



Phage Therapy: A Golden Alternative to Antibiotics in the Era of Multidrug Resistance

A. Hassan ^{a*}, O. O. Mabekoje ^a and H. Majiya ^a

^a Department of Microbiology, Faculty of Natural Sciences, Ibrahim Badamasi Babangida University Lapai, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Phage treatment is a technique that has been used for over a century to treat bacterial illnesses by using bacterial viruses (phages). Antibiotics' overall reduction in efficacy has reignited interest in reexamining this procedure. Phage therapy may be utilized as an alternative to or a complement to antibiotic therapies, according to recent studies on the use of phages and their lytic proteins, specifically against multidrug-resistant bacterial infections.

Keywords: Phage therapy; multidrug resistance; bacteriophage; bacterial infections.

1. INTRODUCTION

“Bacteriophages are bacterium-infecting viruses that kill bacteria without harming human or animal cells. As a result, it is thought that they can be used to treat bacterial infections alone or in combination with antibiotics. Phages are non-living biological entities that are made up of DNA or RNA contained in a protein capsid, phages are

naturally occurring bacterial parasites that are incapable of replicating on their own and must rely on a bacterial host to survive” (Weinbauer, 2004).

“Phages bind to specific receptors on the surface of bacteria, inject their genetic material into the host cell, and then either integrate this material into the bacterial genome and reproduce

*Corresponding author: E-mail: aminhass24@gmail.com;

vertically from mother to daughter cell, or hijack the bacterial replication machinery to produce the next generation of phage progeny and lyse the cell (lytic phage)” (Domingo-Calap and Delgado-Martínez, 2018).

“The lytic proteins become active and hydrolyze the peptidoglycan cell wall, releasing new phage to restart the lytic cycle after a critical mass of phage offspring has been reached, which can range from a few to over 1000 virus particles depending on environmental variables” (Domingo-Calap and Delgado-Martínez, 2018).

Phage therapy, also known as bacteriophage therapy, is the treatment of bacterial illnesses with viruses. According to previous research, phages exclusively fight bacteria and are harmless to humans, animals, and plants. Phage therapy has been used to treat typhoid fever, diarrhea, skin and surgical wound infections, peritonitis, septicemia, urinary tract infections, and external otitis, according to certain research. Phages have been shown to exhibit lytic efficacy against a variety of pathogenic organisms, including *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Acinetobacter baumannii*, *Salmonella* species, *Staphylococcus aureus*, *Enterococcus* spp and *Serratia* spp [1].

Bacteriophages are the most common biological organism on the planet. They can be found in soil and seawater, as well as on oceanic and terrestrial surfaces and in severe habitats with extremely high or low temperatures. They have also been found in hospitals, sewers, and other places where bacteria can dwell, such as animal and human tissues. All participants of phage therapy have been treated empirically to date, with limited information on the phages, appropriate route of administration, dose, duration, and antibiotic compatibility, among other things [2].

“The World Health Organization produced a list of global priority pathogens in 2017 that included 12 bacteria species that were classified as critical, high, or medium priority based on their level of resistance and therapy options” [3]. “Resistance development is outpacing antibiotic discovery and development at an alarming rate, posing a global public health threat. Antimicrobial resistance could kill up to 10 million people per year by 2050, according to estimates” [4]. “While this is a debatable figure [5], it does show the major dilemma we have when it comes to

therapeutic alternatives for multidrug-resistant bacterial infections” [6].

“Bacteriophages, also known as phages, are bacteria's natural predators that are thought to number in the trillions per grain of sand on the planet” [7]. Phages, which evolved in tandem with bacteria, are prospective antibacterial treatment agents against MDR infections.

Inside the host bacteria, phages go through a lytic or lysogenic developmental cycle. Between the attachment of a phage particle to a bacterial cell and the subsequent release of new daughter phage particles, the lytic cycle occurs. It has five stages: phage adsorption to the host cell, phage nucleic acid penetration, transcription and translation, assembly, and departure.

“The lysogenic cycle, on the other hand, involves the replication of phage nucleic acid along with the host genes for many generations without causing the cell to undergo significant metabolic changes, it is referred to as a latent phase of infection since it happens infrequently. In this stage, phage genes may occasionally return to the lytic cycle, resulting in the release of phage particles. Lysogeny is the term for this process, and phages that can evolve both lytically and lysogenically are known as temperate phages” [8].

2. A GOLDEN ALTERNATIVE TO MULTIDRUG RESISTANCE

Antibiotic resistance is, without a doubt, the most serious danger to global health today. Infections are becoming increasingly difficult or impossible to treat, resulting in significant morbidity, mortality, and financial costs. The therapeutic use of bacteriophages, which are viruses that infect and kill bacteria, is well suited to be part of multifaceted antibiotic resistance tactics (WHO, 2021).

Despite the fact that phage therapy was first used than a century ago, it was put on hold after the effective advent of antibiotics, with the rise of antibiotic resistance, phage therapy is getting a much-deserved second wind. The goal of this review is to present a forward-looking view on phage therapy and its role in current society [9,10].

In recent years, bacteriophages have piqued interest as a potential alternative antimicrobial therapy for infectious disorders. Because phage

therapy is the most promising multidrug resistant treatment of this period, its therapeutic effectiveness is a source of concern [1].

Phages have been identified as one of the possible alternatives to antibiotics in animal therapy, prophylaxis, and bacterial load reduction in food products of animal origin in the studies evaluated. Food-borne pathogens such as *E. coli* and *Salmonella* [11], as well as *Campylobacter*, have been successfully eradicated in trials using bacteriophages and farm animals [12].

The global antibiotic resistance dilemma, along with a renewed understanding of the role of the human microbiome, has reawakened interest in phage therapy. The emergence of multidrug-resistant diseases has increased the potential for generating non-conventional medication alternatives.

Bacteriophage treatment is an example of a technique that can be used instead of antibiotics.

Many researchers have noted the importance of live phages in combating fatal infectious and life-threatening infections caused by Gram positive and Gram negative bacteria.

Bacteriophage therapy, which is employed topically, orally, or systemically in research evaluated, is an important alternative to antibiotics in the current era of multidrug resistant bacterial illnesses.

These studies reported effectiveness rates of 80-95%, with minor gastrointestinal or allergy adverse effects. Phage efficacy against *Escherichia coli*, *Acinetobacter* spp., *Pseudomonas* spp., and *Staphylococcus aureus* was also confirmed in British investigations [13].

The concurrent use of antibiotics, according to the studies examined, is a critical issue that may decide the efficacy of phage therapy. There is mounting evidence that such a phage-antibiotic combination has synergistic antibacterial properties. As a result, phages have been employed as a stand-alone therapy in all of the clinical trials that have been completed thus far.

Antibiotics, on the other hand, have been used successfully alongside phages in successful compassionate use approaches. At this moment, it is impossible to rule out the possibility that such combinations could be the most effective treatment for challenging bacterial infections,

therefore a clinical study on phage therapy could include a subgroup of patients who were given both phages and antibiotics. The findings of such a study could provide a definitive response to the question of whether antibiotics combined with phages are preferable to phage therapy alone.

However, this technique of using phages and antibiotics in combination may not be appropriate for all phages and antibiotics: current research suggests that aminoglycoside antibiotics impede Mycobacteriophage DNA replication, interfering with phage pathogen elimination [13].

D'Herelle was the first to use phages in people for medical purposes. He gave phages to four patients at Paris's Hospital des Enfants-Malades in 1919. They had dysentery, an intestinal ailment caused by *Shigella* bacteria that results in bloody diarrhoea, stomach cramps, and a fever. D'Herelle had already proven that phages were safe by giving them to himself, his coworkers, and his family. Each patient was given an oral dose of anti-shigella phages, and within a day, they exhibited symptoms of recovery.

"This was simply the beginning of a series of tests carried out by d'Herelle (d'Herelle). While d'Herelle took his time to publish his findings, Richard Bruynoghe, a physician at Katholieke University Leuven in Belgium, and Joseph Maisin, a student, were inspired enough by his work to try out the use of a phage to treat staphylococcal skin illness in patients. They found clear evidence of recovery within 48 hours after injecting an anti-staphylococcal phage into the patients' infected areas, according to a report published in 1921" [14].

In London, a few European clinical trials have revealed phage therapy against *P. aeruginosa* in chronic otitis; the largest so far, "Phagoburn," a multi-center trial involving 11 clinical partners in Belgium, France, and Switzerland, focused on burn patients with topical use of phage cocktails against *E. coli* and *P. aeruginosa*. There are also phages being produced for a multi-center study aimed at diabetic foot ulcers [15].

In Germany, "Phage4Cure" was initiated in September 2017 with full government funding to show the safety of highly purified phage preparations and, ultimately, to reduce *P. aeruginosa* in chronically infected cystic fibrosis or non-cystic fibrosis bronchiectasis patients through phage inhalation.

AmpliPhi Biosciences Corporation has started a new clinical trial focusing on *P. aeruginosa* in cystic fibrosis patients; preliminary results have been released. Topical phage therapy is very important for the treatment of diabetic ulcers; there are significant efforts underway in the United States [16].

3. MODE OF ACTION OF PHAGE THERAPY

Phages possess a developmental cycle inside the host bacteria which can be lytic or lysogenic. The lytic cycle covers a series of events that occur between attachment of phage particle to a bacterial cell and its subsequent release of new daughter phage particles. It consists of five

stages, namely; adsorption of phage to host cell, penetration of phage nucleic acid, transcription and translation, assembly and exit.

The lysogenic cycle on the other hand involves replication of phage nucleic acid together with the host genes for numerous generations without major metabolic consequences for the cell. This is known as a latent mode of infection which occurs at a very low frequency. The phage genes in this state may occasionally return to lytic cycle, leading to release of phages particles. This phenomenon is known as lysogeny and phages that can develop both lytically and lysogenically are regarded as temperate phages (Benett, 1993).

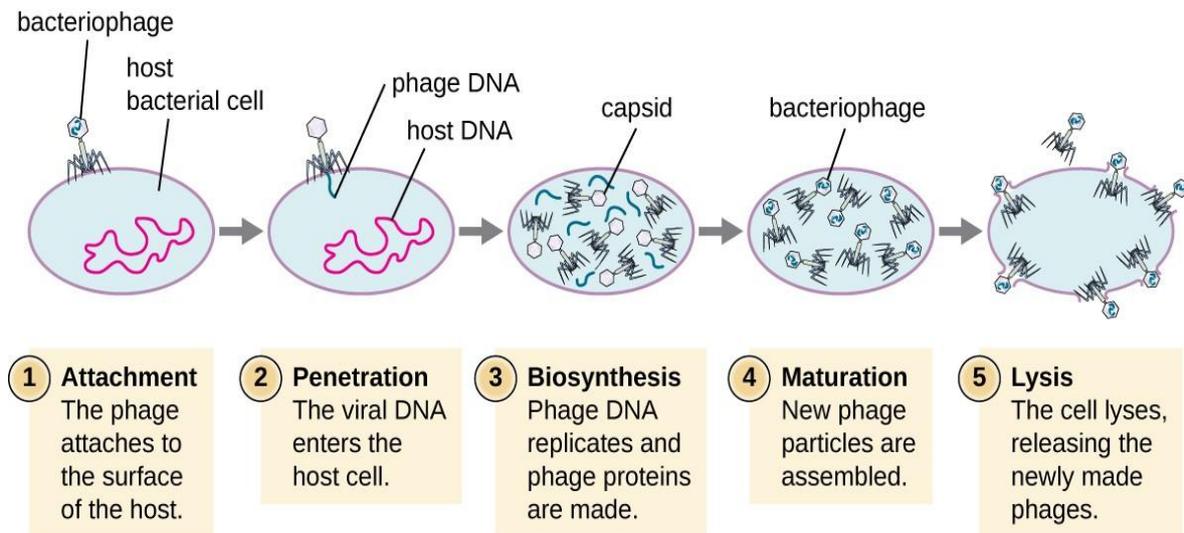


Fig. 1. Diagram showing Lytic phage replication

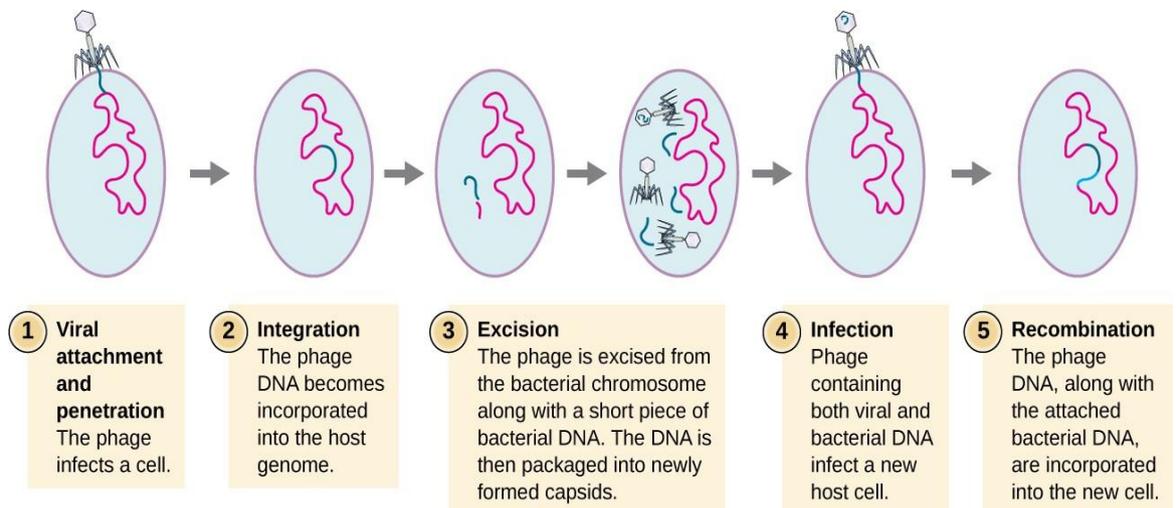


Fig. 2. Diagram showing Lysogenic phage replication

4. SOME OF THE REVIEWED DOSAGE TRIAL

4.1 Oral Phage Trial

“Oral phage treatment for diarrheal *E. coli* in children did not improve acute diarrhea symptoms any more than oral rehydration therapy in a randomized controlled trial, 3 times daily for 4 days, children were given 1.4×10^9 PFU per dose of a Russian commercial 11-phage mix or 3.6×10^8 PFU per dose of a 10-phage cocktail. Oral application did not cause any side effects. *E. coli* was found in less than half of the recipients' stools, accounting for only 5% of total fecal bacteria. The Eliava Institute, on the other hand, discovered that phage treatments, both oral and intrarectal, might cure an MDR *K. pneumoniae* gut infection” [17].

4.2 Intraoperative Phage Trial

Implant-associated MDR and recalcitrant infections have been treated with phages applied intraoperatively. Left ventricular assist devices (LVADs) are becoming a more common life-support technology, although phage treatment of LVAD infections has had inconsistent results.

“After 1-phage local doses of 2×10^{10} PFU delivered through drainage every 12 hours for 7 days, a 62-year-old with fulminant pleural empyema induced by *S. aureus* infection after LVAD installation was healed. After two weeks of daily local treatments with a 4-phage mix at 1.0×10^{10} PFU administered via drainage, a 51-year-old could not be cured of chronic *S. aureus* LVAD infection. The reason for the treatment failure was unknown, but after two weeks there were no symptoms of phage resistance or phage-neutralizing antibodies” [18].

Phages have also been used to treat infections in prosthetic joints (PJIs). After debridement and a single local dose of a 6-phage mix at 6×10^{10} PFU in 20 mL of phosphate-buffered saline, an 80-year-old man with type 2 diabetes was healed of a methicillin-sensitive *S. aureus* prosthetic hip infection. Daptomycin, amoxicillin, and clindamycin were used to suppress *E. faecalis* and *Staphylococcus lugdunensis* (both of which were isolated from the joint), as well as oral amoxicillin/clindamycin for 6 months. For 18 months, the patient's PJI was infection-free [19].

Similarly, “an 80-year-old woman with a *P. aeruginosa* PJI was cleared after receiving a 100-mL intraoperative therapy of a 3-phage cocktail at 1×10^8 PFU, followed by 5×10^8 PFU per dosage every 8 hours by drainage for 5 days. In another case, 4 local administrations of a 4-phage mix of approximately 2.0×10^{10} PFU, colistin, and a compress soaked in approximately 4.0×10^{10} PFU, combined with off-label intravenous ceftolozane/tazobactam cleared a chronic MDR *P. aeruginosa* PJI after removal of sacroiliac joint cement and tissue debridement cleared a chronic MDR *P. aeruginosa* PJI, after 2 weeks of phage intervention, surgical repair was possible” [19].

There were no serious side effects in a patient with severe *P. aeruginosa* osteomyelitis who had intraoperative phage therapy, and the wound infection cleared up without relapse. After thorough debridement and irrigation, the bone/soft tissue defects were washed with 10–40 mL of a 10^7 PFU/mL mix of *S. aureus* and *P. aeruginosa* phages. Before closure, a gentamicin and phage-soaked collagen sponge was placed to the infected bone after a 10-minute contact time. Phage was given 3 times per day for 7–10 days after surgery, with 3 months of antibiotics given concurrently [20].

4.3 Intravenous Phage Trial

In a 72-year-old patient with obesity and a chronic methicillin-resistant *S. aureus* infection, phage therapy under eIND saved a joint prosthesis. Two intra-articular doses of a single phage at 5.4×10^9 PFU in 10 mL of saline were delivered during debridement surgery and insertion of a static vancomycin spacer. The daily infusions of 2.7×10^9 PFU in 50 mL of intravenous saline were maintained. After 3 days, the patient developed transaminitis, prompting the phage treatment to be stopped. With continued treatment of daptomycin alone, aminotransferase and alanine aminotransferase levels reverted to normal.

Despite this, a combination of procedures eradicated the chronic joint infection, allowing for the installation of a distal femoral cemented prosthesis. After multiple failed surgical treatments and protracted antibiotic therapy, a 62-year-old man with a potentially life-threatening chronic *K. pneumoniae* prosthetic knee infection was treated with intravenous phage therapy [21].

4.4 Topical Phage Trial

After daily topical and oral doses of an unknown combination of *Streptococcus*, *Staphylococcus*, *Proteus*, *Escherichia*, *Pseudomonas*, and *Enterococcus* phages, a 16-year-old with Netherton syndrome saw a considerable reduction in atopic eczema. A 20-minute application of sterile gauze soaked in phage mix was followed by phages mixed into ointment cream as part of the daily therapy routine.

In addition, “the patient took 20 mL of the same phage mix. After 2 weeks of continuous daily therapy with 2-week intervals, a second 2-phage cocktail was introduced to address the emergence of phage-resistant *S. aureus* isolates, and after 3 months, a third 2-phage cocktail was introduced to address the emergence of phage-resistant *S. aureus* isolates. Reduced skin irritation and increased joint mobility improved the patient's quality of life” [22].

4.5 Inhalation Phage Trial

“Under the Declaration of Helsinki, a 40-year-old woman with a *K. pneumoniae* lung infection during drug-induced immunosuppression after heart transplantation was successfully cleared after inhalation of a 2-phage cocktail at 2 10⁸ PFU per dose and daily nasogastric ingestion of 1.8 10⁹ PFU per dose for four days. *K. pneumoniae* was undetectable in bronchial lavages after phage therapy, although it remained in the patient's feces” [23].

4.6 Intrarectal Phage Trial

With twice-daily intrarectal phage therapy, three cases of *E. faecalis* chronic prostatitis were successfully cured (phages at 10⁸ to 10⁹ PFU per dose given for approximately 1 month). The prostates of all three individuals were restored to normal and physiologic function at the conclusion of treatment. Similarly, in the 15 months following a kidney transplant, a 60-year-old patient experienced 12 severe episodes of UTI caused by *K. pneumoniae*. After the patient's tenth UTI, phage therapy was started and continued for two more hospitalizations. Despite hospitalization, intrarectal phage injection had no negative consequences on the patient, and the patient did not require hospitalization for UTIs for the next four years [23]. In mice, a mixture of six phages

was found to effectively treat respiratory *P. aeruginosa* infection as well as sepsis in *Galleria mellonella* models [24].

“Another notable advantage of phages over traditional treatments is their ability to penetrate *P. aeruginosa* biofilms” [15], (Waters et al., 2017), “while co-administration of phages and antibiotics has been reported as a mechanism for restoring antibiotic sensitivity” [25]. When phages use components of multidrug efflux pump systems as receptors, mutations conferring phage resistance change the pump mechanism, resulting in antibiotic re-sensitization.

“In a case study of *P. aeruginosa* infection of an aortic prosthetic graft, direct delivery of phage and ceftazidime to the graft was successful in resolving and perhaps eradicating infection” [17].

Finally, phage lysin research is on the rise: Guo et al. [26] described a new endolysin with in vitro action against *P. aeruginosa* and other Gram-negative bacteria on the critical priority pathogens list, with comparable reports from other labs [27].

5. ADVANTAGE OF PHAGE THERAPY OVER ANTIBIOTIC

5.1 Host Specificity

Phage treatment has an advantage over antibiotics in terms of host specificity. Although host specificity has some disadvantages (requiring phage-to-bacterial target matching and/or the production of extremely multivalent phages), it also has the significant advantage of phages not killing other bacteria species.

Phage therapy, for example, is unlikely to destroy the beneficial flora of the intestines, lungs, or urogenital tract, and thus is unlikely to produce the illnesses and fatalities seen when antibiotics promote pathogen overgrowth [28].

5.2 Side Effect

There have been no major negative effects reported. When an infection is cleared, the body can usually destroy phages within seven days. This is due to the fact that after phages have eliminated the bacterium, they cease multiplying.

5.3 Dosage

Because phages grow swiftly throughout the process of destroying bacteria, phage therapy requires fewer doses. This leads in a large concentration of phages in the diseased area, which aids in the bacterial infection's clearance.

5.4 Safety

Antibiotic side effects have been well documented, and include anaphylaxis, nephrotoxicity, cardiotoxicity, hepatotoxicity, and neurotoxicity, as well as gastrointestinal and hematological problems. Anaphylaxis is linked with certain antibiotic classes or is the result of high tissue concentrations in the majority of adverse responses; in these few cases, anaphylaxis is associated with specific antibiotic classes or is the result of high tissue concentrations.

Unlike the extensive literature on antibiotic safety, phage therapy has only lately acquired attention in western medicine, hence much of the current knowledge on phage safety is new. Although oral phage delivery is generally believed to be safe, phage translocation over the intestinal epithelium, where they then circulate in the blood, is a critical problem for phage therapy.

According to some evidence, "phage translocation may benefit the host by inhibiting interleukin-2, tumor necrosis factor, and interferon gamma production, therefore reducing the immune response to native gut microbial antigens. In other research, a host innate immune response aimed at eliminating the phage following injection in mice was discovered" [29].

5.5 Biofilm Penetration

Antibiotic therapy is particularly successful against planktonic bacteria like *Vibrio cholerae* and *Yersinia pestis*, but it is ineffective against biofilm-based bacterial infections. High dosages of antibiotics are often necessary to penetrate dense biofilms and notice any suppression of bacterial growth, but total eradication is unusual, and colony rebuilding occurs after antibiotic treatments are stopped. Although many antibiotics are typically regarded non-toxic at low dosages, large concentrations can cause tissue toxicity.

Gabisoniya et al. [30] from the Eliava Institute of Bacteriophages in Tbilisi, Georgia, discovered

that using phages on *P. aeruginosa* in vitro colonies not only stopped the bacterium from forming new biofilms, but also dissolved existing biofilms. Biofilms produced by *L. monocytogenes*, *P. aeruginosa*, and *Staphylococcus epidermidis* on the surface of medical devices have been eradicated using phage treatments.

6. CONCLUSION

The use of bacteriophages as antimicrobial agents to control pathogenic bacteria has emerged as a potential new method, and phage therapy appears to be a viable alternative to antibiotics. Because phages are abundant in the environment, they have the potential to be used to control pathogenic bacteria in food and animals.

7. RECOMMENDATION

Bacteriophages are being the subject of interest for alternative antimicrobial therapy for infectious diseases in recent years. It is recommended for further extensive research for phage therapy in order to combat the present and future microbial resistant to antibiotics. Based on the research reviewed phages as natural predators of bacteria will play an essential role in escaping such a dreadful future.

Phage therapy can also combat the dilemma of multidrug resistances and maintaining the level of microbiomes due to their specific target to certain infectious or pathogenic organisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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