	6	Female	Yes	13	Cure with sterile urine and weight gain. No recurrent episodes.	5 intravesical injections, 3 subcutaneus injections and 5 oral doses	Other treatments before phage therapy (not mentioned)	24 ; 1929
Staphylococcal	Pyodermitis (9 cases)			Yes		Cure with quick reduction of pain and inflammation	Polyvalent anti-staphylococcal phage preparation made in Rio Grande do Sul		24 ; 1929
Staphylococcal	Furunculosis (32 cases)			Yes		Cure with quick reduction of pain and inflammation, no recurrent episodes	Polyvalent anti-staphylococcal phage preparation made in Rio Grande do Sul		24 ; 1929
Staphylococcal	Septicemia preceded by furunculosis	20	Male	Yes	6 injected + 1 topical	Better after dose 2, negative haemoculture after dose 3	5-7 days interval between doses	Many others (not specified)	27 ; 1934 32 ; 1938
Staphylococcal	Staphylococcal furunculosis for 1 year	4	Female	Yes	Several	Completely cured after a few doses	"Estaphylofagina" product used		28 ; 1935
	Pyelitis, dhiarrea, urine incontinence, daily fever, undernourished	2	Female	Yes		Fever disappeared in 3 days and she completely recovered	"Colifagina" product from Raul Leite laboratory used		29 ; 1935
Not mentioned	Long term pyelitis	3		Yes	6 ampoules	Better after dose 3, cured after dose 6	"Colifagina" product used		30 ; 1935
Not mentioned	Osteomyelitis (unknown cause)	41	Male	Yes	30 injections	Pain gone after dose 3. Suppuration stopped after dose 6. Cured after dose 30.	"Estafilofagina" from Raul Leite Laboratory used	Surgery and scraping of infected tissue	31 ; 1935
Staphylococcal	Septicemia preceded by knee furuncle	12	Male	No	Few drops	Phage therapy discontinued after reaction to	Only a few drops injected	Blood transfusion	32 ; 1938

						a contaminated injection			
Staphylococcal	Septicemia preceded by osteomielytis	10	Male	Yes	5	Negative haemoculture after dose 2		Blood transfusions, trepanation, draining of infected tissue, carbon injections	32 ; 1938
Staphylococcal	Septicemia preceded by osteomielytis	Adult[#]	Male	No	5	Negative haemoculture after dose 1		Blood transfusion, bone surgery, "Staphylococcal anatoxin" imported from the Pasteur Institute	32 ; 1938
Staphylococcal	Septicemia preceded by furunculosis and hemorrhoids abcess		Male	No	6	Negative haemoculture after dose 3		Only phage therapy	32 ; 1938
Staphylococcal	Septicemia preceded osteomielytis	12	Male	No	2	Passed away from surgery complications		Only phage therapy	32 ; 1938
Staphylococcal	Septicemia preceded osteomielytis	Adult[#]	Male	Yes	At least 2	Negative haemoculture after dose 2		Only phage therapy	32 ; 1938
Staphylococcal	Acute septicemia preceded by provoked abortion	around 30	Female	No	1	Did not survive for dose 2 (acute disease)		Only phage therapy	32 ; 1938

[#] Although no precise age was recorded, these designations used to describe the patient gives an idea about the age group.

Grey lines represent several cases mentioned together in the publications, with no individual details.

Supplementary material

Phage research in Brazil: investigating the nature of phages

The first paper mentioning phages in the Brazilian literature was published by Dr Cruz and Dr A. Machado in 1921[1]. The paper makes clear that the true nature of phages was still questionable, and that different points of view existed at the time. Three theories are presented in the paper: a filterable virus capable of parasiting bacteria as considered by Dr d'Herelle ; a mutation in the bacteria provoked by an immunised host which leads to secretion of a bacteriolytic ferment as defended by Dr Bordet ; or by a precursor in the bacteria activated by intestinal kinases as supposed by Dr Kabeshima. The presented results show that the addition of sera against the bacterial host in cultures containing phages and hosts abolished the activity of the phage. Since addition of specific antibacterial sera abolished phage activity, the conclusion was that phages could be a ferment released by dead bacterial cells when lysed in a special way. This idea appears again in other publications by Dr Cruz. In a paper published in 1922, seven phages were isolated from eight stool samples from patients with Shiga bacillus infection[2]. These were tested for host range, adaptation to other strains, growth conditions and resistant hosts were isolated. Then a series of immunological tests using sera from patients and from animals injected with bacteria or with the phages were presented, indicating that the phage adhered to the bacterium by the same specific adsorption process used by antibodies. Although mentioning that most authors believed in Dr d'Herelle opinions, Dr Cruz concludes the paper with the idea that the phage was a ferment, a sort of catalyser present in the bacterium used for the disintegration of membranes that lysis cultures if released by cell death. It is even stated that, "*without any pretension to affirm absolute truths*", that their point of view linked the phenomena of lysis in series to humoral immunity. In a follow-up paper published in the same year he studies sera against the Flexner phage made in rabbits[3]. The results show that specific sera inactivated the phage lytic activity, while sera against the host protected it from phage lysis. He also selected, *in vitro*, a phage resistant to the sera. Interestingly, this phage was not as efficient as the original one in infecting its host. In the end, Dr Cruz mentions that the data could be interpreted from Dr Bordet's point of view, but that Dr d'Herelle's opinion on the viral nature of phages offers an easier explanation. From these publications it is clear that Dr Cruz was divided between opinions on the nature of phages, but his immunology expertise led him to interpret the problem from an immunological point of view.

Dr Cruz's investigations of the nature of phages continued in the following years. One publication describes the influence of electrolytes on phage-mediated lysis, proposing it to be a way to end the controversies regarding the nature of phages[4]. The addition of NaCl, CaCl or NaSOMg improved phage lytic activity, taken as proof that phages were closer to ferments and antibodies than to viruses. This was further investigated in another publication in the same year[5]. The introduction cites many evidence that phages could be living viruses, including the fact that they easily adapt to adverse conditions, but the publication concludes that their other properties have made most researchers reject the idea. After showing that phages, as antibodies, were electrolyte dependent, he compared the activity of phage solutions made in distilled water, passed through filters and the filter eluate by physiological solutions. The conclusion is that electrolytes dispersed phages (as with antibodies), keeping them in the right state to act, while tending to flocculate living beings. Saying that phages are not alive and have the physical properties similar to antibodies, he speculates whether other viruses (chicken pest, smallpox, rabies) would also behave the same. At the same year his connection to the international literature is shown when he responds, with simple experiments and discussion, two other papers that had diverging results or conclusions when compared to his own[6]. The first paper is related to the action of sera on phage activity, and the second to phage-mediated lysis in distilled water. In the same publication a small test of phage resistance to silver is presented, showing that lytic activity was abolished in the right conditions. In a follow-up paper he tested whether acid conditions would make phages precipitate as with antibodies[7]. The conclusion is that they did, strengthening his theory on the nature of phages. He also presented these results to the international community via publications in French[8-11]. In 1929, Dr Eduardo de Araujo (director of the Institute Oswaldo Cruz from Bahia state) presented a note on the presence of phages in different water sources from his state, demonstrating that other researchers from the Institute Oswaldo Cruz were also interested in phage biology[12].

Meanwhile, publications on the nature of phages from the Butantan Institute in São Paulo also appeared in the Brazilian literature, with no clear connection to the work done at the Institute Oswaldo Cruz. The first publication is a paper divided into three sections[13]. The first part describes that Dr d'Herelle's experiments on phages were successfully repeated and confirmed by the isolation of bacteria and phage from human stool samples. The second tests the idea that phages could originate from bacteria, and the third focuses on the nature

of the phage and whether it could lyse red blood cells and influence the bacterial fermentation of sugars. The third section is further investigated by another publication of the same Institute, which concludes that the phage did not act on a hemotoxin not on dysenteric toxins but had an influence on some sugars and on the fermentative activity of a bacillus[14]. Another paper provides an introduction about Dr d'Herelle's work on the microbial lysis in series[15]. It focuses on finding lytic activity from liquid and solid cultures of different bacterial strains, finding discreet lysis for many and hypothesising that lysins secreted by bacterial cells *in vitro* were less active than the ones secreted by bacteria infecting an animal as in the case of Dr d'Herelle's experiments. The fourth paper mentions that the enthusiasm following the Twort-d'Herelle phenomenon resulted in many researchers claiming to have found "*the bacteriophage*" in many organic liquids without taking proper care regarding the basic facts of the "*bacteriophagy*"[16]. The bacteriolytic activity from the venom of different snake species was tested. Although many had lytic activity, the destroyed cells did not result in the "*formation of a new lytic principle*" and the conclusion states that the lysis mediated by venoms was unrelated to phages. In 1923, another paper on the nature of phages was published, with an introduction mentioning the history behind phage discovery and discussions of the nature of phages, acknowledging Dr Twort's role[17]. Inspired by international authors who described lytic activity from river water, such as Ernest Hankin's pioneer study, Dr Monteiro shows that it also happened with waters from the rivers Pinheiros and Tiete in São Paulo[18]. Years later, he investigated the presence of phages in bovines used for the preparation of the Brazilian smallpox vaccine and in the vaccine itself, linking his data to Dr Twort's phage description[19]. The last publication mentioning phages from the Butantan Institute is from 1942, in which phages were used as one of many variables to study the effect of an electrical current in microorganisms[20]. It is interesting to note that another Brazilian Institute related to public health also showed an interest in phages in the past, although only these papers have been retrieved from the literature. A contact with the Documentation Nucleus of the Butantan Institute revealed that there is a folder in their collection named "*Notes and studies on the nature of bacteriophages*" among the documents from Dr Lemos Monteiro, dated from 1920-1935. These documents are not available in digital format and can only be accessed on site after a formal request. Until further information is found, the Butantan Institute did not studied applied phage therapy nor made phage products.

Years later, two other papers investigating the nature of phages appeared, with no affiliation given to the author. The first discusses "*bacteriophagy*", mentioned to be a phenomenon more interesting from the biological than from the therapeutical or microbiological point of view[21]. It states that understanding the mechanism of the lytic phenomenon would result in solving several problems related to it, with profitable rewards for therapeutic use. The author believed that phages came from the bacteria and acted by digesting the bacterial cells, resulting in "*bacteriophagy*". The difference among viruses, phages and toxins would be only the level of biochemical complexity, with phages and viruses being at the same level. In the second paper, the nature of phages and phage mediated lysis is studied from an interaction between liquids and solids perspective[22]. The hypothesis was that mixing phages and bacteria would lead to three states: only bacteria after phage assimilation (minimum hydration state), only phages after complete lysis (maximum hydration state), or mixed cultures (intermediate). The "*bacteriophagic virulence*" is mentioned, phages are described as a proteinaceous substance, and Dr d'Herelle is cited on many occasions, so the author could have been a defender of the viral hypothesis. He also investigates the influence of water on phage activity and studies bacterial resistance to phages, correlating it to cell morphology. The conclusion is that phages were a product of the hydrolytic disintegration of the bacterial cell, resulting in a virus capable of reproducing in series (an analogy to the Rous sarcoma virus was made). The paper presents a drawing depicting phages as spheres surrounded by water, and it may be one of the earliest attempts to represent a phage structure in the literature (**supplementary Figure 1**). Also, in 1929, Dr Cruz presented an extensive review on the nature and properties of phages in a South American medical conference[23].

In 1940 Dr Cruz published a review on the properties, nature and therapeutic use of phages[24]. The introduction mentions Dr Twort and Dr d'Herelle, highlighting that the latter was responsible for the description of the "*bacteriophagy*" phenomenon. Then he argues against Dr d'Herelle view on the viral nature of phages, discussing that the "*serial transmission*" was not an inherent characteristic of living beings, pointing out flaws in experiments made to adapt phages to different conditions, besides citing his own results with salts and specific sera. He concludes that phages belonged to the group of ultra-microscopic viruses, which comprised agents of varied nature, biotic and abiotic. Dr Cruz mentions that phytopatologists at the time considered that some plant diseases, such as tobacco mosaic, could be caused by processes not parasitic in nature and discusses literature on this subject. In 1943, a summary from a paper published in 1942 appeared describing seasonal variations of typhoid-paratyphoid phages in rivers from the city of São Paulo[25]. Phages were found all year long, but in less abundance in colder months and during rainy periods. Phage load varied between the two rivers

sampled, and phage quantity from the rivers was directly proportional to the number of human disease cases in the same period. This is the latest publication concerning the nature of phages on the literature and timeframe searched.

The number of publications investigating the nature of phages published in the Brazilian literature can be seen as proof of how this subject was intriguing at the time. Although many important observations were made, some Brazilian researchers who worked on the subject missed the nature of phages as viruses, including Dr Cruz. In hindsight, it is easy to detect flaws, but we must remember that at the time phages were a mystery for microbiologists, and the confirmation of their viral nature only came in the 1940s with the advent of the electron microscope[26].

Origins of the phages used for phage therapy in Brazil

Two independent publications, one from the Institute Oswaldo Cruz in Rio de Janeiro and the other from the Institute Butantan in São Paulo, described the isolation of phages from Brazilian samples in 1922[1,13]. However, already in 1921, Dr Cruz made his first clinical tests against dysentery at the city of Barbacena. In addition, Dr Monteiro mentioned that the Institute Butantan had been working with phages for about a year at the time of his 1922 publication. Thus, phages were already present at both Institutes before their publications describing phage isolation in 1922. Were these phages isolated from Brazilian samples in unpublished experiments or were they obtained from abroad?

There is no clear evidence that Brazilian doctors had any direct collaboration with phage researchers from abroad, such as Dr d'Herelle or Dr Eliava. However, it is clear that there was a certain degree of intellectual and material exchange between Brazilian and foreign doctors at the time. Dr Cruz himself studied in Portugal before starting his medicine course in Rio de Janeiro. Besides, it was stated that Dr Jules Bordet* once said that Dr Cruz was the most brilliant student he ever had[27]. However, when exactly Dr Cruz interacted with Dr Bordet is not known. Dr Cruz's obituary mentions that he was in Europe in 1925 and 1931 for improvement studies, but no details are given regarding which Institution or what the focus of the studies was[28]. From the Institute Butantan side, one publication from 1922 reveals that one of the authors (Professor R. Kraus) studied phages in Buenos Aires, Argentina[13]. There is also evidence of material exchange between Brazil and the Institute Pasteur, although the material in these documented cases was not phage related. For treating a difficult septicaemia case in the 1930s, the Institute Oswaldo Cruz received a sample of staphylococcal anatoxin by plane from the Pasteur Institute[29]. Brazilian samples were also sent to Paris by Dr Cruz[27].

Phage exchange was mentioned a few years later. In 1923 Dr Barbosa revealed that the phage used to treat staphylococcal infections in Brazil was isolated by Dr Andre Gratia of the Institute Pasteur of Brussels in 1921, and obtained by Dr Madeira from Professor Bordet[30]. Then it was adapted to white and golden staphylococci isolated from Brazilian patients for phage therapy use. In his 1938 publication, Dr Cruz acknowledges that the phage used against staphylococcal septicaemias was the "staphylococcal phage H isolated by Gratia"[29]. This phage was able to infect all isolates from the clinical cases treated, but how it was obtained by Dr Cruz (a second exchange between Brazil and Europe, or given by Brazilian colleagues working with Dr Barbosa) is not mentioned.

Although it is clear that the phages used in the first Brazilian phage therapy cases against staphylococcal infections came from abroad, could the phages from an international collection, such as the one from Dr d'Herelle from the Institute Pasteur in France, be the ones used for the 1921 clinical cases in Barbacena? If the phages used were not adapted to Brazilian host strains, this could explain the failure. Then, after isolating phages himself, Dr Cruz would have used Brazilian phages for the successful 1923 testing. However, this is only speculative. The differences between the 1921 and 1923 phage therapy testings are not clear. The origin of the phages used in each is not mentioned, there are no details about the number of phage treated patients in 1921, and no information about the better working conditions of the 1923 cases is given. However, in a 1940 review, Dr Cruz revealed that, in 1921, he gave the phage ampoules to the medical staff responsible to treat the outbreak, and only later received the report that the results were negative[24]. The location of the 1921 cases, which was far from the Institute Oswaldo Cruz, could have posed a problem for keeping the phage preparations stable between production in Rio de Janeiro and transport until its final use in Barbacena. A combination of Dr Cruz not being directly involved with the treatments, the composition of the early prototype product and its

stability during transportation and storage could have influenced the clinical outcome. In 1923, the product could have received improvements in its formulation, the outbreak location was close to the production site, and Dr Cruz was directly involved in the cases. Regardless of the origins, the phages used for the successful trial in 1923 were tested for one year and in 1924 they were used as the base for the *Bacteriofagina disenterica* product.

* Dr Jules Bordet mentioned is probably the Belgian immunologist and Nobel Prize winner in 1919 for his work on antibodies and the complement system.

International collaborations

As mentioned above no direct connection between Brazilian doctors and international phage therapy excellence centres, such as the Eliava and Pasteur Institutes, were found. The closest evidence of phage related exchanges was the staphylococcal phage obtained from the Institute Pasteur of Brussels. However, indirect evidence of international collaborations or at least exchange of intellectual information existed.

Part of Dr Cruz work was published in French and cited by phage researchers of the time, including Dr d'Herelle, demonstrating that Brazilian phage therapy efforts were reaching Europe and other leading experts in the field. Dr Eliava himself mentions Brazilian phage therapy practice as a successful example when requesting funds from the Soviet authorities for expanding his own Institute in Georgia, in the 1930s[31]. Dr Kraus, from the Institute Butantan in São Paulo, was for some time in Buenos Aires (Argentina) studying phages, but no details are given regarding in which Institution[13]. In 1923, Argentinian phage work was acknowledged by Dr Cruz when he mentioned Dr Pico's studies[6]. Phage use in Argentina is mentioned again by Brazilians in 1924, against colites, nephrites, pielites, typho, dysentery, septicaemia, endocarditis and rheumatism[32]. This may indicate an intense exchange of information and maybe collaborations between Brazil and Argentina in the early 1920s. Also, it points that Argentina may have been another unacknowledged South American hotspot for phage therapy and research in the past. During the *IV South American Conference of Hygiene, Microbiology and Pathology* held in 1929, Dr Cruz (Institute Oswaldo Cruz) and Dr Pereira (Medicine School of Porto Alegre) presented data about their own experiences with phage therapy[23,33]. The conference had delegates from at least Chile, Argentina and Uruguay, proving that the Brazilian experience with phage therapy was being shown to doctors from other South American countries.

Much later, in 1950, an author from the Hygiene Municipal Institute in Warsaw published a therapeutic note in the Brasil-Medico journal[34]. Written in Portuguese as a recommendation to the Brazilian medical community, it focuses on the prophylactic use of phages to protect new-borns against diarrhoea. The note starts with an introduction about the colonisation of new-born's digestive systems, followed by a description of the Polish experience in protecting children by giving phages as the first liquid after birth, a "*simple and harmless*" prophylactic mean to protect against intestinal infections. Although this suggestion to use phages preventively came from Poland in 1950, no indication exists that it was considered or followed by Brazilian doctors. Also, no other evidence about collaborations between Brazil and Poland at the time was found.

Producers of phage preparations in Brazil

Based on the researched literature, the first Brazilian phage preparation designed for mass human use was produced by the Institute Oswaldo Cruz. This preparation targeted bacterial dysenteries and started to be produced on a large scale around 1923, under the auspices of Dr Cruz and was named *Bacteriofagina disenterica*. An advertisement from 1928 (**Figure 2A, main text**) shows that the production site was in Manguinhos (Rio de Janeiro). Part of the Institute Oswaldo Cruz, it produces biological products for human use to this date.

The success of this phage preparation probably raised interest in particular laboratories, which also started to produce phage preparations with varying qualities, as noted by Dr Pacheco in 1939[35]. One particular laboratory, often mentioned in clinical case descriptions and advertisements, is the Raul Leite Laboratory. While checking the Brasil-Medico journal archives, what might have been a flyer named "*A visit to the Raul Leite Laboratories*" was found[36]. Located in Rio de Janeiro, it was founded as a flour factory in 1921 and became an industry for

biological and pharmaceutical products twelve years later. It is highlighted that investments in advertising led to increased revenue and the consequent diversification of products. The Raul Leite Laboratory was also known for its product line named "medicine for the poor", which consisted of cheap packages with smaller amounts of the medicine inside. Product quality was said to be the same, but the price of the packing and smaller quantities were more accessible to people with a lower income (who could buy the medicine in parts during the treatment, instead of buying the whole dose at the same time). The text does not mention phages, but lists as products oral and injectable medicine, vaccines, hormones, sera and as future product prospects medicinal plants plus veterinary products. The text states that Dr Pacheco, from the Institute Oswaldo Cruz, would manage the production of the veterinary products. In 1935, an advertisement of a Raul Leite Laboratory phage product (**Figure 2B, main text**) mentions Dr Cruz's role in its production. Another advertisement from 1936 reveals that six phage products from the Raul Leite Laboratory microbiology section were being sold in São Paulo (**Figure 2D, main text**). Publications on the clinical use of the Raul Leite Laboratory phage preparations appeared in 1935[37-40]. Taken together, it is possible to speculate that the appointment of Dr Pacheco as manager of a section of the Raul Leite Laboratory in 1934 quickly led to the production of phage products, with the participation of his colleague Dr Cruz. These products were advertised in medical journals, used in clinical publications, and sold outside Rio de Janeiro. An advertisement from 1947 (**Figure 3D, main text**) shows that the Raul Leite Laboratory continued to expand and became a national distributor for the penicillin-G produced by the Commercial Solvents Corporation (C.S.C., New York, USA).

Protocols for phage production and administration in Brazil

The recorded phage products in early twentieth century Brazil includes the *Bacteriofagina disenterica* product from the Institute Oswaldo Cruz, the Raul Leite Laboratory preparations (*Estafilofagina*, *Colifagina*, *Disenterifagina*, *Tifofagina*, *Estreptofagina* and *Plurifagina*, for oral or injected use) and the association between polyvalent phages and a lactic probiotic from the Vicente Amato Sobrinho Company (*Lactozym Alfa*). The advertisements and trademarks suggest that these were readily made products, probably prepared using reference strains and bought from pharmacies, and not tailor made for each patient bacterial pathogen. Despite the information gathered on the existence and use of these products in Brazil, no specific details could be found about the technology used for mass scale production and purification. However, some information on how these products were used and also of patient-specific phage preparations can be found in the literature.

During the successful 1923 trial against dysentery in Rio de Janeiro, patients were treated using the gastric route, with a hydrocarbonated diet recommended to promote phage-mediated lysis[41]. A subsequent publication recommends that the phage ampoules were to be diluted in 200 millilitres of water and drank twice a day[42]. This is probably how the *Bacteriofagina disenterica* preparation was used around Brazil. In Porto Alegre, doctors made their own polyvalent phage preparations containing a mixture of anti-dysentery phages and these were given to patients mixed with water, with five-hour intervals between doses[33]. It is noted that there were no restrictions to phage use, but an adequate diet was important and milk was not recommended.

Staphylococcal infections were often treated by local or intravenous injections of the phage preparations, with descriptions of topical use in certain cases. In some cases phage products not adapted to the patient pathogen were used with success[30], but growing the phages on the patient bacterial isolate for making personalised phage preparations was often considered key for curing the infections. Dr Cruz published detailed protocols for the production of tailored-made staphylococcal phage preparations and their use[29]. Each phage preparation was adapted to the bacterial strain isolated from the patient who was to be treated. During routine diagnostics of septicaemia cases, haemocultures were made. An aliquot of the bacterium was taken from the patient's haemoculture, grown overnight in inclined agar, resuspended in broth the next day and used to inoculate one 200ml and four 10ml flasks containing peptoned-water. A half millilitre of a stock phage was added to three of the 10ml flasks while the fourth 10ml flask was left as control. On the next day, the 10ml flasks inoculated with the phages were added to the 200ml flask containing a liquid culture of the bacterium. After incubation (often 48h), the lysate was filtered in Chamberland filters, aliquoted into 20ml ampoules and sealed for later use on that patient. At the time of use, one ampoule was opened and 15ml of the phage lysate were diluted in 250ml of physiological solution. This mixture was injected slowly (gotta-injector Seabra) into the patient's bloodstream, at forty drops per minute, with the whole volume taking two hours to be injected. Injections were repeated every five days until the patient was cured, with clinical sign evaluation and negative haemocultures used to demonstrate that the treatment was effective. Immune reactions to the phage injections often occurred around

the third dose and were taken as an indication that the treatment was working. Other doctors used similar protocols[43]. The topical use of staphylococcal phage preparations was also made in cases of localised infections, and even oral doses of these phages were used[44]. In the case of oral use, the recommendation was to take the phages diluted in cold water in the morning, with an empty stomach, during a three to four week-long treatment. Furthermore, one publication mentions, without many details, intra-uterine phage therapy and also hypodermical treatments in cases of puerperal infections[32].

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Supplementary figure legends

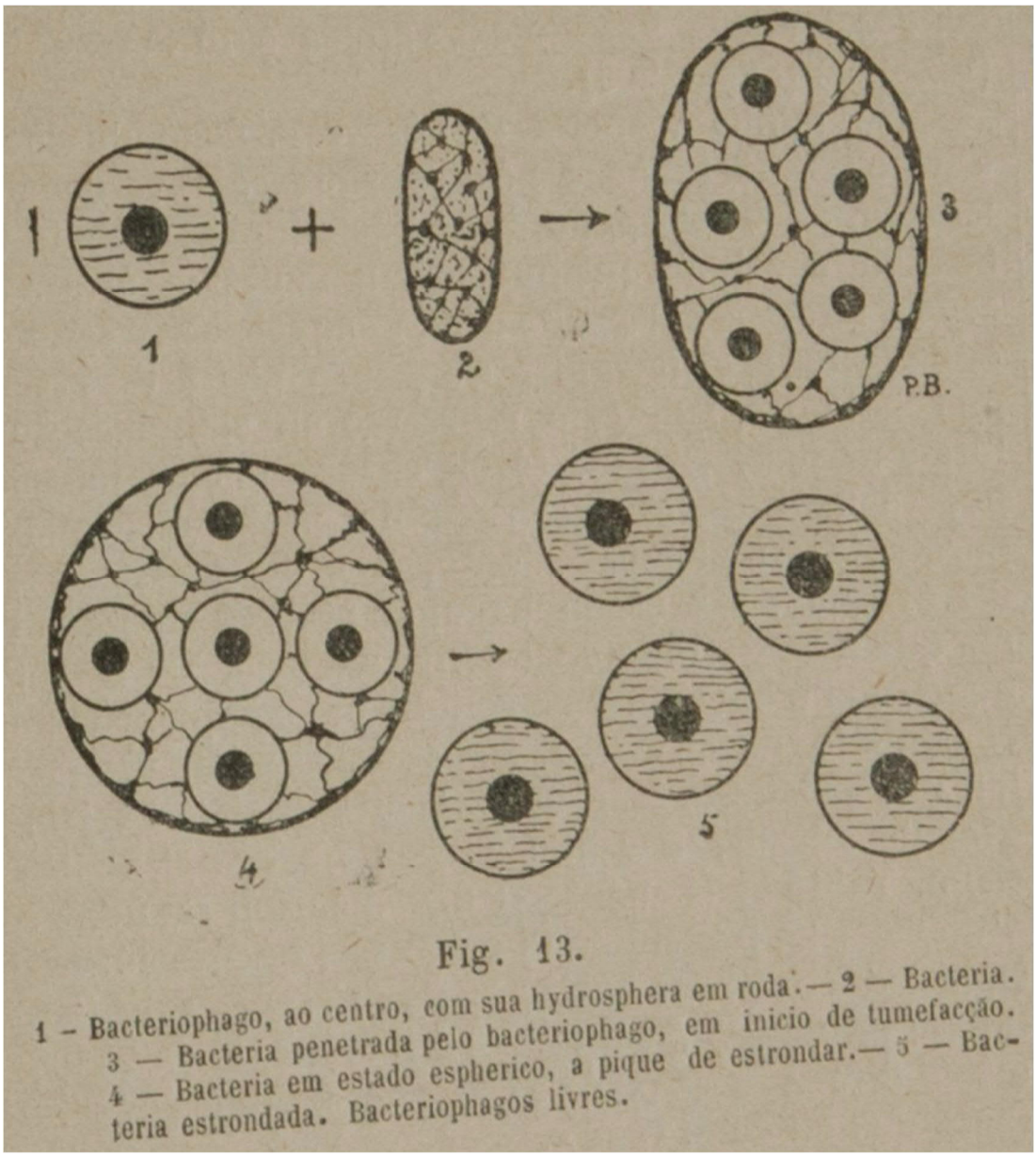

Fig. 13.

1 – Bacteriophago, ao centro, com sua hydrosphera em roda. — 2 — Bacteria. 3 — Bacteria penetrada pelo bacteriophago, em inicio de tumefacção. 4 — Bacteria em estado espherico, a pique de estrondar. — 5 — Bacteria estrondada. Bacteriophagos livres.

Supplementary figure 1: An early attempt to represent phages published in 1929[22]. This image is in public domain and thus can be reproduced here.