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Bacteriophage Infections of Biofilms of Health Care-Associated Pathogens: *Klebsiella pneumoniae*

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Abstract

Members of the family *Enterobacteriaceae*, such as *Klebsiella pneumoniae*, are considered both serious and urgent public health threats. Biofilms formed by these health care-associated pathogens can lead to negative and costly health outcomes. The global spread of antibiotic resistance, coupled with increased tolerance to antimicrobial treatments in biofilm-associated bacteria, highlights the need for novel strategies to overcome treatment hurdles. Bacteriophages (phages), or viruses that infect bacteria, have reemerged as one such potential strategy. Virulent phages are capable of infecting and killing their bacterial hosts, in some cases producing depolymerases that are able to hydrolyze biofilms. Phage therapy does have its limitations, however, including potential narrow host ranges, development of bacterial resistance to infection, and the potential spread of phage-encoded virulence genes. That being said, advances in phage isolation, screening, and genome sequencing tools provide an upside in overcoming some of these limitations and open up the possibilities of using phages as effective biofilm control agents.

BIOFILMS AND THE *ENTEROBACTERIACEAE*

Biofilms are communities of microorganisms, often associated with a surface, that are encased in a self-produced extracellular polymeric substance (EPS) matrix (1). Biofilm development has three main stages: attachment, maturation, and dispersion (2). The overall process, however, is far more complex, with many factors contributing to its progression. The physiological transition from planktonic, or free-swimming cells, to their sessile, surface-associated counterparts is one that involves highly regulated gene expression (3, 4) that is unique to the organism and its environment. The biofilm phenotype provides members of these communities a greater level of protection, particularly when environmental conditions are highly variable or challenging. Biofilm cells are notably different from their planktonic counterparts in that they exhibit reduced rates of metabolic activity as well as increased tolerance against antimicrobials and host defenses (5, 6).

Biofilm formation in the *Enterobacteriaceae* is well documented. In their comparison of biofilm formation by *Klebsiella pneumoniae, Salmonella enteritidis,* and *Escherichia coli*, Jones et al. demonstrated that all three bacterial species were able to produce robust biofilms

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within 24 h on chlorinated polyvinyl chloride (CPVC) pipe surfaces. Furthermore, metabolic activity and production of EPS were pronounced over the course of 48 h for both *K. pneumoniae* and *S. enteritidis* (7). Similarly, biofilm composition among different genera and species can be highly variable and dependent on the environment. For example, biofilms of *E. coli* and *Salmonella* species exhibit a greater abundance of curli amyloid fibers (8), with other components such as polysaccharides (e.g., cellulose and colanic acid) (9, 10) and extracellular DNA also playing key roles in surface adhesion and matrix stability. An in-depth microscopic analysis of biofilms formed by clinical isolates of *K. pneumoniae* by Birarda et al. demonstrated the localization of extracellular DNA and proteins at middle to lower portions of the biofilm, while polysaccharides were more widely distributed, suggesting a scaffolding-like role in the matrix (11). In the food processing industry, bacterial biofilms of *Enterobacteriaceae* are a source of contamination, particularly on equipment and preparation surfaces. Biofilms of Enterobacter spp. such as *E. cloacae*, grown on stainless steel surfaces, have been shown to be highly resistant to chemical disinfection (12). Contamination of food products, such as powdered infant formula by *Cronobacter* spp., has been tied to cross-contamination of processing equipment as well as prolonged survival in desiccated formula products (13, 14).

KLEBSIELLA BIOFILMS AND THEIR ROLE IN INFECTIONS AND ANTIBIOTIC RESISTANCE

Klebsiella are Gram-negative, encapsulated, nonmotile bacteria that are members of the family *Enterobacteriaceae*. They can be found in a number of environments, including water and soil, and in association with both plants and animals (15). In humans, *Klebsiella* can be found in the intestinal tract; however, certain species, such *K. pneumoniae*, have been known to cause respiratory and urinary tract infections as well as bloodstream infections (15, 16). Virulence factors such as capsular polysaccharides, pili, and adhesins are major contributing factors to infections (17). These factors also play a role in biofilm formation.

Biofilm formation in *Klebsiella* species, for example, is closely associated with the production of type 1 and 3 fimbriae, which aid in attachment and biofilm production. Ghasemian et al. demonstrated an association of the presence of adhesin-encoding genes, biofilm formation, and resistance to non-β-lactam antibiotics in *Klebsiella oxytoca* isolates (18). Also, in experiments using a β-lactamase-deficient mutant *K. pneumoniae* strain exposed to ampicillin, Anderl et al. demonstrated a marked reduction in killing of biofilm cells compared to their planktonic counterparts (19). In a separate study, Vuotto et al. found a positive correlation between antibiotic resistance profiles and the biofilm-forming capability in extensively drug-resistant *K. pneumoniae* strains, also noting the involvement of genes associated with efflux pumps, lipopolysaccharide and EPS production, and quorum sensing, among other factors (20). The biofilm mode of growth is a critical factor in the colonization of indwelling medical devices (21), leading to device-associated infections. *K. pneumoniae* and *K. oxytoca* were ranked among the top 10 pathogens associated with cases of central line-associated bloodstream infections, catheter-associated urinary tract infections, ventilator-associated pneumonia, and surgical site infections reported to the National Healthcare Safety Network from January 2006 to October 2007 (22).

Carbapenemase-producing *K. pneumoniae* (CPKP), a member of the carbapenem-resistant *Enterobacteriaceae*, has been identified as an urgent public health threat by the Centers for Disease Control and Prevention (CDC) (23). The global dissemination of *K. pneumoniae* carbapenemase (KPC) strains such as KPC-2 and KPC-3 (24) coupled with documented mortality rates as high as 48% in patients presenting with infections (25) has contributed to the urgency in finding alternative methods to dealing with CPKP. As stated previously, treatment of *K. pneumoniae* infections is often complicated by the formation of biofilms. In a recent survey of clinical samples from tertiary care hospitals in Indonesia, researchers found that nearly 55% of *K. pneumoniae* isolates were multidrug resistant, with 85% of the isolates being biofilm producers (26). In a separate study, investigators looking for an association with biofilm formation and extended-spectrum beta-lactamase (ESBL) production found that nearly 84% of biofilm-positive *K. pneumoniae* strains evaluated were able to produce ESBLs (27). In the isolates from sputum and urine samples from that study, nearly 45% formed biofilms and approximately 45% produced ESBLs. In a survey of various regions in Brazil, investigators found that up to 18% of CPKP isolates recovered were also hypervirulent (i.e., exhibiting a phenotype capable of causing invasive infections, particularly in healthy individuals), which contributes to colonization and persistence in patients and the hospital setting (28). Collectively, these various findings highlight the importance of searching for alternative treatment strategies, including the use of phages, to counteract the spread of multidrug-resistant *Enterobacteriaceae.*

BACTERIOPHAGES AS THERAPEUTIC AGENTS

Bacteriophages (phages) are bacterial viruses. They can be found in the same diverse environments as their hosts and are thought to be the most abundant life forms on the planet. Phages can be classified as virulent or temperate based on their life cycle. Infection by either is characterized by an adsorption step in which the phage associates with specific receptors on the host's cell surface, followed by injection of the phage genetic material. Virulent phages quickly take over the host's metabolic machinery, leading to the production of phage virions. The infection culminates in lysis of the host cell and release of viral progeny. Temperate phages go through a lysogenic cycle in which their genetic material incorporates into that of the host and is replicated through subsequent cycles of cell division. Under select conditions, these phages can enter the lytic cycle and proceed through to cell lysis and release.

Phages have long been considered as potential therapeutic and biocontrol agents against bacterial infections. Since their discovery in 1915 to 1917, the use of bacteriophages for the treatment of a wide range of bacterial infections has been investigated. Phages may provide unique advantages as therapeutic agents. For example, phages are relatively abundant and can generally be harvested from the same environments as their bacterial hosts (29, 30) and propagated to a high titer. A single phage infection can result in the production of multiple lytic progeny phages within a relatively short lytic cycle. Furthermore, they can exhibit specificity toward a narrow or broad (31, 32) range of hosts and can be combined as cocktails to target multiple strains of species (33). The narrow host range of phages can be used to target pathogens or antimicrobial-resistant organisms while potentially sparing nonpathogenic organisms in the community (as in the gut microbiome). Their use could

help reduce the reliance on antimicrobials that further contribute to the global spread of antimicrobial resistance. Certain phages have been demonstrated to produce enzymes that degrade capsular and EPS matrix polymers (depolymerases), enhancing the association of phage particles with their host bacterial cells in the biofilm. Several *in vitro* and *in vivo* studies have demonstrated the efficacy of phages against bacterial infections (34–37).

There are, of course, drawbacks to phage therapy. If a phage has a narrow host range, it could be an issue when treating infections caused by polymicrobial biofilms, such as a device-associated infection. In this case, the development of a phage "cocktail" containing multiple phage strains could broaden the effective host range of the treatment. Bacteria will develop resistance to their respective lytic phages as a result of alteration or loss of the receptor sites, because these bacterial cells undergo lysogeny, or because the organisms have acquired the CRISPR-Cas adaptive immune system. CRISPRs (clustered, regularly interspaced short palindromic repeats) are part of an adaptive immune system in bacteria that can be programmed to reject DNA molecules that have not been previously encountered (38). CRISPRs are separated by short spacer sequences that match bacteriophage or plasmid DNA sequences and specify targets of interference. These targets may be phage or plasmid DNA (including plasmids encoding antimicrobial resistance).

Yosef and colleagues (39) suggested a different approach incorporating bacteriophages to target antimicrobial-resistant bacteria based on the use of CRISPR-Cas technology. Bacterial cells acquiring these spacer sequences by transduction would acquire resistance to phages encoded by the spacer sequence and be cured of antimicrobial resistance-encoding plasmids at the same time. The result of this would be that the target bacteria would be resensitized to antibiotic treatments.

PHAGE INFECTION OF BIOFILMS

The use of phages for biocontrol purposes is versatile in the sense that they can be used before, during, and after biofilms have already formed (40). Doolittle et al. showed very early on that lytic infections of *E. coli* biofilms by phage T4 could take place (41). As may be expected, the infection kinetics of biofilm-associated bacteria are predictably different from those of their planktonic counterparts. Biofilm architecture, for example, plays an important role in biofilm defense against phage attack. The biofilm matrix, primarily composed of EPS, represents one of the major physical barriers that phages must overcome in order to reach their hosts. In a comparison of *E. coli* biofilm mutants, Vidakovic et al. demonstrated that the absence of curli amyloid fibers resulted in biofilms being susceptible to phage attack (42). The curli localize between cells and upper portions of the biofilm, providing protection from phage attack. In an *in silico* analysis of phage-biofilm interactions, Simmons et al. identified three parameters influencing phage spread in biofilms: (i) environmental nutrient concentration, (ii) phage infection probability, and (iii) relative diffusivity of phages within biofilms (43). In their simulations, they found that biofilms growing under low nutrient availability could resist phage attack as a result of slower growth; this, coupled with sparse biofilm cluster formation and proximity, led to isolated phage infections without much spread. The effects of nutrient limitation have also been shown to impact lytic development of phage in *E. coli* (44). Łoś et al. found that

phage development could be inhibited by initiating a carbon-source starvation very early in the phage infection cycle. It should be noted, however, that nutrient limitation does not preclude cells from phage attack or infection. In fact, T4 phages have been shown to infect stationary-phase *E. coli* cells, entering what has been described as a "hibernation" state in which lysis of the cell resumes once nutrients become available (45). Studies by Woods (46) and Shrader et al. (47) also demonstrated phage lytic activity against stationary-phase (*Achromobacter* spp.) and starved organisms (*Pseudomonas aeruginosa*). Phage lytic activity in soil, aquatic, and marine environments, where microorganisms can be nutrient limited, would also support the position that phage infection and cell lysis can still proceed.

It stands to reason then that phage infection of biofilms may occur more efficiently when phages have access to newer, more metabolically active clusters of cells in close proximity (48). Additionally, mechanical (49) or enzymatic (50, 51) debridement of biofilms could play an important role in enhancing access of phages to deeper portions of the biofilm, particularly when it comes to wound infections. To that end, some phages have evolved to overcome these barriers through the production of enzymes, such as depolymerases, which are able to degrade components of the biofilm matrix, thus giving better access for virions to reach their targets (52). These enzymes are also capable of degrading bacterial capsules, which have also been shown to resensitize bacteria to antimicrobial treatments as well as the innate immune response (53). A schematic of these concepts is summarized in Fig. 1. A study by Verma et al. found that eradication of older *K. pneumoniae* biofilms could be enhanced by treatment with a depolymerase-producing phage coupled with ciprofloxacin, potentially through a disruption of the biofilm matrix (54). The production of these enzymes, however, is not a sure-fire way of gaining access to any given host. Biofilm matrices, especially those of multispecies composition, could render these target-specific enzymes (e.g., depolymerases targeting polysaccharides) less effective due to the heterogeneous nature of the EPS components (52). It should be noted that entrapment of phages in the EPS matrix, although seemingly counterproductive to biofilm clearance, could, in the presence of uninfected hosts, lead to further waves of infections. This concept was put forward by Kay et al. in their observation that phages trapped in the EPS matrix remained infective and were released from the EPS by treatment of the biofilms with Tween 20 (55).

POLYMICROBIAL BIOFILMS AND PHAGES

Biofilms are likely to be polymicrobial in composition, which may lead to difficulty in targeting a specific host. A critique or perceived limitation of phage therapy is that some phages exhibit a narrow host range and thus could limit the treatment of polymicrobial biofilms. However, the use of polyvalent phages, or phages that are able to infect hosts of various genera and/or species, could play an important role in their application for biofilm-associated pathogen control (56–58). This was demonstrated by Yu et al., where they showed that a polyvalent phage, able to infect both *E. coli* and *Pseudomonas putida*, was able to suppress *E. coli* in a mixed biofilm to a greater degree than an *E. coli*-specific phage alone (59). Furthermore, they showed that interspecies competition further suppressed *E. coli* in a synergistic manner. The method by which phages are isolated can also impact the spectrum of the host range, and thus, several studies have looked into modified enrichment strategies in order to achieve this goal (31, 60, 61). This includes enrichment of phages from

environmental sources using multiple bacterial hosts, instead of a single host, as well as selecting phages that exhibit highly lytic activity, thus improving the chance that both highly lytic and broad-spectrum phages are obtained. Alternatively, the use of phage cocktails, or multiple phage types, is yet another widely explored method for the improvement of treatment outcomes (33). Using an *in vivo* mouse model, Maura et al. used a cocktail of virulent phages to target an enteroaggregative strain of *E. coli* colonizing the intestinal tract (62). They reported that phages penetrated the bacterial aggregates (i.e., biofilm), reducing the bacterial concentration in the ileal section of the intestines, without much interference from the mouse gut microbiota. In a separate study, the same group detected replication of their phage cocktail after 2 weeks in a similar *in vivo* mouse intestine model (63).

BACTERIOPHAGE AGAINST *KLEBSIELLA* BIOFILMS

Studies using phages for the control of biofilms formed by K. pneumoniae and other members of the *Enterobacteriaceae* are listed in Table 1. *K. pneumoniae* phages of the order *Caudovirales*, families *Siphoviridae* (64–66), *Podoviridae* (67, 68), and *Myoviridae* (69), have been reported. K. pneumoniae produces a capsular polysaccharide which may also be released into the extracellular environment to comprise the biofilm EPS matrix. Some bacteriophages of *Klebsiella* have evolved to produce depolymerases, which depolymerize these capsular polysaccharides and provide access of the phage particles to biofilm-associated *K. pneumoniae* cell clusters and to the cell surface (65, 67, 70–72). Brzozowska et al. characterized a tubular tail protein of *K. pneumoniae* bacteriophages (TTPA gp31) that exhibited a dual function as a structural protein involved in binding to the bacterial cell surface and as an enzyme hydrolyzing capsular and biofilm polysaccharides (70).

Different strategies have been employed against *K. pneumoniae* biofilms, including the use of phage cocktails and combination therapies. For example, Verma et al. used the lytic phage KPO1K2 to sensitize older *K. pneumoniae* biofilms to ciprofloxacin (54). They hypothesized that phage depolymerase activity contributes to matrix destabilization, allowing for better penetration of ciprofloxacin. Older biofilms (5 days) treated concomitantly with ciprofloxacin and a depolymerase-producing phage resulted in a greater log reduction of bacterial counts compared to each agent alone. The authors noted that although the heterogenous structure of the biofilm matrix may contribute to impaired antimicrobial penetration, the depolymerase activity of the phage aids in overcoming this challenge. In another study by this group (73), treatment of *K. pneumoniae* biofilms with phage plus ciprofloxacin resulted in a smaller number of ciprofloxacin-resistant morphological variants, and variants that were recovered exhibited reduced biofilm formation, smaller quantities of cell-associated capsular polysaccharides, and increased susceptibility to macrophages. Wu et al. (71) isolated a putative bacteriophage tail fiber protein (Dep42) that demonstrated enzymatic activity against the capsule of a multidrug-resistant *K. pneumoniae* strain and also enhanced the activity of polymyxin B against biofilms of this organism.

In other studies investigating combination treatments, Chhibber and colleagues coupled an iron-antagonizing molecule, in the form of copper sulfate, with a depolymerase-producing

lytic phage. Since iron plays an essential role in biofilm formation for many bacteria, the authors hypothesized that by reducing iron availability, biofilm development could be further impaired. They showed a greater reduction in viability of biofilm-associated cells in both younger (1 day) and older (7 days) biofilms compared to biofilms treated with each agent alone (67). In a polymicrobial biofilm model using *K. pneumoniae* and *P. aeruginosa*, a combination of phages, specific to each organism, was paired with xylitol in a synergistic manner, leading to significant reductions in each of the bacteria (74). Xylitol, a sugar alcohol, is believed to accumulate as a toxic, nonmetabolizable by-product, inhibiting bacterial growth as well as the production of stress proteins. Verma et al. (54) demonstrated that treatment of *K. pneumoniae* biofilms with phage or phage plus 10 μg/ml ciprofloxacin was more effective than ciprofloxacin alone. Singla and colleagues demonstrated that liposome-entrapped *K. pneumoniae* phages significantly reduced biofilms (up to 94.6% for biofilms up to 7 days old) and enhanced the efficacy of 40 μg/ml amikacin (68). Across these various studies, one common factor cited as contributing to the reductions observed was the activity of the phage depolymerases in destabilizing the biofilm matrix.

Phage cocktails have been considered by some to be a valuable tool as antimicrobial treatments, particularly in the treatment of chronic wounds and infections. Biofilms are often associated with chronic wounds and implanted medical devices; therefore, studies that model these conditions are of particular value. In a study using a murine model for a burn wound infection, Kumari et al. demonstrated the efficacy of a cocktail of *K. pneumoniae* phages. Animals that were burned, infected, and treated with phages had an 80 to 100% rate of survival over the course of 72 h, whereas untreated animals only experienced a survival rate of 5.53% (75). A similar burn wound study by Chadha and colleagues demonstrated a superior protective effect of a phage cocktail over a single-phage treatment (76). It should be noted that in these two studies, no control group with conventional antibiotic treatments was used, as their focus was on using phages as an alternative treatment altogether. A recent communication from researchers in Shanghai, China, described the use of a phage cocktail of *K. pneumoniae* phages in combination with a “nonactive” antibiotic (sulfamethoxazole-trimethoprim) in the treatment and clearance of a recurrent urinary tract infection caused by *K. pneumoniae* (77). Their report suggests that the phage cocktail potentiates a high-dose administration of the antibiotic, which was previously tested and found to be ineffective at preventing the growth of the urinary tract infection isolate.

ROLE OF PHAGES IN THE PREVENTION OF HEALTH CARE-ASSOCIATED INFECTIONS

The use of phages for therapeutic (i.e., clinical) and environmental applications is of particular interest due to their versatility and unique properties as replicating entities, their abundance in the environment, and a broad history of use as antimicrobial agents (78). In a historical sense, a great deal of knowledge of the use of phages for therapeutic purposes comes from institutes such as the Eliava Institute of Bacteriophages, Microbiology, and Virology (Republic of Georgia) and the Hirszfeld Institute of Immunology and Experimental Therapy (Wroclaw, Poland), whose focuses vary from the production of phage cocktails for general use to customized phage preparations (79). It is well established that health

care-associated infections (HAIs) such as central line-associated bloodstream infections, catheter-associated urinary tract infections, and ventilator-associated pneumoniae (22, 80, 81) lead to increased morbidity and mortality in addition to increased health care costs (82, 83); thus, there is growing interest, particularly in Western medicine, to incorporate alternative strategies in dealing with these dangerous and costly infections. Biofilms may play a role in HAIs (21, 84), and as such, strategies that have the potential to target biofilm-associated organisms are needed.

A number of studies focused on the use of phages to reduce the microbial burden on the surfaces of these devices have demonstrated various levels of efficacy (85–87). Melo et al. used a urinary catheter biofilm model to test a phage cocktail of two virulent *Proteus mirabilis* phages and demonstrated a reduction in biofilm formation over the course of 168 h compared to untreated catheters (88). The authors noted that the crystalline nature of *P. mirabilis* biofilms in catheters may play a role in reducing the efficacy of the phages. Similarly, Curtin and Donlan (86) and Lehman et al. (87) demonstrated that pretreatment of catheter lumens with phage cocktails targeting *Staphylococcus epidermidis* or *P. mirabilis* and *P. aeruginosa* were effective at reducing biofilm formation by both organisms over the course of 24 or 48 to 72 h, respectively (Fig. 2). *Enterobacter* spp. (e.g., *E. aerogenes* and *E. cloacae*) are recognized to be opportunistic pathogens of concern, particularly due to their involvement in lower-respiratory tract infections in patients in intensive care units (89). Work done by Jamal et al. highlighted the efficacy of phage MJ2, isolated from wastewater, against biofilms of a multidrug-resistant strain of *E. cloacae* grown on stainless steel coupons over the course of 120 h (90). The authors noted significant reductions in biofilms grown under both static and dynamic conditions after a 4-h treatment using their phage. It should be noted that although several studies have been identified regarding the isolation and characterization of phages for *Enterobacter* spp., relevant models looking at the efficacy of phages toward biofilms of *Enterobacter* spp. are still lacking.

The use of phages in the biocontrol of pathogens in the health care environment (e.g., patient rooms, hand-washing sinks, shower units) should also be considered in order to reduce the incidence of HAIs. Biofilms that are present in these environments can be colonized and ultimately serve as reservoirs for pathogens (Fig. 3). In an epidemiologic investigation of a hospital outbreak of carbapenem-resistant *K. pneumoniae*, researchers noted that along with patient-to-patient transmission due to geographical overlap with the index patient, transmission from the environment (e.g., sink drains and ventilators) likely contributed to additional patient colonization by the outbreak strain (91). A number of recent studies have highlighted the role that the built environment plays in the dissemination of pathogens in the health care environment (92–94). Droplet dispersal (95), sink positioning, and the presence of biofilm communities in sink p-traps (96, 97) further highlight the need for novel intervention strategies. A recently published study by Santiago et al. used an *in vitro* sink p-trap model to test the ability of a four-phage cocktail to target a KPC-producing strain of *K. pneumoniae* which colonized a six-species drinking water biofilm community (98). They demonstrated a significant reduction in the viable counts of biofilm-associated *K. pneumoniae* organisms relative to untreated controls, without a significant effect on the other members of the biofilm community, suggesting that targeting of specific pathogens in mixed communities is possible.

Another avenue of phage research that could have some potentially beneficial outcomes in regard to the control of HAIs is engineered phages. The use of engineered phages could help to overcome some of the limitations that may be encountered by naturally derived phages, such as narrow host specificity, stability, and biofilm degradation capabilities (99). Likewise, engineered phages can be used as biosensors to aid in the detection of relevant health care pathogens through a variety of methods and applications (100, 101).

SUMMARY

The expanding threat of antibiotic resistance in health care pathogens requires novel strategies to overcome treatment challenges. Biofilms may contribute to a chronic state of infection (102–104). Bacteriophages have reemerged as potential therapeutic agents to overcome some of the treatment challenges posed by widespread antibiotic resistance. Additionally, growing evidence points to the efficacy of bacteriophages against biofilm-associated bacteria either as customized single or cocktail applications or in combination with existing empirical treatments. Although there is a significant body of knowledge in regard to phage-host interactions and potential therapeutic and environmental applications in planktonic bacteria, future studies should continue to focus on phage-biofilm interactions as well as innovative strategies for biofilm control using phages.

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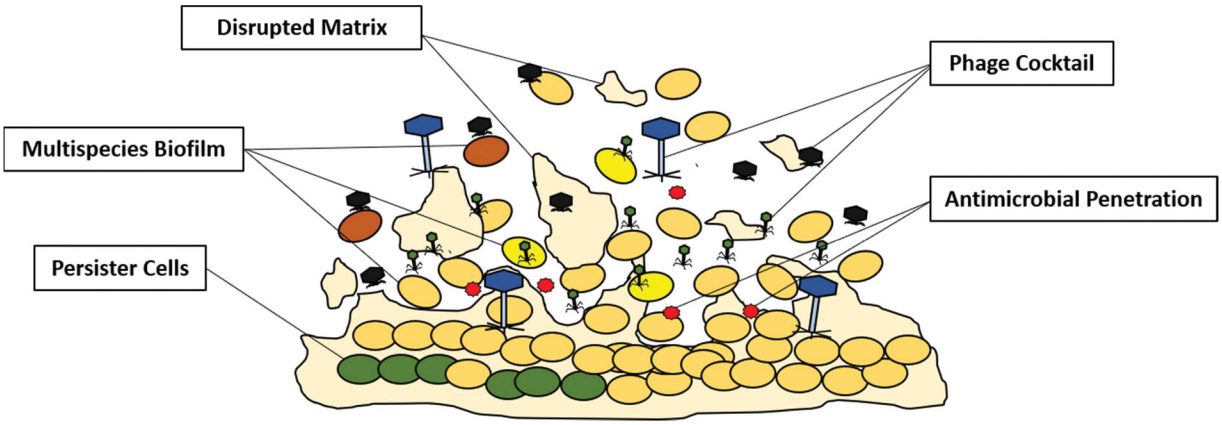

Figure 1. Schematic representation of phage interaction with biofilm.

Three phage types (shown as blue, green, and black phage particles) represent the use of phage cocktails for increased efficacy. Disrupted matrix represents depolymerase activity. Improved antimicrobial penetration is represented by red markers diffusing further into the biofilm. Various bacterial species are represented by brown, yellow, and light orange cells. Persisters are represented by green cells.

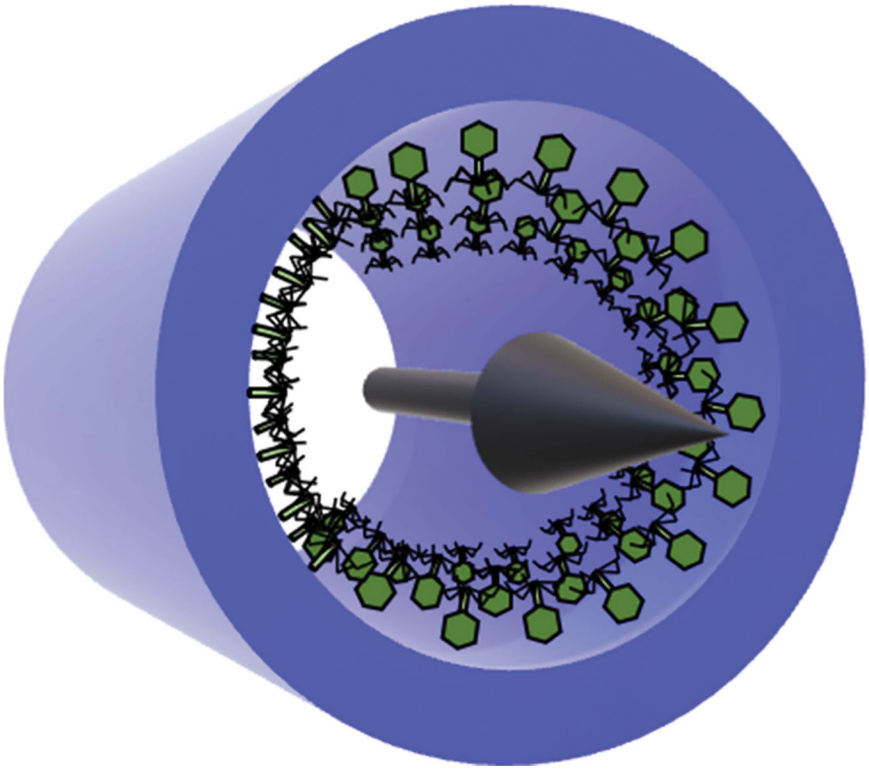

Figure 2. Schematic representation of phage-loaded urinary catheter lumens for the prevention of biofilm formation.

The catheter segment is represented in blue. Phage particles are represented in green. The black arrow represents the flow of urine.

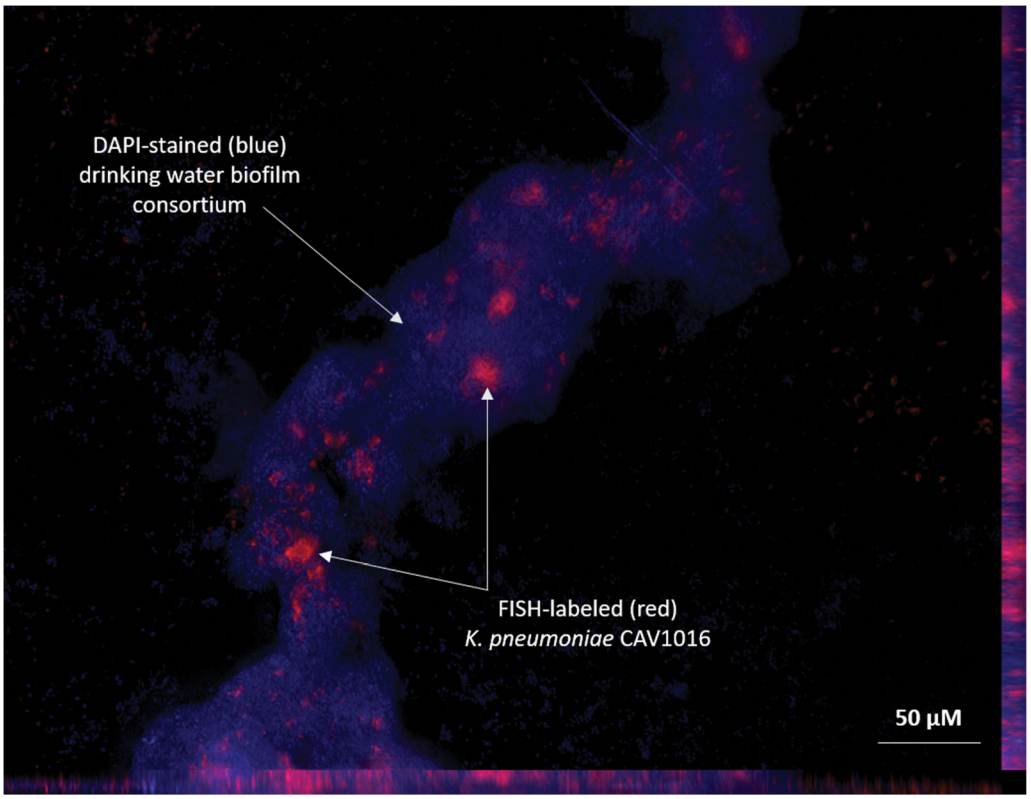

Figure 3. Multispecies drinking water biofilm colonized by *Klebsiella pneumoniae*.

Biofilms consisting of six drinking water bacteria were allowed to form on stainless steel coupons in a CDC biofilm reactor p-trap model for 28 days and then were inoculated with *K. pneumoniae* CAV1016 and grown for an additional 21 days. The biofilm was stained with a *K. pneumoniae* species-specific 23S rRNA fluorescent *in situ* hybridization probe and counterstained with 4′,6-diamidino-2-phenylindole (DAPI), visualized using a Zeiss Axioplan epifluorescence microscope with an Axiocam monochrome camera and ×40 oil immersion objective, and rendered using Axiovision image analysis software (Carl Zeiss). The biofilm consortium cells in the image are blue, and *K. pneumoniae* cells are red. The scale bar represents 50 µM.

TABLE 1

List of bacteriophages used in studies investigating antibiofilm properties

Phage	Family	Genome size (bp)	Host	Source	Ref.
KPV15	*Myoviridae*	167,034	*K. pneumoniae*	Wastewater	105
KPV811	*Podoviridae*	42,641	*K. pneumoniae*	Wastewater	105
ZCKP1	*Myoviridae*	160,000	*K. pneumoniae*	Freshwaters	69
KPO1K2	*Podoviridae*	~ 42,000	*K. pneumoniae*	Sewage	54
Kpn5	*Podoviridae*	23,100	*K. pneumoniae*	Sewage	106
Kpn12	*Podoviridae*	23,600	*K. pneumoniae*	Sewage	106
Kpn13	*Podoviridae*	24,000	*K. pneumoniae*	Sewage	106
Kpn17	*Podoviridae*	23,100	*K. pneumoniae*	Sewage	106
Kpn22	*Podoviridae*	23,100	*K. pneumoniae*	Sewage	106
vB_EcoM-UFV017	*Myoviridae*	~43,000	*E. coli*	Sewage	107
λW60	*Siphoviridae*	NA	*E. coli*	NIH stock	55
ACG-C40	*Myoviridae*	167,396	*E. coli*	Sewage	108
ACG-M12	*Siphoviridae*	46,054	*E. coli*	Sewage	108
ACG-C91	*Podoviridae.*	43,731	*E. coli*	Sewage	108
vB_EcoP-EG1	*Podoviridae*	39,919	*E. coli*	Sewage	109
F20	*Siphoviridae*	51,500	*E. aerogenes*	Aquatic	110
vB_EaeM_φEap-2	*Siphoviridae*	40,491	*E. aerogenes*	Hospital sewage	111
UZ1	*Podoviridae*	~ 34,000	*E. aerogenes*	Hospital sewage	112
SF153b	NA	NA	*Enterobacter agglomerans*	Sewage	52