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Bacteriophages isolate of stream water in Brazil lyses the superbugs *Klebsiella spp*

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Bacteriófagos aislados de un arroyo en Brasil lisan superbacterias *Klebsiella spp*

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Ingrid Emanoelly Oliveira Camilo
ORCID: https://orcid.org/0009-0004-1093-9481
State University of Minas Gerais, Brazil
E-mail: ingridoliveiracamilo21@gmail.com
Marcely de Oliveira Peixoto
ORCID: https://orcid.org/0009-0003-7670-0759
State University of Minas Gerais, Brazil
E-mail: marcelyoliveira603@gmail.com
Eduarda Geovana Coelho dos Santos
ORCID: https://orcid.org/0009-0003-5965-1350
State University of Minas Gerais, Brazil
E-mail: eduardageovanacoelho@gmail.com
Fernanda Prieto Bruckner
ORCID: https://orcid.org/0000-0003-4927-1779
State University of Minas Gerais, Brazil
E-mail: fernanda.bruckner@uemg.br
Marisa Cristina da Fonseca Casteluber
ORCID: https://orcid.org/0000-0001-8821-8749
State University of Minas Gerais, Brazil
E-mail: marisa.casteluber@uemg.br

Abstract
This study aimed to evaluate bacteriophages isolated from a stream in Brazil that lyse *Klebsiella spp.* superbugs. *Klebsiella pneumoniae* is a Gram-negative bacterium associated with high infection rates worldwide and is currently listed by the World Health Organization (WHO) as a critical priority pathogen for the development of new antimicrobials. The indiscriminate use of antibiotics has accelerated the emergence of multidrug-resistant strains, underscoring the urgent need for alternative therapeutic strategies such as phage therapy. In this study, two bacteriophages (vB_MC_KP1 and vB_MC_KP2) were isolated from stream water in Minas Gerais, Brazil, a site receiving domestic sewage and used for irrigation and other human activities. Both phages specifically infected *Klebsiella spp.* and were characterized by *in vitro* assays assessing host range and physicochemical stability. They demonstrated high specificity, lytic activity against multiple *Klebsiella* species, and remarkable stability under a wide range of temperatures and pH values. These findings indicate that the isolated phages are environmentally persistent and hold potential for both therapeutic applications against multidrug-resistant *K. pneumoniae* and future use in environmental or food safety interventions. Further molecular characterization and *in vivo* studies are warranted to validate their application.
Keywords: Food microbiology; *Klebsiella pneumoniae*; Phage therapy; Phage stability; Water quality.

Resumo
Este estudo teve como objetivo avaliar bacteriófagos isolados de um riacho no Brasil que lisam superbactérias *Klebsiella spp. Klebsiella pneumoniae* é uma bactéria Gram-negativa associada a altas taxas de infecção em todo o mundo e atualmente está listada pela Organização Mundial da Saúde (OMS) como um patógeno de prioridade crítica para o desenvolvimento de novos antimicrobianos. O uso indiscriminado de antibióticos acelerou o surgimento de cepas multirresistentes, ressaltando a necessidade urgente de estratégias terapêuticas alternativas, como a terapia fágica. Neste estudo, dois bacteriófagos (vB_MC_KP1 e vB_MC_KP2) foram isolados de água de riacho em Minas Gerais, Brasil, um local que recebe esgoto doméstico e é usado para irrigação e outras atividades humanas. Ambos os fagos infectaram especificamente *Klebsiella spp*. e foram caracterizados por ensaios *in vitro* que avaliaram a gama de hospedeiros e a estabilidade físico-química. Eles demonstraram alta especificidade, atividade lítica contra múltiplas espécies de *Klebsiella* e notável estabilidade sob uma ampla faixa de temperaturas e valores de pH. Esses resultados indicam que os fagos isolados são ambientalmente persistentes e apresentam potencial tanto para aplicações terapêuticas contra *K. pneumoniae* multirresistente quanto para uso futuro em intervenções ambientais ou de segurança alimentar. Caracterização molecular adicional e estudos *in vivo* são necessários para validar sua aplicação.
Palavras-chave: Microbiologia de alimentos; *Klebsiella* pneumoniae; Terapia fágica; Estabilidade fágica; Qualidade da água.

Resumen
Este estudio tuvo como objetivo evaluar bacteriófagos aislados de un arroyo en Brasil que lisan superbacterias del género *Klebsiella spp. Klebsiella pneumoniae* es una bacteria gramnegativa asociada con altas tasas de infección a nivel mundial y actualmente está catalogada por la Organización Mundial de la Salud (OMS) como un patógeno crítico prioritario para el desarrollo de nuevos antimicrobianos. El uso indiscriminado de antibióticos ha acelerado la aparición de cepas multirresistentes, lo que subraya la urgente necesidad de estrategias terapéuticas alternativas como la fagoterapia. En este estudio, se aislaron dos bacteriófagos (vB_MC_KP1 y vB_MC_KP2) del agua de un arroyo en Minas Gerais, Brasil, un sitio que recibe aguas residuales domésticas y se utiliza para riego y otras actividades humanas. Ambos fagos infectaron específicamente a *Klebsiella spp.* y se caracterizaron mediante ensayos *in vitro* que evaluaron el rango de hospedadores y la estabilidad fisicoquímica. Demostraron alta especificidad, actividad lítica contra múltiples especies de *Klebsiella* y notable estabilidad en un amplio rango de temperaturas y valores de pH. Estos hallazgos indican que los fagos aislados son ambientalmente persistentes y tienen potencial tanto para aplicaciones terapéuticas contra *K. pneumoniae* multirresistente como para su uso futuro en intervenciones ambientales o de seguridad alimentaria. Se justifica una mayor caracterización molecular y estudios *in vivo* para validar su aplicación.
Palabras clave: Microbiología de los alimentos; *Klebsiella pneumoniae;* Terapia fágica; Estabilidad fágica; Calidad del agua.

1. Introduction

In 2019, it was estimated that one in every eight deaths worldwide was caused by bacterial infections, with *Klebsiella pneumoniae* being included among the five pathogens responsible for more than 50% of deaths. Alone, it accounted for 790 000 deaths (Rocha & Andrade, 2022). *K. pneumoniae* is a Gram-negative, non-motile, facultative anaerobic enterobacterium in bacillus form. This microorganism is found in almost all environments such as nature, animals, poorly sanitized food, hospitals, and humans. It is considered an opportunistic microorganism that affects immunocompromised individuals and pediatric patients, causing infections such as pneumonia, meningitis, hepatic abscesses, intestinal and urinary tract infections (Martin & Bachman, 2018).

The indiscriminate use of antimicrobials contributes to the selection of drugs resistance genes among bacteria. The main mechanisms of bacterial resistance include alterations in membrane permeability, efflux proteins that pump out the antibiotics, mutation, production of enzymes that destroy antibiotics, among others. Also, the emergence of new genes through mutation may occur (Costa & Silva Junior, 2017).

In recent years, antibiotic therapy options have become increasingly limited regarding bacteria such as *K. pneumoniae*. Different strains of this bacterium have become multidrug-resistant (MDR) due drugs misuse of antibiotics such as aminoglycosides, quinolones, polymyxins, beta-lactams, and tigecycline, and today it is considered a public health problem (Souza et al., 2019; Herridge et al., 2020). Different antibiotic resistance mechanisms can be found in *K. pneumoniae* strains. The resistance to β-lactam agents, associated with β-lactam ring hydrolysis by β-lactamases, is one of the main, which have a greater impact on infection treatment effectiveness (Herridge et al., (2020).

Also, liposaccharide and polysaccharide capsules production, as well as biofilm synthesis, hinders antibiotics action and immune responses, besides fixing it on surfaces that can become areas of contagion. These characteristics make *K. pneumoniae* more virulent. Due to *K. pneumoniae* increasing antibiotics resistance, new drugs capable of inhibiting these microorganisms are extremely need (Souza et al., 2019).

In addition to new antimicrobials, which have been the subject of extensive studies worldwide to combat multidrug-resistant bacteria, phage therapy also emerges as a possibility in the treatment of bacterial infections. Phages are capable of infecting and lysing specific bacteria, making the treatment more accurate and effective (Loganathan et al., 2021).

Félix d'Herelle described, in 1917, the bacteriophage as an obligatory intracellular organism, whereas in 1915, F.W. Twort observed lysis plaques but did not associate them with a virus (Summers, 2016). They are viruses that infect and parasitize

only bacteria and can present cycles that determine the type of infection in the infected bacterium. Each phage is specific to a particular genus or bacteria species (Reina and Reina, 2018).

Lytic phages can bind to bacterial surface, inject their genetic material into the bacterial cell's cytoplasm, and multiply within the infected cell. When viral particles reach their maximum volume within the bacterium and the cytoplasmic environment is conducive to lytic proteins activation, they rupture the bacterial wall, releasing new phages and triggering the lytic cycle (Loganathan et al., 2021). This type of phage is most recommended for use in phage therapy, since they cause lysis in the host cell at cycle end. Additionally, because they replicate exclusively in a specific bacterial type, the quantity of phages administered in treatment is exceedingly small (Tan et al., 2019).

The first tests with phage therapy in humans were successful in clinical cases such as pediatric dysentery, cholera, and bubonic plague, which generated greater interest in the use of therapy by European countries such as France, Georgia, Russia, and Poland (Caflisch, Suh & Patel, 2019).

Studies have demonstrated the success of using phages *in vitro* and *in vivo* in the treatment of birds and humans with *Salmonella* spp. Infections (Li et al., 2020), and against a wide range of bacteria (Loganathan et al. 2021). Phage therapy offers an advantage over antibiotic therapy since lytic phages are used, there is high specificity of phages for their hosts, their use depends on a single dose due to their high replication capacity, and they do not require large administrations (Chang et al., 2018).

Environmental waters act as important reservoirs for multidrug-resistant bacteria and their bacteriophages. The detection of *Klebsiella*-specific phages in aquatic ecosystems directly or indirectly indicates the presence of their bacterial hosts, highlighting the role of streams receiving sewage or agricultural runoff as potential foci of persistence and dissemination of pathogenic strains. This fact is exemplified in the study by Wee and Yap (2022), who isolated *Klebsiella pneumoniae* from urban drainwater. This isolated harbored genes for resistance to 10 different classes of antimicrobials and genes for resistance to metals.

Untreated water used to irrigate vegetables consumed raw also represents a critical route for bacterial spread in the food chain. Contamination of resistant bacteria in food and packaging has generated growing concern in recent years, highlighting the importance of monitoring phages in aquatic environments related to agricultural and food production practices. ListShield is a product made from phages that infect *Listeria monocytogenes* that aims to control and eliminate contamination of foods such as lettuce, cheese, salmon, and frozen dishes (Perera *et al*. 2015).

The present study isolated two bacteriophages, hereafter referred to as vB_MC_KP1 and vB_MC_KP2, from water samples collected from the Corredor Stream in Mário Campos, Minas Gerais, Brazil. This stream receives sewage from the surrounding population and is used for irrigating vegetables that are consumed raw. The aim of this investigation was to evaluate the isolated bacteriophages. The studied phages were able to lyse a multidrug-resistant strain of *Klebsiella pneumoniae* as well as other genotyped strains. They remained stable and infectious under different physicochemical conditions of pH and temperature. The data obtained suggest the potential application of these phages in phage therapy assays against *K. pneumoniae*.

2. Methodology

An experimental laboratory study was carried out, combining a qualitative methodology for plate identification (Pereira et al., 2018) with a quantitative approach based on statistical analysis (Vieira, 2021).

2.1 *K. pneumoniae* cultivation

The target bacterium used in this study was *Klebsiella pneumoniae* (ATCC 700603), which produces beta-lactamase SHV-18, being used as a control for extended-spectrum beta-lactamase production (Rasheed et al., 2000). A simple streak of this

bacterium was performed on nutrient agar (according to the manufacturer's recommendation). The plate was then incubated at 37°C for 18 hours. After this period, a colony from the plate was transferred to a flask containing 15mL of nutrient broth, which was then incubated for 4 hours at 37°C. Subsequently, the cell concentration of the broth was adjusted using spectrophotometry to an optical density of 0.4 at 600 nm, and this concentration was used in all subsequent tests. All experiments in this study were done in triplicate.

2.2 Isolation of bacteriophages from environmental samples

Water samples from Corredor Stream in Mário Campos, Brazil, were collected in sterile screw-capped bottles. The water was centrifuged for 30 minutes, and the supernatant filtered through a polyethersulfone membrane with a pore size of 0.22µm. In an Erlenmeyer flask, 10mL of Luria Bertani medium (Bertani, 1951), 30mL of the filtered sample, and 1mL of fresh bacterial culture were added.

After 18 hours of incubation, the culture was centrifuged for 15 minutes at 3500rpm. The supernatant underwent further filtration through a 22µm pore membrane, followed by serial dilution and plating for observation of lysis plaques using the overlay method. To phage isolation, four successive passages were performed on bacterial cells. To each passage, an isolated lysis plaque was removed using a pipette tip and transferred to a microtube containing 180µL of SM buffer (5.8 g NaCl, 2.0 g $MgSO_4 \cdot 7H2O$, 50 ml 1 M Tris-HCl pH 7.4, 0,01% gelatin (v/w) in 1 liter of dH_2O).

From this first tube, serial dilutions were made by transferring 20µL from this tube to the next until reaching a dilution of 10^{-9}. Various lysis plaques formed were then isolated and inoculated onto new Petri plates containing *K. pneumoniae* cultures.

2.3 Host Range Assay

For the host range determination, six bacteria were used: *Escherichia coli* (ATCC 25922), *Enterobacter hormaechei* (ATCC 700323), *Pseudomonas aeruginosa* (ATCC 15442), *Klebsiella pneumoniae* (ATCC 700603), *Klebsiella oxytoca* (ATCC 13182), and a clinical sample of *Klebsiella pneumoniae* isolated from a lung infection in patient of Clinical Hospital of Belo Horizonte, Brazil, generously donated by Laboratory of Basic Virology of Federal University of Minas Gerais, Brazil. All these bacteria underwent the same procedure as *K. pneumoniae* (ATCC 700603) to achieve an optical density of 0.4, as described previously. After cultivation, each bacterium was plated in triplicate using the overlay method.

2.4 Testing viral stability at different pH levels and temperatures

The bacteriophages suspension, with a viral title of 10^{17} UFP/ml were exposed to different temperatures: 25°C, 37°C, and 60°C. The phages were serially diluted in SM buffer and exposed to each of the specified temperatures for 10 minutes. Then, the dilutions were plated using the overlay method. For the pH test, SM buffers were prepared at different pHs: 5.5, 6.5, 7.0, and 8.0. From each buffer, serial dilutions of each bacteriophage were made. The last 5 dilutions were plated using the overlay method on Petri dishes containing nutrient agar.

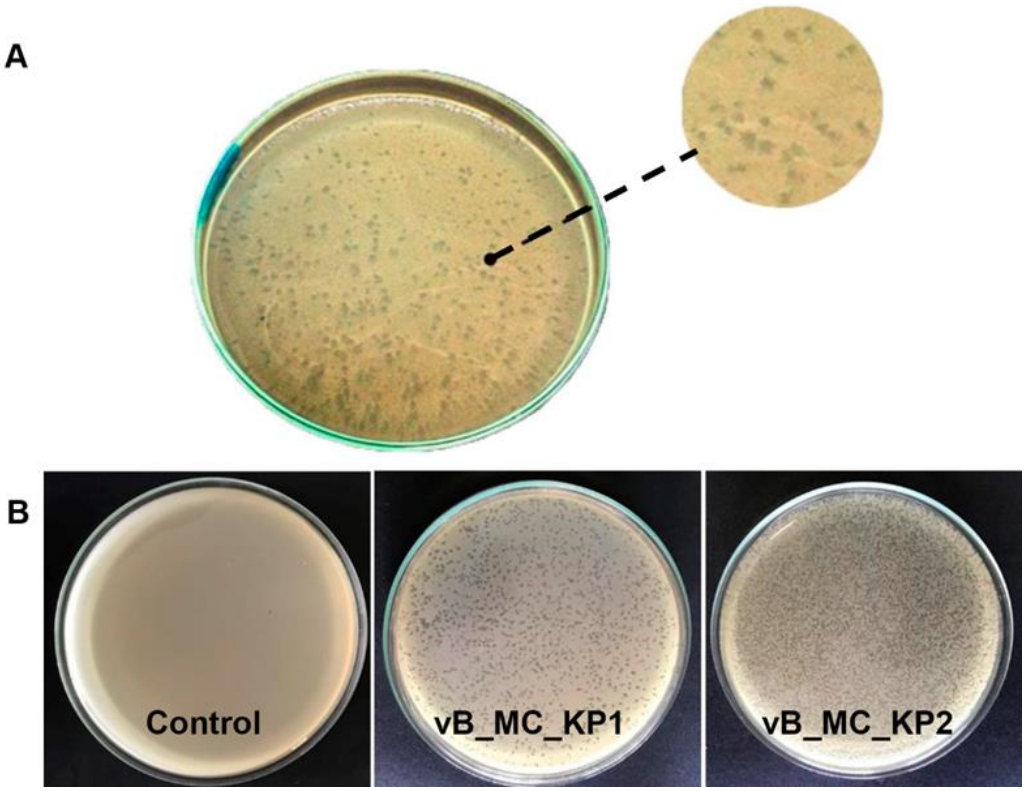
After inoculation, the cultures were incubated at 37°C for 24 hours. In both tests, counting was performed on plates with a minimum of 30 and a maximum of 300 PFU. The results obtained from temperature and pH tests were calculated and expressed in plaque-forming units per milliliter (PFU/mL). Statistical tests were conducted to compare the data of each bacteriophage, confirm the normal distribution, and verify the confidence intervals using the GraphPad Prism.

3. Results

3.1 Isolation of bacteriophages and host range test

In the process of phage isolation, two distinct patterns of lysis plaques were observed (Figure 1). One phage showed a small lysis plaque, while the other a large lysis plaque. These two patterns indicated the presence of more than one bacteriophage in the collected water sample. The isolated phages were named vB_MC_KP1, and vB_MC_KP2.

Figure 1. Lyses plaques of B_MC_KP1 and vB_MC_KP2 in *K. pneumoniae* (ATCC 7000603). A- Lyses plaques observed prior to phages isolation. B- Separation of phages according to the morphology of lyses plaques formed.



Source: Authors' private collection (June, 2024).

Both phages were capable of infecting and lysing *K. pneumoniae* (ATCC 700603), the hospital strain of *K. pneumoniae*, and *K.oxytoca* (ATCC 13182). Plates containing *E. coli, P. aeruginosa,* and *E. hormaechei* showed no lysis despite presence of bacterial growth (Table 1).

Table 1. Host range of phages isolated from water samples of Corredor Stream.

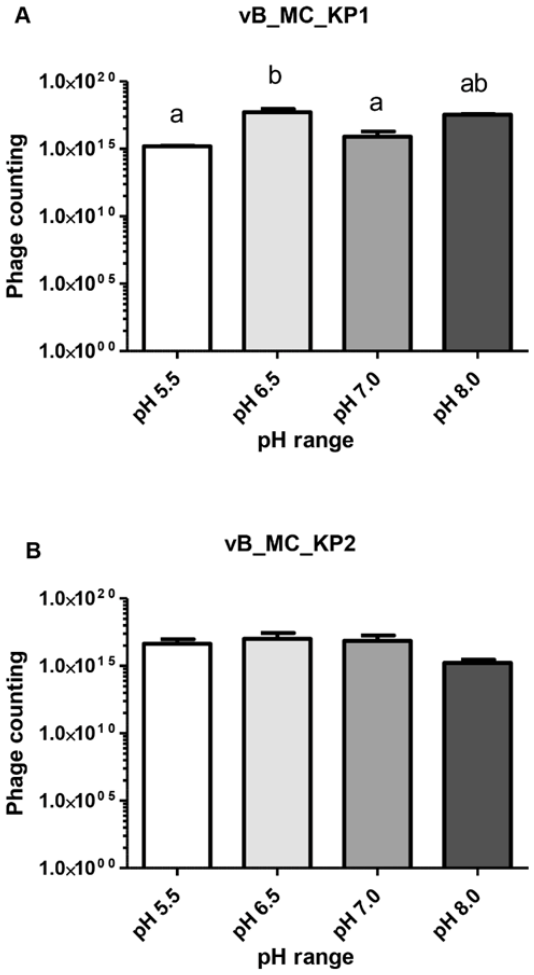
Bacterial strains tested	VB_MC_KP1	VB_MC_KP2
*K. pneumoniae K6 (*ATCC 700603)	+	+
Clinical *K. pneumoniae*	+	+
E. coli (ATCC 25922)	-	-
*P. aeruginosa (*ATCC 15442)	-	-
*E. hormaechei (*ATCC 700323)	-	-
*K. oxytoca (*ATCC 13182)	+	+

Note: The intensity of lysis is represented as follows: "+" for clear lysis; "-" for no lysis. Source: Authorship (2024).

3.2 Bacteriophages stability

The stability tests were performed using *K. pneumoniae (*ATCC 70006) because it was the bait strain for phage isolation. Lysis by vB-MC_KP1 was observed at all analyzed temperatures without statistical difference (Figure 2). In vB_MC_KP2, lysis was observed at 25°C and 37°C without statistical difference; no lysis halos were observed at 60°C ($p<0,001$). These results indicate that vB_MC_KP2 is more sensible to high temperatures than vB_MC_KP1, as observed at 60°C. However, both isolates were stable and viable in room and body temperature. This finding is important for proposing future applications using these viruses.

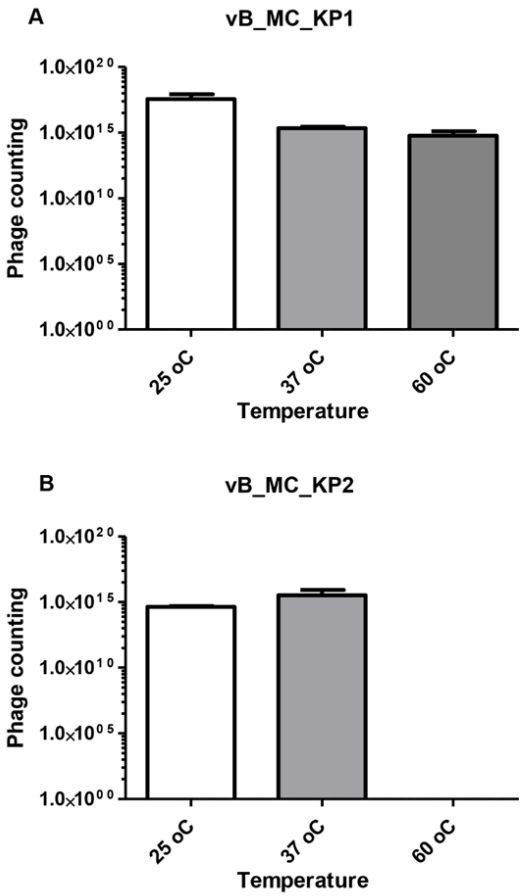
Figure 2. Bacteriophage viability at different pH levels. A) Plate counting of vB_MC_KP1. B) Plate counting of vB_MC_KP2. The Y-axis indicates phage counting on a logarithmic scale. Error bars indicate standard deviation. The letters above the bars indicate statistical correspondence by ANOVA and Tukey test, with a p-value < 0,05.



Source: Authorship (2024).

Both vB_MC_KP1 and vB_MC_KP2 remained stable and viable across all tested pH values (Figure 3). Phage vB_MC_KP1 showed its optimal pH at 6.5, exhibiting a statistically significant difference compared to the other evaluated pH levels (Figure 3A). For phage vB_MC_KP2 no statistical difference between the analyzed pH was observed (Figure 3B). This finding about infection of two superbugs and the ability to induce lysis in different temperatures and pHs makes vB_MC_KP1 and vB_MC_KP2 promising candidates for molecular characterization and further exploration of biotechnological potential.

Figure 3. Viability of bacteriophages at different temperatures in *K. pneumoniae* (ATCC 700603). A) Plate counting of vB_MC_KP1. B) Plate counting of vB_MC_KP2. The Y-axis indicates phage counting on a logarithmic scale. Error bars indicate standard deviation. The statistical analysis was performed by ANOVA with a p-value < 0,05.



Source: Authorship (2024).

4. Discussion

Phages that lyse *K. pneumoniae* have been employed as a treatment with excellent results when used against multidrug-resistant strains. In 2018, phage therapy was applied as a treatment method in a victim of a bombing incident, in which wounds became infected by *K. pneumoniae* despite the use of antibiotics. As the infection persisted in the victim even after two years, bacteriophages combined with antibiotics were administered intravenously, and within weeks, the wounds, which extended to the femur, healed (Eskenazi et al., 2022).

In this study, the specificity and stability of two phages, vB_MC_KP1 and vB_MC_KP2, recently isolated from water samples from Corredor Stream in Mário Campos, Brazil, were analyzed. The water from this stream is used for irrigation of vegetables consumed raw, for animal husbandry, among other uses by the local population. The presence of these phages indicates the presence of *Klebsiella* in the water. In this case, the findings may demonstrate the risk of using untreated water, which can serve as a vehicle for dissemination of infection by multi-resistant bacteria.

Rodrigues et al. (2025) evaluated the microbiological and physicochemical parameters of stream water and reported the detection of bacteriophages specific to *Escherichia coli*. The occurrence of these viruses can be attributed to fecal contamination of the water, combined with surface runoff resulting from rainfall, which promotes the transport of organic matter derived from

animals inhabiting the surrounding area, thus serving as a potential source for the dissemination of both the phages and their bacterial hosts.

Liu et al. (2024) also reported the isolation of a novel bacteriophage specific to *Klebsiella quasipneumoniae* from karst waters impacted by industrial and agricultural activities. In agreement with their findings, the detection of environmental phages serves as indirect evidence that the bacterial host is, or has recently been, present in the aquatic ecosystem. This observation underscores the potential risk of pathogen dissemination through multiple routes, including the consumption of raw vegetables irrigated with contaminated water and the subsequent contamination of the local food chain.

Moreover, environmental phages are not only indicators of the presence of their bacterial hosts, but may also participate in horizontal gene transfer, contributing to the environmental dissemination of antimicrobial resistance determinants.

vB_MC_KP1 presents clear rounded lysis, while vB_MC_KP2 presents clear and slightly elongated lysis, indicating a lytic cycle, which means that the bacteriophages studied here are classified as virulent. Jurczak-Kurek et al. (2016) analyzed the lysis morphologies of 83 distinct phages and found that those with clear morphology would be lytic phages and those with halos around them would also be lytic.

The host range results showed lysis in *K. pneumoniae* (ATCC 700603), in the clinical sample of *K. pneumoniae*, and in *K. oxytoca (*ATCC 13182). The other enterobacteria studied was not infected by any of the phages, confirming the specificity of vB_MC_KP1 and vB_MC_KP2, which probably only bind to host receptors presented by bacteria of the *Klebsiella* genus. It is interesting to note that these phages can lyse different *Klebsiella* species, which suggests it may have a broad spectrum of action against several members of this genus specifically (Rohnelt, 2020).

Unlike antibiotic therapy, phage therapy does not alter the individual's intestinal microbiota (Cully, 2019), thus avoiding dysbiosis and future secondary infections or autoimmune diseases [Cully, 2019; Drulis-Kawa, et al*.,* 2012). The results obtained in the present research were promising, as it suggests the possibility to phage therapies, they would remain stable and maintain their lytic activity in thermal variations; this also allows for various methods and forms of storage and transport of bacteriophages, as well as enabling an approach with phages for environmental and industrial control.

Bacteriophages belonging to the former families Siphoviridae, Myoviridae, and Podoviridae were analyzed in culture with different strains of *K. pneumoniae (*Zurabov and Zhilenkov, 2021). These phages were resistant, like vB_MC_KP1, to temperatures of 25°C and 60°C, but when subjected to temperatures above 65°C, they were found to be unable to infect the analyzed bacterial cells like observed to vB_MC_KP2.

Other bacteriophage infecting *Pseudomonas cichorii* remained stable at temperatures between 5°C and 45°C, and after 60°C, the titer decreased drastically (Alves, 2021); these results resemble the pattern observed in vB_MC_KP2. Too, different bacteriophages (Φ i*Lp*84 and Φ i*Lp*1308) infecting *Lactobacillus paracasei* showed a better adsorption rate at 37°C with minimal efficiency of adsorption at 0°C and an increasing with the temperature up to 37°C. However, temperatures higher than 45°C had little effect on the adsorption of Φ i*Lp*84, but it greatly affected that of Φ i*Lp*1308. These phages exhibited a lower adsorption rate when subjected to temperatures above 60°C (Mercanti, Ackermann & Quiberoni, 2015). Since these phages are derived from complex samples, such as stream water, where the temperature can vary from 15°C to 26°C at certain times of the year, it is expected that they would show better viability at temperatures close to ambient. The same pattern was seen in phages isolated from sewage effluents in Pakistan, when tested at temperatures up to 50°C, and they withstand higher temperatures as in this work up to 60°C (Ullah et al., 2022).

Rodrigues et al. (2025) observed that the pH of the stream water ranged from 6.2 to 8.9 between February and July. This condition may account for the stability of the bacteriophages studied here across a broad pH spectrum and suggests their potential persistence in the environment throughout this period.

The fact that phages maintain their ability to form lysis plaques at different pHs suggests that, in cases of alkalosis, blood acidosis, or pH changes in the solution they are inserted into, they will remain viable and capable of lysing *K. pneumoniae.* Ni *et al*. (2021) stated that stability of phages can be observed in both basic and acidic pH, indicating that different phages exhibit different resistances to alkalinity or acidity (Tey et al., 2009). Results of bacteriophage tested in mice infected with *K. pneumoniae* (Anand et al., 2020) showed better activity when subjected to basic pH. Phages capable of lysing *K.* pneumoniae were stable at pHs ranging from 5.0 to 11.0 (Obradović et al., 2023). vB_MC_KP1 and vB_MC_KP2 follow the patterns found in other studies with different phages. Meanwhile, a diversity of phages tested for *K. pneumoniae* infection performs better activity when cultivated in pH 5 to 8; however, when incubated at pH 4, the viral titer decreased significantly (Kęsik-Szeloch et al., 2013). This reduction in activity may be observed in the performance of phage vB_MC_KP1 evaluated in this study,as at pH 6.5, it showed better performance with reduced efficacy at pH 5.5. Although the growth of the phage at pH 4.0 was not evaluated, the data suggest that the growth at this pH value could be even lower.

Herridge *et al*. (2020) also highlighted the use of phages as an alternative therapy for patients infected with *K. pneumoniae*, *P. aeruginosa*, *Staphylococcus aureus* and *E. coli*. They demonstrated significant effect with oral administration of phages in humans, whose average body temperature ranges from 31.6°C to 37.2°C. The data found in this study may suggest that phages vB_MC_KP1 and vB_MC_KP2, which showed better survival rates at room temperature, could be used in future *in vivo* tests.

Other studies showed a significant reduction in *K. pneumoniae* MTCC109 (Anand et al., 2020) pulmonary burden in mice after a single intranasal administration of phages. According to Tan et al. (2019), hypervirulent lytic bacteriophages should be selected for *K. pneumoniae* treatment, as this bacterium presents resistance mechanisms such as biofilm formation.

In addition to phage therapy, interests from the food industry are observed when discussing bacteriophages. Contaminated foods are responsible for causing illnesses in 600 million people annually (Al Sharif, 2021). In artisanal foods ready for consumption, 75 isolates of *Klebsiella* were found, with 52.69% being *K. oxytoca* and 23.31% *K. pneumoniae* (Crippa et al., 2023). In this context, vB_MC_KP1 and vB_MC_KP2 could be applied as a means of food control and quality assurance. Studies evaluated bacteriophage P100 applied in the food industry for the biocontrol of *Listeria monocytogenes* (Rossi and Almeida, 2010), concluded that phages could be used as biocontrol agents, thus preventing ingestion of contaminated foods and related health problems.

5. Conclusion

The viruses vB_MC_KP1 and vB_MC_KP2 represent promising candidates for the control of *Klebsiella* infections, exhibiting strong *in vitro* lytic activity against both *K. pneumoniae* and *K. oxytoca* strains. Beyond their medical potential, these phages could play a significant role in environmental and food safety contexts, such as reducing bacterial contamination in water sources, soil, and food products. Their capacity to specifically target and eliminate pathogenic bacteria highlights their potential for biotechnological applications aimed at minimizing the spread of *Klebsiella* in both ecological and alimentary settings. Further studies are warranted to fully explore their efficacy, stability, and practical implementation in diverse environmental and food-related scenarios.

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