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Article

Phages Can Be More Effective and Specific Than Antibiotics in Combating Bacteria

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SUMMARY

Every year, tens of thousands of people die of infections from antibiotic-resistant bacteria. Phage therapy has been suggested as an alternative because bacteria resistant to antibiotics may still be susceptible to phages. Furthermore, phages have the advantage of being more specific and thus less likely to harm beneficial bacteria. However, phages may have limited effectiveness in combating bacteria since bacteria possess several antiviral defense mechanisms and can quickly develop resistance to phages. The purpose of this study was to compare the effectiveness and specificity of antibiotics and phages in combating bacteria. To this end, we exposed strains of the bacteria *Escherichia coli* to T4 bacteriophages and antibiotics and assessed effectiveness and specificity of bacterial killing. Fission yeast, a unicellular eukaryotic organism, was used as a control. Cell growth, phage plaque formation, or cell concentrations were observed or measured. It was found that T4 phages are more specific and effective in fighting or inhibiting both antibiotic-resistant and sensitive bacteria than antibiotics. One phage can kill at least 4000 actively growing bacterial cells within two hours. Therefore, the data suggest that phage therapy can be developed as an efficient tool to combat antibiotic-resistant bacteria.

INTRODUCTION

Due to the overuse of antibiotics in the healthcare and food industry, antibiotic resistance in bacteria has become a global problem. Every year, approximately 700,000 people die from antibiotic-resistant infections worldwide (1). If this trend continues, by 2050, a projected 10 million people will have died from antibiotic resistant infections at a cost of $100 trillion unless new and effective treatments are quickly developed (1). In the U.S. alone, tens of thousands of deaths are annually attributed to drug-resistant strains of the bacterium *Escherichia coli* (2). Some bacteria, such as *Neisseria gonorrhoeae* and *Staphylococcus aureus*, have developed resistance to essentially all classes of antibiotics (3-5). Unfortunately, very few new classes of antibiotics have been recently discovered and approved (3, 6, 7).

Most *E. coli* strains (**Figure 1A**) are non-pathogenic bacteria that inhabit the intestines and normally aid with food digestion. However, pathogenic *E. coli* with virulence factors can be contracted from a variety of fairly common sources. *E. coli* can be found in improperly sanitized produce, unpasteurized liquids, and contaminated meats (8). Even healthcare equipment, such as endoscopes, has also led to some outbreaks of *E. coli* superbugs in hospitals. The rapid growth rate is one reason why a bacterial infection can be so dangerous. When provided with optimal growth conditions (such as nutrients and optimal temperature), *E. coli* cells are capable of doubling their population every 20 minutes (9). At this alarming rate, a single *E. coli* cell can produce 4,722,366,482,869,645,213,696 (2^{72}) progeny in a single day. Bacterial pathogens can still proliferate very fast even in people with healthy immune system, so many infections need to be treated urgently.

E. coli has led to many deaths; the H30Rx *E. coli* superbug alone has resulted in thousands of deaths in the United States. Drug resistance in *E. coli* is steadily rising especially for antibiotics such as the commonly prescribed fluoroquinolones and certain generations of cephalosporin (10). In addition, an increasing number of strains are also showing resistance to antibiotics like ampicillin, penicillin, and streptomycin (11). Both classical *E. coli* pathogens and commensal opportunistic *E. coli* strains can become antibiotic-resistant. The H30-Rx strain, a clinical isolate, was identified twenty years ago when a strain of *E. coli* developed mutations resulting a new strain, H30R, which is resistant to the antibiotic Ciprofloxacin (12). This in turn paved the way for H30Rx, which is resistant to several antibiotics (12). H30Rx also has the capability to disseminate into the bloodstream from urinary tract infections causing systemic inflammation, which can become life-threatening (12). Unfortunately, H30-Rx is not the only deadly superbug that exists. Carbapenem (a last resort antimicrobial) resistant bacteria has a ~50% mortality rate according to one report (13).

Due to the dramatic uptick in drug resistance, many people have suggested using phage therapy as a solution to combat bacteria (1, 14). Phage therapy is a method in which bacteriophages (or phages) are used to kill pathogenic bacteria (14). Phages, discovered a century ago, are viruses that lyse and kill bacteria (**Figure 1B**). They are found everywhere on the earth and an estimated 30 billion phages are absorbed into our bodies via our intestines each day (15). Phages are essential to maintaining a healthy microbial ecosystem in human digestive tracts. They were mentioned as a tool for curbing antibiotic resistance threats in a 2014 status report by the National Institute of Allergy and Infectious Diseases (16). Phages have been safely used to treat bacterial infections since the 1920s, but they fell out of favor due to the discovery of

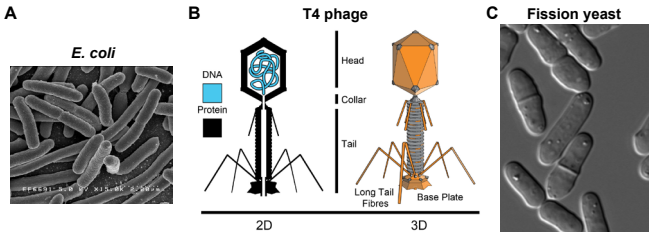


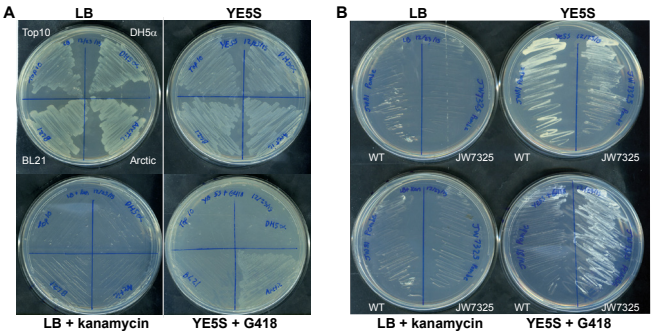
Figure 1. Images of *E. coli* (A), T4 phage (B), and the fission yeast *S. pombe* (C). The images of *E. coli* (credit: Rocky Mountain Laboratories, NIAID, NIH) and T4 phage (credit: authors Adenosine and Pbroks13) are from Wikipedia website.

antibiotics. However, there is now a renewed interest in phage therapy thanks, in part, to escalations in antibiotic resistance and the relative safety of phages. For example, Listex, a phage based solute used to kill *Listeria* and *Salmonella* in foods, was approved by the FDA in 2006 (17, 18). UCSD psychologist Thomas Patterson, who was infected with a bacterium resistant to all the available antibiotics, and five other people were successfully cleared of bacterial infections with phage cocktails by a UCSD team (19). Proponents believe that phage therapy is more precise than antibiotics, which are typically broad-spectrum and kill or inhibit both pathogenic and beneficial bacteria, since most phages specifically target certain species of bacteria. Due to this specificity, phages also cause less side effects in humans. Despite these advantages and the recent successes, phage therapy is still rarely used in the US and still has several obstacles to overcome. Research has shown that certain phages can help transfer drug resistance and lysed bacterial cells may release toxins (20). Moreover, bacteria also have several antiviral mechanisms to protect themselves from phages, such as the famous CRISPR/Cas defense systems (21-24). Therefore, bacteria may eventually develop immunity to phages. This will be problematic if more and more people decide to use phages over antibiotics. However, unlike antibiotics, phages may be able to evolve alongside these bacterial pathogens, avoiding some of the issues with resistance.

Here, we tested the specificity and effectiveness of traditional antibiotics compared with phages on limiting growth of *E. coli*. Fission yeast (**Figure 1C**), a genetically tractable unicellular model organism with cell-division cycle and many proteins/genes conserved in human cells (25-28), was used as a eukaryotic control. Wildtype fission yeast cells are sensitive to many antibiotics including geneticin (G418), hygromycin, and nourseothricin (clonNAT) (29). We compare the efficiency and specificity of T4 phage and several antibiotics in inhibiting or killing yeast and *E. coli* strains on agar plates or in liquid culture. It was hypothesized that T4 phages are more effective than antibiotics since they will be able to target and infect antibiotics-resistant bacteria. In addition, phages will remain active resulting from their proliferation even after the antibiotics are depleted, so this may also make them more effective. Indeed, we find that T4 phage is more specific and efficient in killing both antibiotic-sensitive and antibiotic-resistant bacterial cells. We predict that therapies employing both phages and antibiotics will be ideal to combat antibiotic-resistant bacteria.

RESULTS

First, we tested the specificity of antibiotics by observing the viability and growth of bacteria and fission yeast on LB (standard medium for bacteria) and YE5S (rich medium for fission yeast) plates with broad spectrum antibiotics kanamycin or geneticin (G418), respectively. Kanamycin binds to the 30S subunit of prokaryotic ribosomes and interferes with protein synthesis. It is effective in treating severe bacterial infections including tuberculosis, but it may have side effects including hearing and kidney problems. G418 inhibits the elongation step of protein synthesis in both prokaryotic and eukaryotic cells. As shown in **Figures 2A** and **2C**, *E. coli* strains Top10, DH5α, BL21, and ArcticExpress were inviable in LB medium with kanamycin, unlike the normal growth in LB medium without kanamycin (**Figure 2A**, left). The only strain that grew well on the medium with G418 was the ArcticExpress cells (**Figure 2A**, right). Thus, all the tested *E. coli* strains are sensitive to kanamycin and G418 except ArcticExpress cells, which is resistant to G418 **(Figure 2C**). Fission yeast cells did not grow well on LB medium even without kanamycin (**Figure 2B**, left), but YE5S + kanamycin plates were not available. Due to these limitations, it was difficult for us to determine if the yeast cells are sensitive to kanamycin or not (**Figure 2C**). However, we confirmed that yeast strain JW7325 (*kanMX6-*



C

Medium	Top10	DH5α	BL21	Arctic	WT yeast	JW7325
LB	+	+	+	+	?	?
LB + kanamycin	-	-	-	-	?	?
YE5S	+	+	+	+	+	+
YE5S + G418	-	-	-	+	-	+

Figure 2. Antibiotics can kill both prokaryotic *E. coli* cells and eukaryotic fission yeast cells. (A) *E. coli* strains Top10 (top left), DH5α (top right), BL21 (bottom left), and ArcticExpress (bottom right) as marked were re-streaked onto LB, LB + kanamycin, YE5S, YE5S + G418 plates and grown at 37°C for 1 day before scanning. (B) Fission yeast strains wildtype (WT; left) and JW7325 (right) were re-streaked onto LB, LB + kanamycin, YE5S, YE5S + G418 plates and grown at 25°C for 2 days before scanning. Two replicates were performed for each experiment. (C) Summary of antibiotic sensitivity of *E. coli* and fission yeast cells as shown in (A) and (B). +, growth or resistant to the antibiotic; -, no growth or sensitive to the antibiotic; ?, unknown due to poor growth of yeast on LB medium.

Pypt3-tdTomato-ypt3) but not wild-type yeast is resistant to G418 (**Figures 2B** and **2C**). Taken together, this data confirms that an antibiotic can target and kill many different kinds of cells.

Next, we tested the specificity of the T4 phage by observing phage plaque formation on lawns of bacteria and fission yeast. Plaques are formed on lawns of cells when they are lysed and cleared by phages. Both antibiotic-sensitive and resistant *E. coli* strains were susceptible to being lysed by T4 phages, as evidenced by the various sizes of phage plaques (cleared zones without bacteria) with different amount of phages (**Figure 3A**, examples marked by arrows). In contrast, no phage plaques were formed on the lawns of either G418-sensitive JW81 or resistant JW7325 fission yeast cells (**Figure 3B** and data not shown). Thus, it can be concluded that the T4 phages are more specific than antibiotics because they cannot infect yeast while antibiotics kills both bacteria and yeast.

Then, we investigated the efficiency of how phages attack and lyse bacterial cells. Ten-fold serially diluted phages were added to 300 µl DH5α cells (with OD_{600} = 0.75; ~1.8 x 10^8 bacterial cells given that 1 OD_{600} = 8 x 10^8 cells/ml), incubated for ~1 hour, and then plated on agar plates. After incubation at 37°C overnight, more phage plaques were formed with increasing concentrations of phages **(Figure 4A**). At 10^{-3} dilution of T4 phages (4 x 10^9 phages/ml x 10^{-3} x 10 µl x 10^{-3} ml/µl = 40,000 phages), ~90% area of the plate had no or significantly reduced DH5α cells (**Figure 4A**). Thus, one phage (from the starting stock) can kill ~4000 bacterial cells (~1.8 x 10^8 bacterial cells x 90% / 40,000 phages), which is highly efficient. Interestingly, at 10^{-2} phage dilution (400,000 phages/per plate), approximately hundreds to thousands of single bacterial colonies were formed on each plate (**Figure 4A**), which suggests that phage-resistant cells can quickly take over the culture once the sensitive bacteria die off. At 10^{-3} dilution of T4 phages, phage-sensitive cells may have used up the nutrients before they died so that the resistant cells have no chance to proliferate and form colonies. The results were confirmed using BL21 cells (**Figure 4B**). Together, it was determined that T4 phages are highly efficient at killing bacterial cells, but resistance to phages can also develop or be selected quickly.

Lastly, we directly compared the effectiveness of killing/inhibiting bacteria between phages and antibiotics in terms of their weights. The molecular weights of ampicillin, G418, and T4 phages are 371, 693, and 1.93 x 10^8 g/molar (30), respectively. In this experiment, G418-resistant but ampicillin and kanamycin-sensitive ArcticExpress *E. coli* cells were mixed with either phages or antibiotic ampicillin or G418 at time zero. Cells were grown at 37°C and OD_{600} was measured every 30 minutes (**Figure 5A**). We found that the phages were highly effective in killing bacteria compared to the antibiotics. Phages at concentrations of both 10^{-3} dilution (phages :

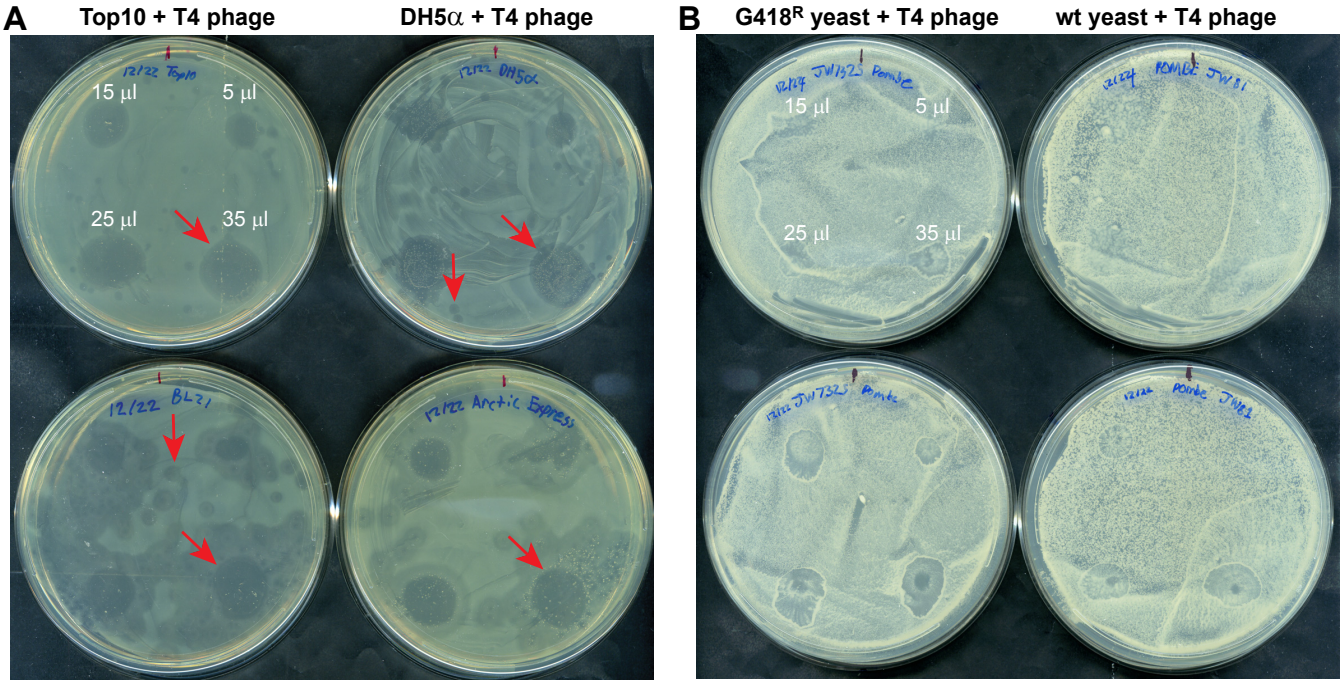


Figure 3. T4 phage can lyse *E. coli* cells but not fission yeast cells. (A) Formation of phage plaques (examples marked with arrows) on LB plates with fresh lawns of *E. coli* cells, which were inoculated with 5, 15, 25, and 35 µl (counterclockwise starting from top right as shown on the first plate) undiluted T4 phages (4 x 10^9 phages/ml) for each strain and incubated at 37°C. (B) Insensitivity of wildtype fission yeast JW81 and G418-resistant JW7325 ($G418^R$) stains to T4 phages. Fresh lawns of yeast cells on YE5S plates were inoculated with 5, 15, 25, and 35 µl (counterclockwise starting from top right as shown on the first plate) undiluted T4 phages for each strain and incubated at 36°C. Images from two repeated experiments were shown. On some plates yeast cells were displaced by the phage solution, as evidenced by more cells on the edge of the spots with phages. Similar experiments were performed for four times.

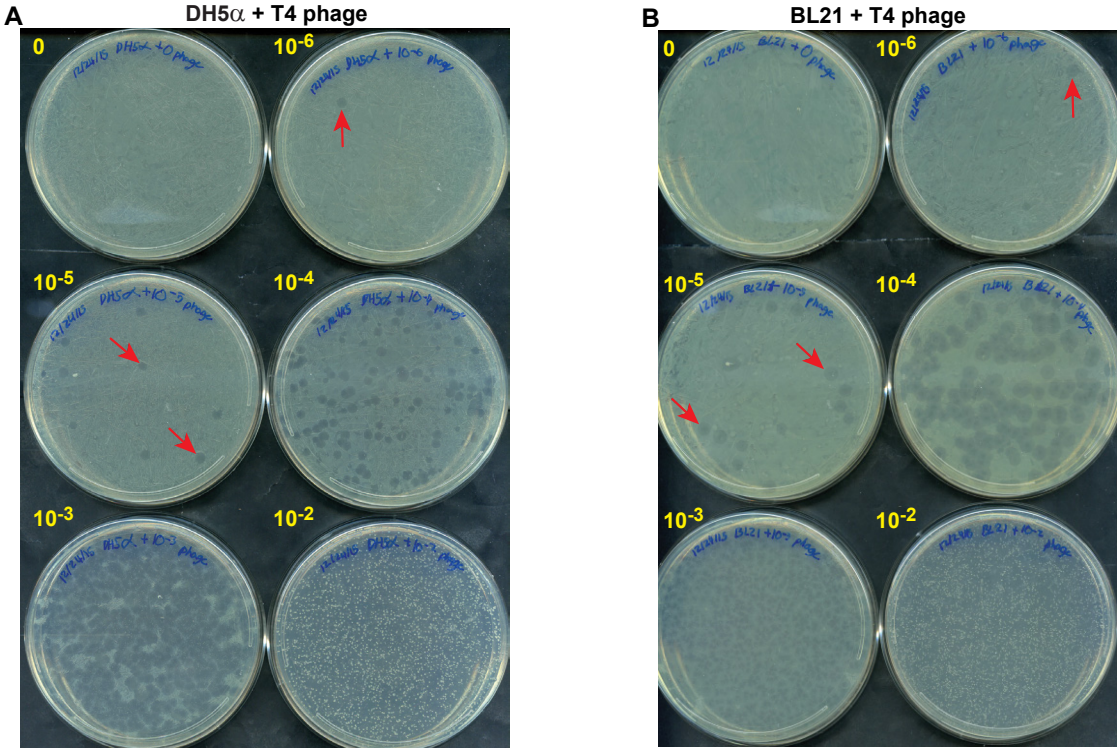


Figure 4. T4 phages efficiently lyse actively proliferating bacterial cells. Active growing DH5α (A) and BL21 cells (B) were mixed with 10 µl different dilutions of T4 phages as indicated, incubated for ~1 hour, and then plated evenly on plates, and incubated at 37°C overnight before scanning. Examples of phage plaques were marked with arrows. Three replicates were performed for each experiment.

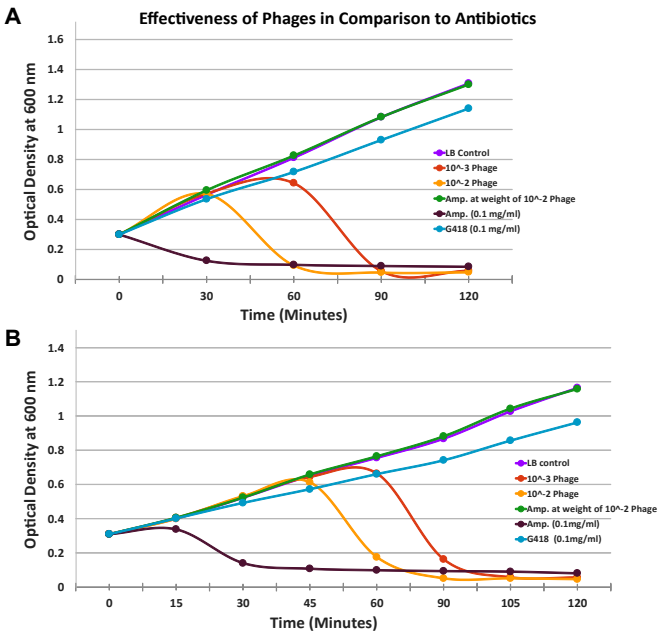


Figure 5. Phages are more effective than antibiotics in killing or inhibiting bacteria. Density of ArcticExpress cells treated with different concentrations of T4 phages and antibiotics (or 40 µl LB medium as control) was measured every 30 (A) or 15 minutes (B) and plotted using Excel. The experiment was performed twice.

bacteria = 1 : 4,500) and 10^{-2} dilution (phages : bacteria = 1 : 450) lysed essentially all the *E. coli* cells after a 30 to 60 minutes delay as the OD_{600} of bacterial cells increased initially but then dropped to almost zero and the culture became clear in about 90 minutes. This confirms that a phage can kill >4000 actively growing bacterial cells within 2 hours. Ampicillin added with a weight equivalent of the 10^{-2} phage dilution had no effect on bacterial growth. The ampicillin stock solution was functional since 0.1 mg/ml of ampicillin (normal lab working concentration, which is 590,000 times higher than 10^{-2} phage equivalent) quickly inhibited cell growth in ~30 minutes. As expected, 0.1 mg/ml of G418 only mildly slowed cell growth. The experiment was repeated by measuring the OD_{600} every 15 minutes. Essentially identical results were obtained (**Figure 5B**). Thus, the T4 phage is much more effective in killing G418-resistant bacteria than ampicillin if applied at the same weight, although each phage is 520,000 times heavier than one ampicillin molecule. Taken together, our data indicate that T4 phage is more specific and effective than antibiotics in killing both antibiotic-sensitive and resistant-bacteria.

DISCUSSION

In this study, we found that the T4 phage is significantly more effective than antibiotics in fighting bacteria if measured by their weight, even though a phage is 520,000 times heavier than an ampicillin molecule and thus much less was used in terms of molar concentration, which makes phages much

more potent (**Figure 5**). This is partly because the phages continue to infect bacteria and proliferate even after antibiotics have already been degraded or depleted. Phages are also more selective than antibiotics since they only target one or a few specific types of bacteria via cell surface receptors, not fungal or human cells. This is confirmed because they do not kill eukaryotic yeast, despite the fact that G418 inhibits growth of wildtype yeast cells. Moreover, the T4 phage is also effective against G418-resistant bacterial strain ArcticExpress. Thus, our data support the hypothesis that phages are more effective and specific than antibiotics in combating bacteria.

In the future, a study can be conducted using phages and antibiotics in conjunction to test if both antibiotic-resistant and phage-resistant bacteria will be eliminated. Moreover, the ED50 (median effective dose) for phages should be measured in future studies. Limitations of this study were the sample size and availabilities of materials. The effect of phages on bacterial populations in liquid cultures was only done twice. However, since both trials produced nearly identical results, an increase in trials may not yield any differences. No YE5S + kanamycin plates were available to test if yeast cells are sensitive to kanamycin. Moreover, we did not have access to tissue culture cells so that we could not test the effectiveness and specificity of phages and antibiotics in treating bacterially-infected human cells.

Phages are crucial to the global ecosystem and human microbial community by regulating bacterial abundance, balance, and diversity (31). For example, it was estimated that there are $>10^{31}$ phages on earth and that one third of marine surface bacteria are wiped out by phages every day (32). In a given ecosystem, approximately 80% of bacterial death is caused by phage infection (31). Phages are enriched >4 times in our mucus layers compared to the adjacent environment (15). Billions of phages enter human tissues via our intestines each day and they may modulate our immune system (15). Thus, it is possible to harness this powerful control on bacterial populations by phages and use it to help fight bacterial infections with minimal side effects. In fact, phages or phage cocktails have already been shown to be successful in treating chronic infections (33), antibiotic-resistant bacterial biofilms (34), and multi-drug resistant bacterium *Acinetobacter baumannii* (19). In general, phage cocktails contain a variety of phages, each with different host targets, which ensure their effectiveness, minimize the development of phage resistance, and lower the risk of harming patients' microbiome (31). Engineered phages with a broader spectrum and minimal host immune reactions will thus be more effective and should be developed urgently. Ideally, phage banks with wildtype and engineered phages against all known bacterial pathogens should be established at hospitals. Bacterial pathogens from patients can be identified within hours by rapid DNA sequencing using powerful sequencers. Then the corresponding phages will be administered immediately to cure or save patients from bacterial infections. Because phages can only reproduce inside bacteria, the phages will stop proliferating once pathogens are cleared and may be eliminated by our immune system over time. Thus, the administered phages should not cause many side effects.

Our research provides useful insights into the potential of phage therapy and could help design better treatments for many diseases caused by antibiotic-resistant bacteria. We found that one T4 phage can kill >4000 bacterial cells in 2 hours. This result challenges the conclusion from previous studies that significant numbers of phages (or huge phage-to-bacterium ratio) are needed to efficiently combat bacteria such as *E. coli* (35, 36), which is one of main obstacles for phage therapy. Actually, phage resistance may develop faster if the phage concentration is too high, as shown in **Figure 4**. Our data also suggest that phages are most effective in killing actively growing bacteria when the two are mixed together. Thus, the timing of phage delivery to bacteria is important. One disadvantage of phage therapy is that endotoxins (more likely to be released by lysing bacteria) may trigger immune responses such as fever or toxic shock in some patients. However, phages have much less overall side effects and thus higher therapeutic index than antibiotics because they only infect/target bacteria and archaea but not eukaryotes such as yeast and humans (33). Phages can be used to treat multiple-drug resistance bacteria, which cause severe problems for patients with suppressed or compromised immune systems and threaten to return humankind to the era before antibiotics (37, 38). Antibiotics, besides their well-known side effects, have the disadvantage of killing both pathogenic and beneficial microflora, which may disrupt the microbial balance and trigger severe secondary infections and allergic reactions in humans. In addition, commonly prescribed antibiotics such as Cipro and Levofloxacin have disabling side effects in some people whose mitochondria may be sensitive and damaged by the antibiotics, since mitochondria were evolved from symbiotic bacteria-like cells (39). However, no evidence shows that phages target or damage mitochondria since mitochondria have no bacterial surface receptors targeted by phages. Moreover, while it may take several years to develop a new antibiotic, phages are capable of adapting to phage-resistant bacteria within a few weeks (38). Thus, phage therapy is a fluid form of treating bacterial infections because it could evolve and be selected alongside bacteria as a flexible solution to combat resistance. Thus, many diseases, such as sepsis, could soon have more treatment options.

Despite the greater effectiveness phage therapies are still limited by resistant strains of bacteria. This problem could be addressed if antibiotics and phages are used simultaneously to kill the resistant strains. Lower doses of antibiotics may be enough if applied with phages. Indeed, it has been shown that phages can restore antibiotic sensitivity in multi-drug resistant *Pseudomonas aeruginosa* by using its multidrug efflux systems as receptor-binding site and phages can suppress bacterial immune system by expressing anti-CRISPR proteins (40, 41). More efforts and studies should be launched to improve phage therapy so that we will be able to combat the ever-increasing

threat of antibiotic resistance.

MATERIALS AND METHODS

Bacterial and Fission Yeast Media and Strains

Standard bacterial and yeast media were used. LB medium (984 mL ddH_2O, 10g Bacto tryptone, 5g Difco yeast extract, 10g NaCl, and 100 µL 10 M NaOH for one liter medium; plus 15g agar for plates) was used for bacteria. Antibiotics ampicillin and kanamycin were used at final concentrations of 100 mg/L and 50 mg/L, respectively. YE5S medium (980 mL ddH_2O, 5g Difco yeast extract, 30g dextrose, and 18g agar for one liter medium) with or without 100 mg/L G418 (geneticin) was used for the fission yeast *S. pombe*. Non-pathogenic versions of bacterium *E. coli* (**Figure 1A**) strains used were: ArcticExpress RIL (230193, Agilent Technologies), BL21 (69451, Novagen), Top10 (Invitrogen), and DH5α (Invitrogen), which were generous gifts from the Wu lab at The Ohio State University.

The T4 phage (**Figure 1B**) was purchased from Carolina Biological Supply Company (item # 124335) with a titer of 4 x 10^9 plaque-forming particles/ml and stored at 4°C before use. We simply assumed that a plaque-forming particle represented a phage in this study.

Fission yeast (**Figure 1C**, the image was taken using a Nikon microscope) strains used were G418 sensitive strain wildtype strain JW81 (*h- ade6-M210 leu1-32 ura4-D18*) and G418 resistant strain JW7325 (*h- kanMX6-Pypt3-tdTomato-ypt3 ade6-M210 leu1-32 ura4-D18*) from the Wu lab collection.

METHODS

Sterile techniques were used throughout the experiments to avoid contamination. These techniques included sterilizing the bench with 70% ethanol and turning on a Bunsen burner when handling cells. *E. coli* strains of ArcticExpress, DH5α, BL21, and Top10 and fission yeast strains JW81 and JW7325 were cultured from -80°C storage. Bacterial cells were grown on LB plates at 37°C and yeast cells on YE5S plates at 25°C. The cells were then streaked onto plates with or without antibiotics using sterile tooth picks to test their sensitivity.

Three experiments were performed to test specificity and efficiency of the T4 phage in impeding yeast and *E. coli* cells. First, *E. coli* or yeast cells were inoculated into sterile culture tubes with 4 mL of LB or YE5S liquid medium, and then grown at 37 (*E. coli*) or 25°C (yeast) overnight. A 300 µL aliquot of cells of each strain was placed on each LB or YE5S plate and spread evenly using sterile glass beads (3 mm in diameter and approximately 4 beads per plate). The plates were incubated at 37 or 25°C incubator until a lawn of cells formed. Then different amounts (5, 15, 25, and 35 µL) of undiluted T4 phages (4 x 10^9 phages/mL) were placed onto bacterial or yeast lawns to observe phage plaque formation after further incubation at 37˚C (*E. coli*) or 36°C (yeast). Second, 300 µL *E. coli* or yeast cells grown at exponential phase were mixed with different concentrations of T4 phages, incubate for ~1 hour, and then plated evenly on LB or YE5S plates using sterile glass beads and incubated at 37˚C (*E. coli*) or 36°C (yeast). OD_{600} (optical density at 600 nm wavelength) of the liquid cultures was: 0.51 for yeast and 0.75 for DH5α and BL21 cells. The T4 phage stock was serially diluted by adding 10 µL phages to 90 µL of LB or YE5S liquid medium. Then the process was repeated with the resulting dilution to get a dilution series of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. We added 10 µL diluted phages to 300 µL cells and spread cells using glass beads. Yeast plates were incubated at 25°C and *E. coli* plates at 37°C. The plates were scanned using Epson Perfection V350 Photo scanner. The area with cells was measured using ImageJ (National Institutes of Health). Third, ArcticExpress cells were grown in liquid LB medium in sterile flask at 37°C to OD_{600} = 0.3. The culture was divided into 6 flasks with 30 mL in each flask. T4 phages, ampicillin, and G418 at indicated concentrations were added to each flask. Cells were grown at 37°C. Samples were taken every 30 minutes to measure cell density at OD_{600} using a spectrophotometer (Beckman Coulter DU730). The experiments were repeated by measuring the OD_{600} every 15 minutes.

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REFERENCES

1. Lin et al. “Phage Therapy: An Alternative to Antibiotics in the Age of Multi-Drug Resistance.” *World J Gastrointest Pharmacol Ther* 8(3), 2017, pp. 162-173.
2. Ventola CL. “The Antibiotic Resistance Crisis: Part 1: Causes and Threats.” *P & T* 40(4), 2015, pp. 277-283.
3. Rossolini GM, Arena F, Pecile P, & Pollini S. “Update on the Antibiotic Resistance Crisis.” *Curr Opin Pharmacol* 18, 2014, pp. 56-60.
4. Lestari ES, Severin JA, & Verbrugh HA. “Antimicrobial Resistance among Pathogenic Bacteria in Southeast Asia.” *Southeast Asian J Trop Med Public Health* 43(2), 2012, pp. 385-422.
5. Magiorakos AP*, et al.* “Multidrug-Resistant, Extensively Drug-Resistant and Pandrug-Resistant Bacteria: an International Expert Proposal for Interim Standard Definitions for Acquired Resistance.” *Clin Microbiol Infect* 18(3), 2012, pp. 268-281.
6. Kostyanev T*, et al.* “The Innovative Medicines Initiative’s New Drugs for Bad Bugs Programme: European Public-Private Partnerships for the Development of New Strategies to Tackle Antibiotic Resistance.” *J Antimicrob*

Chemother 71(2), 2016, pp. 290-295.
7. Fernandes P & Martens E. "Antibiotics in Late Clinical Development." *Biochem Pharmacol* 133, 2017, pp. 152-163.
8. Newman MJ. "Food Safety: Take Life Easy; Eat, Drink and Be Merry." Luke 12: 19b. *Ghana Med J* 39(2), 2005, pp. 44-45.
9. Fossum S, Crooke E, & Skarstad K. "Organization of Sister Origins and Replisomes during Multifork DNA Replication in *Escherichia coli*." *EMBO J* 26(21), 2007, pp. 4514-4522.
10. Collignon P. "Resistant *Escherichia coli* - We Are What We Eat." *Clin Infect Dis* 49(2), 2009, pp. 202-204.
11. Chudobova D*, et al.* "Effect of Ampicillin, Streptomycin, Penicillin And Tetracycline on Metal Resistant and Non-resistant *Staphylococcus aureus*." *Int J Environ Res Public Health* 11(3), 2014, pp. 3233-3255.
12. Banerjee R & Johnson JR. "A New Clone Sweeps Clean: the Enigmatic Emergence of *Escherichia coli* Sequence Type 131." *Antimicrob Agents Chemother* 58(9), 2014, pp. 4997-5004.
13. Righi E*, et al.* "Global Prevalence of Carbapenem Resistance in Neutropenic Patients and Association with Mortality and Carbapenem Use: Systematic Review and Meta-Analysis." *J Antimicrob Chemother* 72(3), 2017, pp. 668-677.
14. Keen EC. "Phage Therapy: Concept to Cure." *Front Microbiol* 3, 2012, Article 238.
15. Guglielmi G. "Do Bacteriophage Guests Protect Human Health?" *Science* 358(6366), 2017, pp. 982-983.
16. National Institute of Allergy and Infectious Diseases. NIAID's Antibacterial Resistance Program: Current Status and Future Directions 2014, pp. 1-15.
17. Soni KA & Nannapaneni R. "Bacteriophage Significantly Reduces *Listeria monocytogenes* on Raw Salmon Fillet Tissue." *J Food Prot* 73(1), 2010, pp. 32-38.
18. Soni KA, Nannapaneni R, & Hagens S. "Reduction of *Listeria monocytogenes* on the Surface of Fresh Channel Catfish Fillets by Bacteriophage Listex P100." *Foodborne Pathog Dis* 7(4), 2010, pp. 427-434.
19. Servick K. "U.S. Center Will Fight Infections with Viruses." *Science* 360(6395), 2018, pp. 1280-1281.
20. Loc-Carrillo C & Abedon ST. "Pros and Cons of Phage Therapy." *Bacteriophage* 1(2), 2011, pp. 111-114.
21. Labrie SJ, Samson JE, & Moineau S. "Bacteriophage Resistance Mechanisms." *Nat Rev Microbiol* 8(5), 2010, pp. 317-327.
22. Koonin EV, Makarova KS, & Zhang F. "Diversity, Classification and Evolution of CRISPR-Cas Systems." *Curr Opin Microbiol* 37, 2017, pp. 67-78.
23. Mali P, Esvelt KM, & Church GM. "Cas9 as a Versatile Tool for Engineering Biology." *Nat Methods* 10(10), 2013, pp. 957-963.
24. Jiang F & Doudna JA. "CRISPR-Cas9 Structures and Mechanisms." *Ann Rev Biophys* 46, 2017, pp. 505-529.
25. Nurse P. "Universal Control Mechanism Regulating Onset of M-phase." *Nature* 344(6266), 1990, pp. 503-508.
26. Norbury C & Nurse P. "Controls of Cell Proliferation in Yeast and Animals." *Ciba Found Symp* 150, 1990, pp. 168-177; discussion pp. 177-183.
27. Fruhmann G*, et al.* "Yeast Buddies Helping to Unravel the Complexity of Neurodegenerative Disorders." *Mech Ageing Dev* 161(Pt B), 2017, pp. 288-305.
28. Balasubramanian MK, Bi E, & Glotzer M. "Comparative Analysis of Cytokinesis in Budding Yeast, Fission Yeast and Animal Cells." *Curr Biol* 14(18), 2004, pp. R806-R818.
29. Brown SD & Lorenz A. "Single-step Marker Switching in *Schizosaccharomyces pombe* Using a Lithium Acetate Transformation Protocol." *Bio Protocol* 6(24), 2016, e2075.
30. Dubin SB, *et al*. "Molecular Weights of Coliphages and Coliphage DNA. II. Measurement of Diffusion Coefficients Using Optical Mixing Spectroscopy, and Measurement of Sedimentation Coefficients." *J Mol Biol* 54(3), 1970, pp. 547-556.
31. Mirzaei MK & Maurice CF. "Ménage à trois in the Human Gut: Interactions between Host, Bacteria and Phages." *Nat Rev Microbiol* 15(7), 2017, pp. 397-408.
32. Wittebole X, De Roock S, & Opal SM. "A Historical Overview of Bacteriophage Therapy as an Alternative to Antibiotics for the Treatment of Bacterial Pathogens." *Virulence* 5(1), 2014, pp. 226-235.
33. Wernicki A, Nowaczek A, & Urban-Chmiel R. "Bacteriophage Therapy to Combat Bacterial Infections in Poultry." *Virol J* 14(1), 2017, Article 179.
34. Pires DP, *et al*. "Phage Therapy as an Alternative or Complementary Strategy to Prevent and Control Biofilm-Related Infections." *Curr Opin Microbiol* 39, 2017, pp. 48-56.
35. Huff WE*, et al.* "Prevention of *Escherichia coli* Respiratory Infection in Broiler Chickens with Bacteriophage (SPR02)." *Poult Sci* 81(4), 2002, pp. 437-441.
36. Zimmer M, Scherer S, & Loessner MJ. "Genomic Analysis of *Clostridium perfringens* Bacteriophage ϕ3626, Which Integrates into *Guaa* and Possibly Affects Sporulation." *J Bacteriol* 184(16), 2002, pp. 4359-4368.
37. Nigam A, Gupta D, & Sharma A. "Treatment of Infectious Disease: Beyond Antibiotics." *Microbiol Res* 169(9-10), 2014, pp. 643-651.
38. Sulakvelidze A, Alavidze Z, & Morris JG, Jr. "Bacteriophage Therapy." *Antimicrob Agents Chemother* 45(3), 2001, pp. 649-659.
39. Marchant J. "When Antibiotics Turn Toxic." *Nature* 555(7697), 2018, pp. 431-433.
40. Chan BK*, et al.* "Phage Selection Restores Antibiotic Sensitivity in MDR *Pseudomonas aeruginosa*." *Sci Rep* 6, 2016, Article 26717.
41. Bernheim A & Sorek R. "Viruses Cooperate to Defeat Bacteria." *Nature* 559(7715), 2018, pp. 482-484.