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Susceptibility of *Achromobacter* spp. to bacteriophages and phage/antibiotic combinations

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Achromobacter infections pose significant challenges to people with Cystic Fibrosis (CF). It is a growing problem in people with CF because it is difficult to eliminate with antibiotics, leading to chronic lung infections that increasingly acquire antibiotic resistance. Here we identified a cadre of 26 bacteriophages that target clinical Achromobacter isolates. We tested these phages against a collection of 56 Achromobacter isolates and found that all were susceptible to multiple of these phages. We also evaluated whether we could restore the activity of certain antibiotics by using them as adjunctive therapy to phages in vitro. We found that when meropenem (a beta lactam antibiotic) was added to the phages, improved susceptibility (decreased MICs) was demonstrated in many isolates regardless of whether the Achromobacter was initially susceptible to meropenem. The data presented here suggests that combination therapy using meropenem with an active phage should be considered for treatment of Achromobacter infections regardless of pre-existing antibiotic susceptibility.

Importance

Achromobacter infections are a rising threat to those with Cystic Fibrosis and other chronic pulmonary disease due to their ability to acquire resistance following exposure to antibiotics. Achromobacter accumulates resistance to antibiotics because resistant strains of Achromobacter persist in the same individual over time while becoming increasingly multi-drug resistant (MDR) or extensively drug resistant (XDR) following exposure to new antibiotics, driving the need to develop alternative therapies for subsequent treatment. We identified a large group of bacteriophages that kill Achromobacter and found that they killed all clinical isolates within our MDR and XDR collection. Moreover, we also tested whether the combination of antibiotics and phages could result in the reversion of meropenem MICs from "resistant" to "susceptible" or just improving meropenem MICs when isolates were not resistant. Antibiotic-phage combinations resulted in conversion to meropenem susceptibility based on assessment of MICs in many instances as long as phage susceptibility was present in vitro. This indicates that prior meropenem susceptibility was not a requirement to achieve antibiotic-phage synergy. Thus, phage treatments for Achromobacter infections can potentially be instituted in combination with antibiotics such as meropenem regardless of antibiotic susceptibility testing results.

Introduction

Antibiotic resistant bacteria present a serious and growing challenge as their numbers continue to increase across the globe[1]. Nonfermenting gram negative bacilli are among the most antibiotic-resistant[2]. The constant evolutionary battle between competing bacteria has promoted the emergence of several mechanisms of antibiotic resistance[3], including enzymatic destruction of antibiotics and mutations in the intracellular targets within bacteria; changes in cell wall structure to prevent antibiotic entry; and efflux pumps that allow the organisms to move potentially lethal compounds out of the cytoplasm. *Achromobacter* spp. contain virtually all these mechanisms of antibiotic resistance[4].

Patients with chronic infections, particularly those with Cystic Fibrosis (CF), provide anatomic locations within the lower respiratory tract in which bacteria like Achromobacter can exist and evade host defenses and antibiotic entry[5,6]; the bronchiectatic lung in CF may contain large pockets of infected mucus within injured lung. Mucus plugs may also create conditions

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Table 1 | Genome characteristics and isolation sources for phages identified in this study

Phage name	Genome size (bp)	Total proteins	% hypothetical proteins	Isolation Source	GeneBank ID
Papale	45,488	76	92%	Wastewater from PA, USA	PV441885
Thup	61,349	93	45%	UCSD wastewater, CA, USA	PV340829
Taco	44,326	75	92%	Wastewater from PA, USA	PV340830
Barbera	93,528	472	63%	UCSD wastewater, CA, USA	PV340831
Kaach	50,395	89	83%	Imperial Beach, CA, USA	PV340832
MochiFu	45,993	137	81%	Wastewater from PA, USA	PV340833
MayoRox	46,092	77	95%	Mayo Rox, OH, USA	PV340834
Malbec	65,576	101	56%	UCSD wastewater, CA, USA	PV340835
Kiitash	41,767	60	72%	San Diego River, CA, USA	PV340836
Fargo	43,287	77	70%	Los Peñasquitos, CA, USA	PV324754
Ewill	45,694	69	94%	Imperial Beach, CA, USA	PV340837
BuckLyse	45,974	74	93%	UCSD wastewater, CA, USA	PV340838
Barolo	45,903	75	92%	UCSD wastewater, CA, USA	PV340846
Lo1	45,807	73	92%	UCSD wastewater, CA, USA	PV340845
Cava	45,798	73	92%	UCSD wastewater, CA, USA	PV340844
Europa	46,183	67	88%	UCSD wastewater, CA, USA	PV340843
Siren	98,908	147	96%	UCSD wastewater, CA, USA	PV324754
Daedalus	99,063	145	96%	UCSD wastewater, CA, USA	PV340842
Theseus	98,427	219	98%	UCSD wastewater, CA, USA	PV340841
Mallory	62,288	94	91%	UCSD wastewater, CA, USA	PV544237
Sirah	45,812	71	92%	UCSD wastewater, CA, USA	PV943645
Nemas	98,277	156	96%	Imperial Beach, CA, USA	PV340839
Cabernet	45,959	75	93%	UCSD wastewater, CA, USA	PV340840
Kaykay	45,818	71	90%	San Dieguito Lagoon, Del Mar, CA	PX354141
Prosecco	40,288	58	83%	UCSD wastewater, CA, USA	PZ013370
Marine1	225,656	284	97%	UCSD wastewater, CA, USA	PZ013371

Gene IDs correspond to entries in the NCBI taxonomy database.

within airways and lungs that allow bacteria to survive[7], providing the opportunity for repeated invasion of adjacent airways and lung[8,9]. Repeated antibiotic treatment for recurrent infection allows expression and persistence of resistance mechanisms noted above[10], creating antibiotic-resistant pathogens that force us to direct our attention to other means of killing bacteria, including the use of Achromobacter bacteriophages[11]. Pulmonary disease, particularly in people with CF, can be similar in morbidity to *Pseudomonas aeruginosa* infections, and infection with both pathogens can lead to more severe disease[7]. Additionally, the Achromobacter genus is involved in infections other than in those with CF, are circulating in several regions of the world, and phages could serve as therapeutic options[12].

Phages have been used as experimental treatments for antibiotic resistant bacterial infections, usually when they are applied alongside required standard-of-care (SOC) antibiotics, even if the bacterial pathogen is resistant to all SOC antibiotics[13]. In these situations, it often is not known whether there are cooperative effects (additive or synergistic) between the antibiotics and the phages or whether there may be antagonism. There also is very little guidance to suggest which antibiotics and phages to use in combination to treat infections in such patient populations[14].

In this work, we identified 26 phages that infect *Achromobacter* spp. and determined that they kill all 56 isolates in our clinical collection. We tested the effects of phages and antibiotic combinations against *Achromobacter* spp. using several antibiotics with different mechanisms of action and found that certain phages combined with specific antibiotics were likely to result in synergistic interactions against the *Achromobacter*. spp. even when there was no evidence of prior antibiotic susceptibility.

Results

Achromobacter collection

In an effort to identify a diverse collection of Achromobacter strains, we collected a group of isolates from UCSD hospitals between 2022 and 2025. These isolates were identified as *Achromobacter xylosoxidans* via the MALDI-Tof, but no further sequencing or analysis performed to verify their identities[15,16]. They had varying antimicrobial susceptibilities. We collected 56 isolates in total, with 9 qualified as multi-drug resistant (MDR) and 21 qualified as extensively drug resistant (XDR). Many of these were isolated from people with CF, however, a number were isolated from individuals with other acute and chronic conditions (Tables S1 and S2). Many of these Achromobacter infections repeatedly failed antibiotic treatment and were often recurrent in nature, demonstrating the need to develop alternative treatment strategies aside from antibiotics alone. We repeatedly worked from the original stock solutions of the *Achromobacter* spp. isolates to reduce the potential that the bacteria would adapt to laboratory conditions.

Isolation and characterization of phages

While there are phages capable of killing *Achromobacter* spp., we did not have access to any of those phages[11,13,17–19]. We therefore engaged in a phage hunting expedition to identify phages with strictly lytic potential against Achromobacter isolates (Table 1). We identified 26 phages against Achromobacter that demonstrated relatively diverse morphologies. Via electron microscopy, we imaged each of the phages and found most of the morphologies to be consistent with siphoviruses (Fig. 1).

We sequenced the genomes of each of these phages to classify them according to their genetic sequences. We confirmed their genome sizes

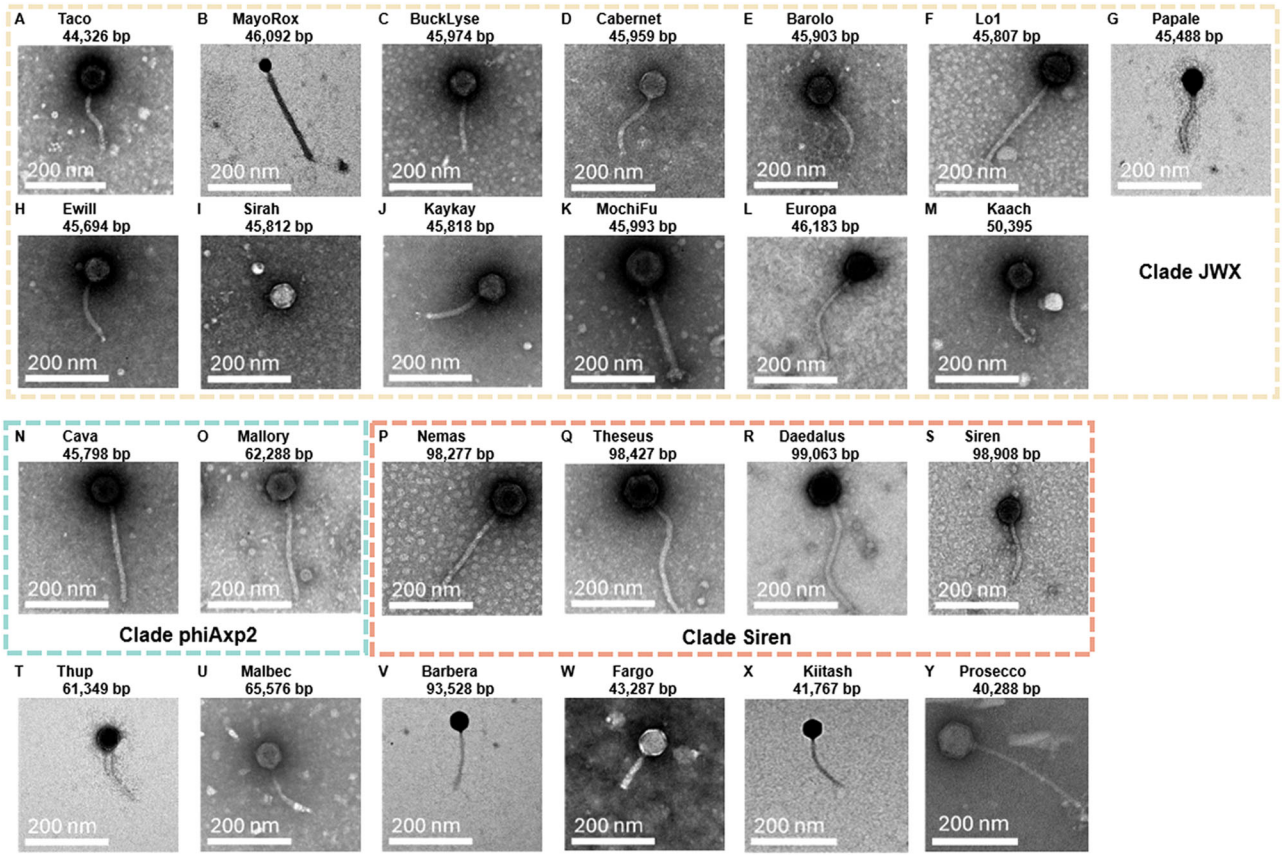


Fig. 1 | Achromobacter Phages Morphology. Transmission Electron Microscopy of 25 phages. Phages are organized by their phylogenetic clades, Clade JWX (**A**-**M**), Clade phiAxp2 (**N, O**), Clade Siren (**P**-**S**), monophyletic phages (**T**-**Y**).

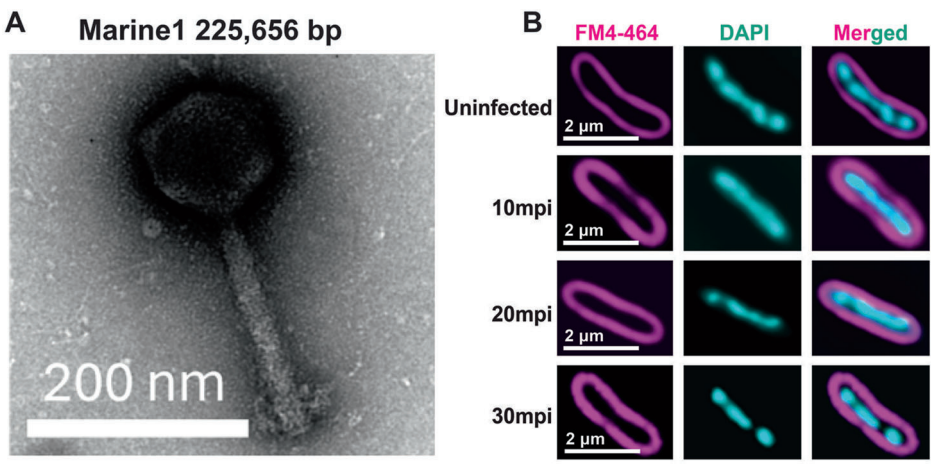


Fig. 2 | Achromobacter jumbo phage Marine1 morphology and infection phenotype. **A** Transmission Electron Microscopy of jumbo phage Marine1. **B** Fluorescence microscopy of *Achromobacter* spp. AX49 cells, either uninfected or infected Marine1. Images were acquired at the indicated time points post-infection. Cell membranes were stained with FM4-64 (magenta) and nucleic acids with DAPI (cyan). Time is denoted as min post-infection (mpi).

ranging from 40.3 kb to 225.7 kb (Table 1). The largest of the phages, Marine1, represented a jumbo phage (Fig. 2–A), but did not have a nucleus-like structure as determined by DAPI staining (Fig. 2–B). We classified each of the phages via phylogenetic analysis and found that many were similar yet distinct from previously identified Achromobacter phages (Fig. 3). They spanned nearly every clade of previously identified Achromobacter phages available. Four phages belonged to a previously undescribed clade that we called Siren. An integrase was identified in phage Thup, but no other integrases or other genes suggesting lysogeny were identified in the other phages. The jumbo phage Marine1 did not form a cluster with a prior Achromobacter jumbo phage Motura. When placed into a phylogenetic structure including only other previously identified jumbo phages (Table S3), it clustered most closely with a Ralstonia jumbo phage RSL2 (Fig. S1). Both Achromobacter and Ralstonia are Betaproteobacteria.

Host ranges

Given the breadth of this phage collection, we evaluated whether they were capable of efficiently killing our Achromobacter isolates. We used quantitative plaque assays to assess the susceptibility of each isolate to each phage. We found that for all of the isolates, there was at least one phage capable of lysing it efficiently (Fig. 4; Supplementary Data 1). Phage Barbera showed the broadest host range, killing 84% of the tested bacteria strains. For most

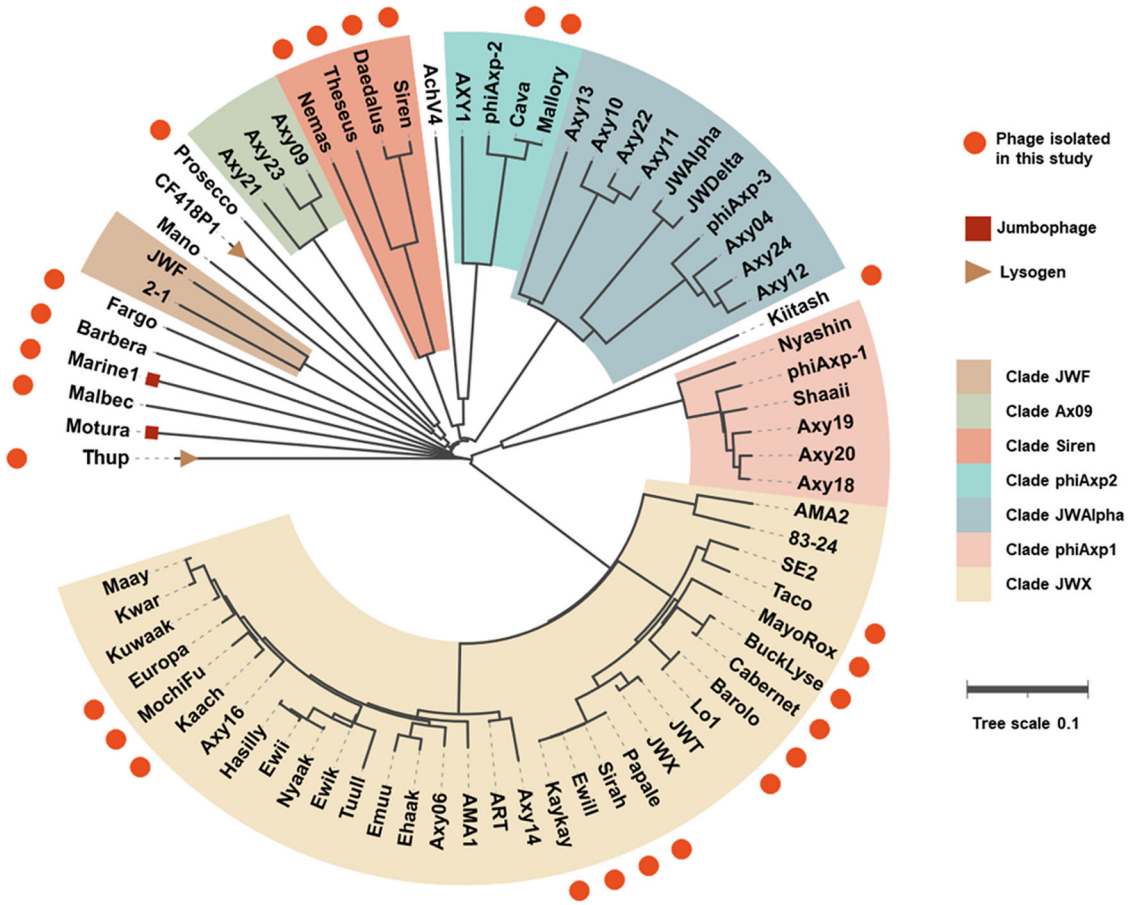


Fig. 3 | Phage Proteomic Tree of Achromobacter phages. Phylogenetic relationships between 73 Achromobacter phages. Including 26 phages isolated in this study (red circle), and 47 genomes of publicly available Achromobacter phages as of March 2025. The Phage Proteomic Tree was calculated using the VIPtree server and edited in iTOL. Six previously described clades are shown, plus the new Clade Siren.

isolates, there were multiple phages capable of killing them, and all of the phage lysis could be diluted to single plaques and recovered, which excluded the potential that there was lysis from without. Interestingly, all of the MDR and XDR isolates could be killed efficiently by phages, suggesting that antibiotic resistance and phage susceptibility were uncoupled.

We also examined efficiency of plating (EOP; Figure S2), which is a measure of a phages ability to infect and create plaques on a host bacteria compared to a reference host[20]. In this analysis, a different set of phages emerged than we might have predicted based on our initial host ranges (Fig. 4). Our primary focus was on phages Barbera, BuckLyse, and Taco because of their broad host ranges, but they were not the phages with the best EOP values that suggested they may be viable for use in phage therapy. The best EOP values were identified for phages Cabernet, Kaykay, and Europa (Fig. S2), although their EOP values were not substantially different than those identified for many other phages. We were surprised that there were relatively few *Achromobacter spp*. isolates killed efficiently by phage Taco.

Phage-antibiotic synergy

Because Achromobacter infections persist in the CF lung despite proper antibiotic therapy[7], we tested whether there might be cooperativity between antibiotics and phages to assist with reducing bacteria burden beyond that achievable with antibiotics alone. We set up grided arrays in 96-well plates where each well had the same number of bacteria, but the amount of phage or antibiotic added differed in concentration gradients across the y-axis and x-axis, respectively (Fig. 5 A–C; Supplementary Data 2). We performed these assays for multiple Achromobacter isolates using multiple different phages and antibiotics.

We focused primarily on the antibiotic meropenem because it represents a common treatment for Achromobacter infections although many of our MDR and XDR isolates demonstrated resistance to it (Tables S1 and S2). Our primary goal was to determine whether we could identify cooperativity between the phages and the antibiotic in a manner that resulted in reduced MICs to meropenem, indicating synergy between the phage and the antibiotic. We found evidence of synergistic interactions for *Achromobacter* spp. strain AX31 with phages BuckLyse and Kaach (Fig. 5, Panels A, B, D, E, G, and H). We did not find evidence of synergy when phage Taco was used with meropenem (Panels C, F, and I). We also did not identify evidence of synergistic interactions with strain AX19, largely due to resistance to phage BuckLyse (Figure S3, Panels A, D, and G), or due to exquisite susceptibility to phages Kaach (Fig. S3 Panels B, E, and H) and Taco (Fig. S3 Panels C, F, and I).

We also tested most of the *Achromobacter* spp. isolates for evidence of synergistic interactions between phages and meropenem (Fig. 6, Panel A; Supplementary Data 3) and found that 77% of them demonstrated evidence of synergy (Fig. 6, Panels B, B', B", C, C', C"). Of the remainder of the isolates that did not demonstrate evidence of synergy, 15% were exquisitely susceptible to the phages making it difficult to demonstrate synergy (Fig. 6, Panels D, D', D"), and 4% were resistant to the phages tested and meropenem. The synergistic interactions between phages and meropenem were interesting because when the combination of antibiotics and phages were used, the MIC for the combination was significantly reduced below the CLSI recommended breakpoint for meropenem (4 mcg/mL). That we identified the phenomenon for multiple Achromobacter isolates, and for Achromobacter phages BuckLyse, Kaach, and Taco, indicates that these results were not specific to an individual phage in our collection.

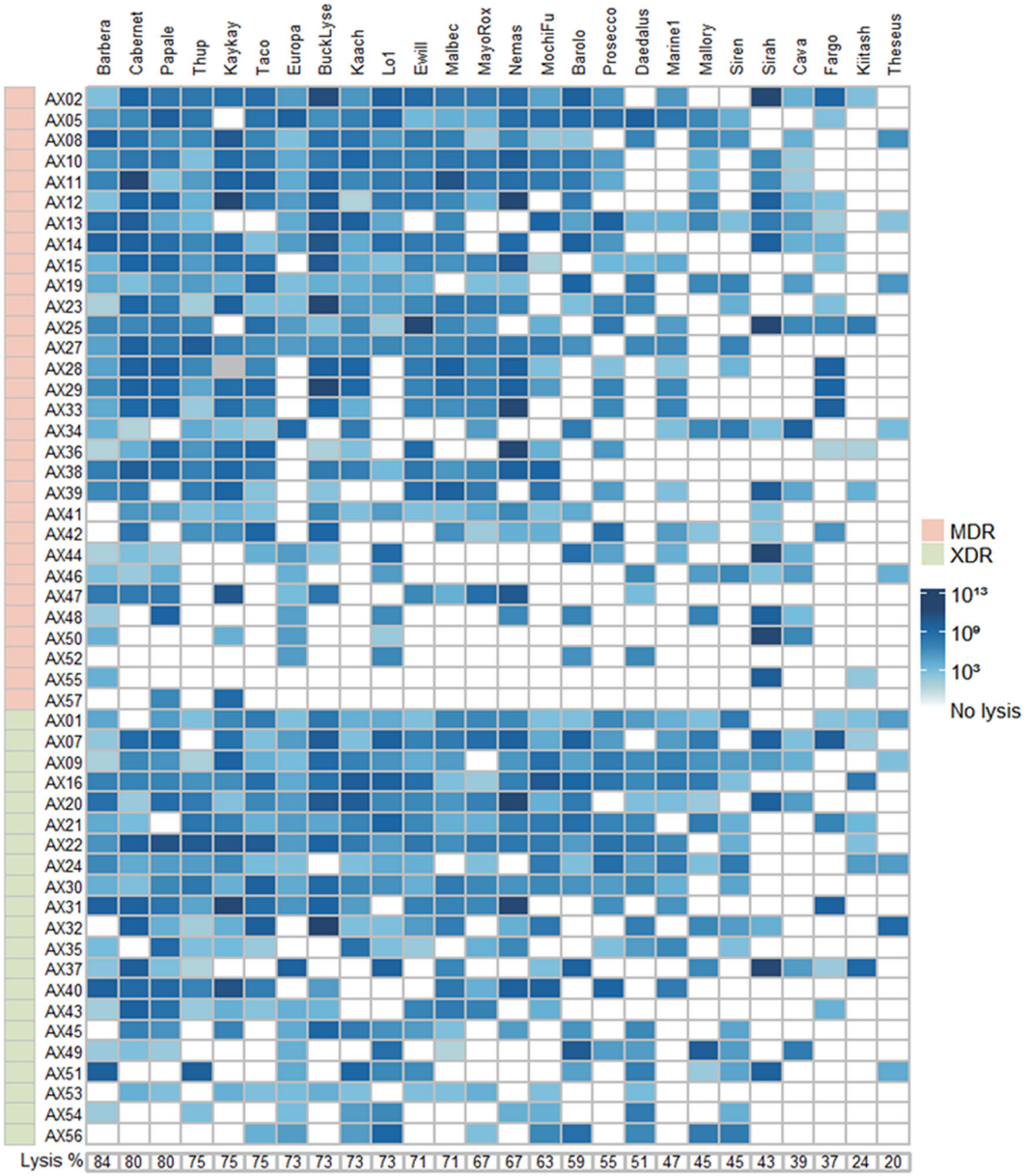


Fig. 4 | Host range matrix of 26 Achromobacter phages vs 50 clinical *Achromobacter* spp. strains. Phage infection was tested in 0.3% LB agar plates using 12 phage dilutions.

Our initial synergy screening included 4 antibiotics: two beta lactam antibiotics that inhibit cell wall synthesis (meropenem and ceftazidime), one antibiotic that inhibits folate production therefore blocking DNA and proteins synthesis (trimethoprim-sulfamethoxazole), and one fluoroquinolone which inhibits DNA replication (levofloxacin). In addition to identifying synergy with meropenem, we also identified synergistic interactions with ceftazidime (Figs.S4 and S5). Some synergistic interactions were observed between phage Kaach and trimethoprim-sulfamethoxazole, but not with other phages (Figs. S6 and S7). We did not identify synergistic interactions between the three phages tested and levofloxacin (Figs. S8 and S9). While these results indicate that synergistic interactions across different antibiotic mechanisms are possible with phages, they were not consistent with any of the antibiotics tested aside from beta lactams.

Discussion

There are a number of pathogens that are prone to acquiring antibiotic resistance and become significant problems in immunocompromised, long-term care, and chronically ill populations such as patients with CF. Even with the use of CFTR modulators there is still chronic bacteria colonization in CF patients[21]. *Achromobacter* spp. has become a significant problem due to its ability to become both MDR and XDR, and particularly in the CF population where it becomes a chronic colonizer; this feature of the organism can result in deadly outcomes[5,7,9]. Because *Achromobacter* spp. accumulates resistance as the human host is exposed to antibiotics over time, it appears inevitable that it will become MDR and/or XDR[8,22]. Phage therapy has the potential to address the unmet need for alternative Achromobacter anti-infective therapy[13,23,24].

To address the *Achromobacter* spp. problem at our institution, we developed a collection of phages that specifically target the organism. The collection of 26 phages showed complete coverage of all the tested patient-

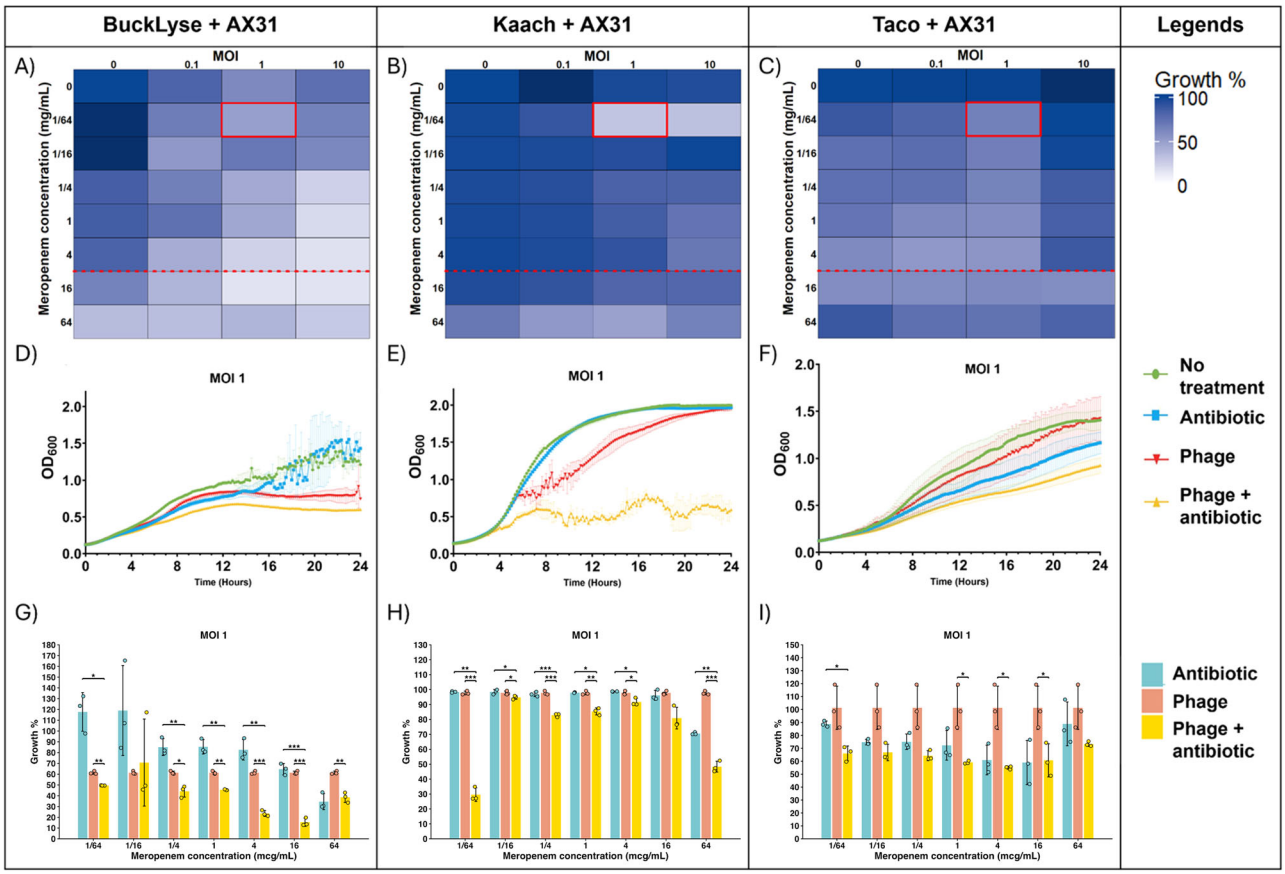


Fig. 5 | Phage-antibiotic-synergy between meropenem and select phages for strain AX31. Multiplicity Of Infections of 0.1, 1 and 10 were tested for all isolates, and meropenem concentrations between 64 mcg/mL to 1/64 mcg/mL were also tested in liquid media at 37°C over 24 h. Three separate independent biological replicates were performed per experimental condition. Percentage growth was calculated relative to the no-treatment (**A**–**C**), and meropenem breakpoints are shown with a red-dotted line. Representative growth curves (**D**–**F**) showing the mean of 3 biological replicates with error bars representing standard deviation. Bar charts display the mean of growth percentage over antibiotic concentrations at MOI = 1 for 3 biological replicates (dots) with error bars representing standard deviation (**G**–**I**). Pairwise differences were evaluated using an independent two-sample t-test, and *p*-values were corrected for multiple comparisons using the Holm method. Significant thresholds are shown as $p \le 0.05$ (*), $p \le 0.01$ (**), $p \le 0.001$ (***), $p > 0.05$ not significant and not shown.

derived MDR and XDR *Achromobacter* spp. in our collection. This finding suggested that naturally occurring phages can provide a solution to the complex problem of MDR and XDR *Achromobacter* spp. infections; we just haven't been developing that solution. Most of these phages had morphologies consistent with siphoviruses (Fig. 1), although it was difficult to discern from images alone. Jumbo phage Marine1 did not possess a nucleus-like structure (Fig. 2), which protects the phage from bacterial immune components[25], that usually increases the host range of the phage. Interestingly, phage Marine1 was one of the lower host range phages in this collection, lysing only 47% of the *Achromobacter* spp. isolates.

We chose phages BuckLyse, Taco, and Kaach for our cocktail/synergy experiments because they demonstrated complementary activity with one another (Fig. 4), and phage BuckLyse originally was our broadest-spectrum phage, lysing ~86% of our isolates compared to 84% for phage Barbera. However, during the course of the experiments, we noted that BuckLyse lost host range and was no longer our broadest host range phage. We retested the host range after the experiments were concluded and found that it only lysed 73% of the *Achromobacter* spp. isolates, suggesting that phage Barbera would have been a more appropriate choice for the cocktail. While our goal here was more targeted towards the in vitro activity of these phages, the examination of EOP (Figure S2) strongly suggests that a different set of phages that may include Europa, Cabernet, and Kaykay might be more appropriate candidates for a cocktail targeting clinical *Achromobacter* spp. isolates. Just examining phage host ranges versus examining efficiency may lead to conclusions that are not well founded. As we have observed in this study, phage Taco is one of our broadest host range phages (kills 75% of the isolates yet is amongst the least efficient isolates; Figs. 4 and S2) yet would be deemed inappropriate for therapy against most Achromobacter isolates based on its EOP.

Just finding phages capable of killing antibiotic-resistant microbes wasn't necessarily the end game. Antibiotics remain the standard of care for bacterial infections and won't be easily replaced by phages. Thus, we evaluated whether there may be complementary interactions between antibiotics and phages. We identified synergistic interactions between antibiotics and phages before[26–28], as have others[29,30], so we anticipated that we may find some complementary interactions between antibiotics and phages in the killing of *Achromobacter* spp. We focused on meropenem (Fig. 6) because it is an often-used anti-Achromobacter drug for serious infections[4], but we observed somewhat similar results for other antibiotics as well (Figs. S4–S7). Synergy between meropenem and phages has been reported in *Klebsiella pneumoniae*[31], *Acinetobacter baumanii*[32], and *Pseudomonas* aeruginosa[33].

We did not attempt to develop mutant *Achromobacter* spp. isolates that were resistant to our phages. In our other works[27], we have successfully been able to develop phage resistant mutants when we attempted to do so; however, we could not guarantee that the mutations would occur in the phage receptors or whether they might alternatively occur in other bacteria components such as mucous layers or other membrane components that render receptors difficult to access. We intend to characterize phage receptors for this collection as part of a separate work.

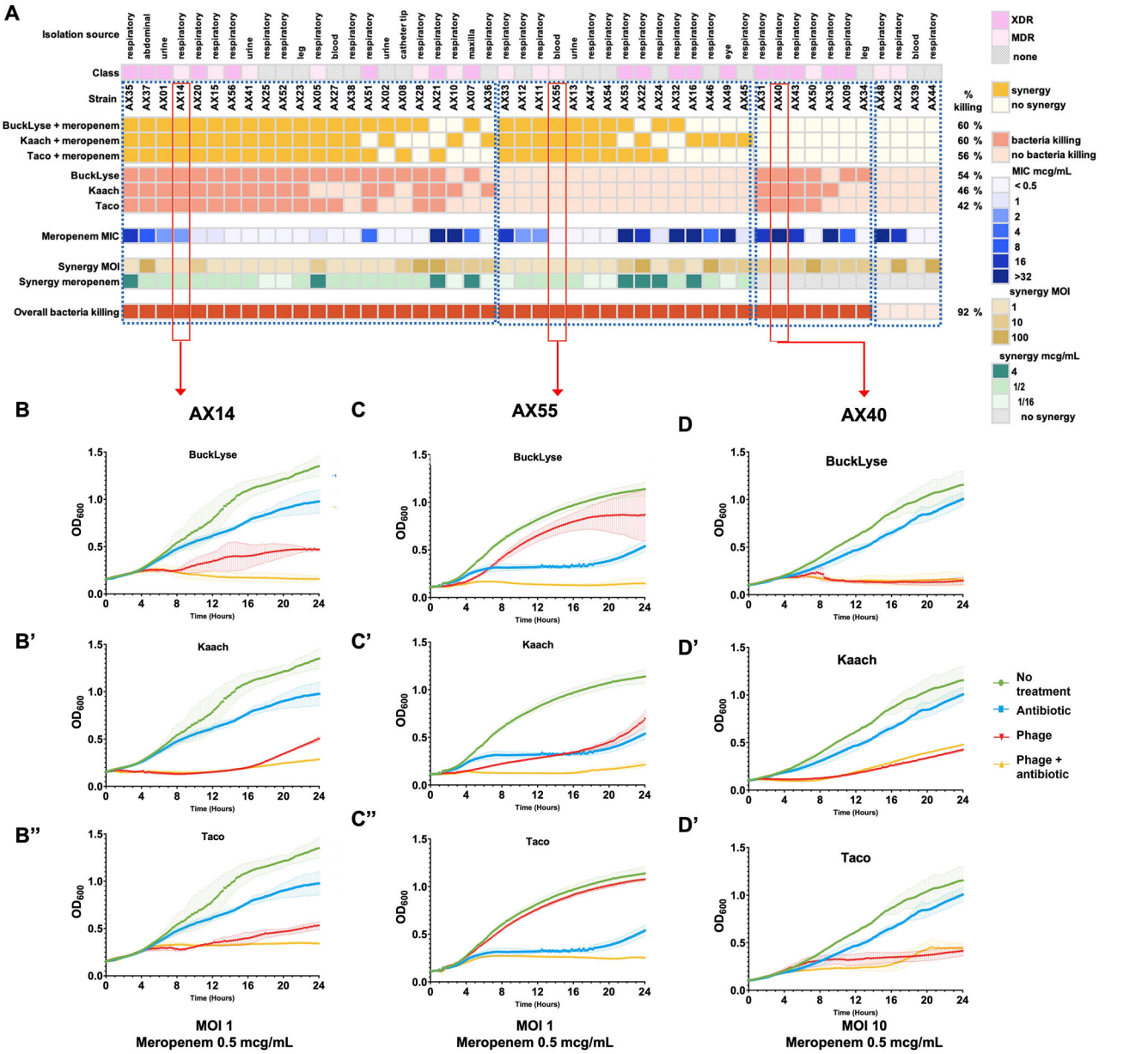


Fig. 6 | Summary of Phage-Antibiotic-Synergy between meropenem and phages BuckLyse, Kaach or Taco in 48 clinical strains. A Bacteria killing by phages and antibiotics (yellow scale) or phages alone (orange scale) in liquid over 24 h; meropenem MIC for each isolate (blue scale); MOI (brown scale) and meropenem concentration (green scale) at which synergy was observed. Overall bacteria killing (red scale). All experiments were performed using $n = 3$ biological independent experiments per condition. **B** Representative growth curves of synergy in which phages plus antibiotics show greater bacteria killing than each one separately. **C** Representative growth curves of synergy in which the phages show moderate to no bacteria killing by themselves, but there is strong bacteria killing with the addition of antibiotics. **D** Representative growth curves of phages capable of bacteria killing, but there is no synergy with the addition of antibiotics. In all growth curves the mean of 3 biological replicates is shown along with standard deviation.

We considered developing cocktails of different phages in this collection consisting of phages with different host ranges to potentially target the vast majority of the *Achromobacter* spp. isolates encountered. We ultimately decided against this strategy because we believe that the primary target of this phage collection will be to treat people with CF with antibiotic-resistant *Achromobacter* spp. infections. Because of the relative difficulties in completely eradicating the microbe from the CF lung, we ultimately decided against a cocktail strategy because we believed that it would likely select for *Achromobacter* spp. isolates that alter their membranes to become less accessible by our phages. In this case, we believe that other strategies such as successive individual treatments[34] may be more likely to be successful.

We observed significant inhibition of the bacteria when antibiotics and phages were used in combination, with 77% (37 of 48 isolates with interpretable results) demonstrating evidence of synergy. In fifteen cases, the bacteria had MICs above measured CLSI breakpoints for resistance to meropenem, and those measured MICs were reduced to below the CLSI breakpoint for susceptibility in each of these instances when used in combination with phages (Fig. 6). This is similar to what we observed in the *Enterococcus* spp. isolates for several different antibiotics[27]. These observations suggest that phage-meropenem combinations may restore meropenem activity against some resistant isolates. While these principles have not been demonstrated in patients or animal studies, they are highly reproducible in vitro and will be the subject of future clinical trials to identify the effects of combination antibiotic-phage treatments on bacterial clearance for hosts such as *Achromobacter* spp.

Methods

Achromobacter isolates and antibiograms

Strains used in this study were isolated from patients at the UCSD Center for Advanced Laboratory Medicine. Their use was approved by the University of California San Diego Administrative Panel on Human Subjects in Medical Research and was categorized as exempt, which means that is does not qualify as human subjects research and includes a waiver of informed consent for the use specimens obtained. Each of the isolates were identified using MALDI-TOF (Bruker, database version 5.1.420.61). After their initial growth, purity plates were made, and a minimum of 6 separate stocks were created from each microbe to ensure that the original non-laboratory-adapted version of the isolates could be used for all future experiments. This allowed us to revert to the original version of the *Achromobacter* spp. isolates with minimal passages of the bacteria rather than work with laboratory adapted versions of the bacteria. These non-laboratory-adapted versions of the bacteria often only survived for several passages, making it difficult to perform antimicrobial susceptibility testing, phage susceptibility testing, and synergy testing without going back to the original stocks of the bacteria to ensure their viability. Antibiotic susceptibility testing was performed on the bacteria isolates using MicroScan NM46 panels combined with e-tests for imipenem, ciprofloxacin, tigecycline, and minocycline[35] (Tables S1 and S2). MDR or XDR classifications were defined as previously described and cutoff values for susceptibility were based on CLSI standards using CLSI M100 Ed35 using cutoff values for Other Non-Enterobacterales[36,37]. Antimicrobial susceptibilities were based only on the antimicrobial susceptibilities tests that were performed and could have changed if other additional antibiotic classes were tested.

Phages isolation

Fifty milliliters of fresh wastewater (municipal sewage or freshly collected water source) was centrifuged at 5000 rpm for 20 min and the supernatant was collected. Wastewater was collected from facilities in Pittsburgh, PA, Mayo Rox, OH, Imperial Beach, CA, San Diego and other suburbs, CA, and the campus of UC San Diego (Table 1). The supernatant was syringe filtered using a 0.2 µm filter. One milliliter of filtered wastewater was incubated at 37 °C for 16 h with 20 µL of 0.2 OD600 bacteria (*Achromobacter* spp. AX19) in 2 mL of LB media supplemented with 10 mM $MgCl_2$ and 10 mM of $CaCl_2$. After co-incubation, the liquid was 0.2 µm filtered, and eight serial dilutions were made. Two microliters of each serial dilution were spotted on a bacteria lawn of 0.3% LB agar supplemented with 10 mM $MgCl_2$and 10 mM $CaCl_2$. The plates were incubated for 16 h at 37 °C. Individual plaques were picked and plaque purified 3 times. After plaque purification, a phage stock was produced in liquid culture.

Jumbo phage isolation

Wastewater (municipal sewage) from UC San Diego campus was centrifuged at 3000 x *g* for 10 min and the supernatant was collected, then centrifuged at 4696 × *g* for 55 min. The supernatant was discarded and the pellet was resuspended in 10 mL of SM buffer and gently vortexed. An overnight culture of *Achromobacter* spp. AX49 was diluted to 0.2 OD600, 200 µL of culture was combined with 4 mL of warmed 0.3% LB top agar and poured over 1% LB plates. The plates were dried for 15 min. Ten microliters of processed wastewater were spotted and dried under an open flame for 30 min. Plates were incubated upside down overnight at 37°C. Phages formed ~1 mm plaques on LB top agar (0.3%) over the bacterial lawn. Phage plaques were picked using a pipette tip and gently stabbed repeatedly into an LB plate with 1% agar. This area was then streaked with a loop, changing loops with each streak. Molten 0.3% LB top agar and 100 µL of overnight 0.2 OD600 AX49 culture were combined and poured onto the plate starting from the lowest dilution point to the stabbed area. The top agar was then allowed to sit at room temperature for 15 min before being incubated overnight at 37°C. Plaques were purified 3 times.

Phage morphological characterization

Phage lysates were concentrated using a 10 KDa MWCO cellulose filter (Amicon, Millipore Cat# UFC9010). Concentrated phage lysates (titer > 10^9 PFU/mL) were used for imaging. A carbon-coated grid (PELCO SynapTek Grids, product# 01754-F) was placed on a drop of 40 µL of phage lysate. Grids were washed with DI water twice and then negatively stained with 2% uranyl acetate (pH 4.0) for 45 seconds. Imaging was performed using JEOL 1400 plus located at the University of California, San Diego—Cellular and Molecular Medicine Electron Microscopy Core (RRID: SCR_022039).

Fluorescence microscopy for phage infection

Fluorescence microscopy was performed in at least two independent biological replicates. For infection, 100 µL of overnight-grown host cells (*Achromobacter* spp. AX48) was infected with 100 µL of jumbo phage Marine1 and incubated for 10 min at 37°C. The fixative was prepared by combining 1 mL of 16% paraformaldehyde with 1 µL of 25% glutaraldehyde and mixing thoroughly. Forty microliters of this fixative were added to 200 µL of exponentially growing cells, along with 8 µL of 1× PBS, followed by gentle mixing. Cells were incubated at room temperature for 20 min.

Following fixation, cells were pelleted at 6000 × *g* for 2 min and washed three times with 1× PBS. The final pellet was resuspended in 20 µL of staining solution prepared in 25% LB, supplemented with DAPI (from a 2.5 mg/mL stock; Thermo Fisher Scientific, Cat# D1306) and FM4-64 (from a 3.75 µg/mL stock; Thermo Fisher Scientific, Cat# T13320). Cells were stained for 5 min at room temperature. Stained cells were then mounted on 1% agarose pads prepared in 25% LB on single concave slides and immediately imaged.

Live-cell imaging was performed using a DeltaVision Elite Deconvolution microscope (Applied Precision, Issaquah, WA, USA). Immediately before imaging, a glass coverslip was placed on top of the agarose pad. For static fluorescence microscopy, imaging pads were stained with 8 µL of dye mixture (25 µg/mL DAPI and 3.75 µg/mL FM4-64) at room temperature for 1 minute prior to imaging (DAPI: Thermo Fisher Scientific, Cat# D1306; FM4-64: Thermo Fisher Scientific, Cat# T13320). Images were deconvolved using DeltaVision SoftWoRx software (version 6.5.2).

Phage genome characterization

Two hundred microliters of phage lysate were treated with 3 µL of DNaseI (2000 u/ml) and 30 µL of DNAse buffer and incubated at 37 °C for 30 min, followed by 10 min at 70 °C. DNA extraction was performed using Monarch Mag Viral DNA/RNA extraction kit (NEB, #T4010S), the final elution was performed in 100 µl of nuclease free water. DNA was quantified with Qubit HR DNA (cat #Q33231, Invitrogen). Fifty microliters of fragmented DNA were used for sonication using Covaris (microTUBEs, duty cycle 10%, intensity 5, cycles per burst 200, time 180 s). Sequencing libraries were prepared using NEBNext Uktra II DNA library prep kit for Illumina (NEB #E7645S) using 50 µL of fragmented DNA as input and 10 amplification cycles. Libraries were sequenced in an Illumina iSeq instrument as PE150. Reads were de novo assembled using SPAdes through the webserver BV-BCR[38]. Assembly graphs were inspected and, in most cases, a single contig was present. When multiple contigs were present, the closest reference genome was used as scaffold and manually assembled using MAUVE[39] visualization. Phage CDS were identified using PHANNOTATE[40] and annotated through the webserver BV-BCR[38]. Average Nucleotide Identity (ANI)[17] was calculated between the sequenced phages, if two phages share more than 99% ANI, they were considered the same phage and the phage stock with the higher genome coverage was kept for further analysis. Phage genome annotations were screened for integrases, excisionases and toxin genes (Table 1).

Phage proteomic tree

Newly isolated Achromobacter phage genomes were compared to publicly available Achromobacter phages ($n = 47$, Table S1) through a Phage Proteomic Tree approach using VIPtree[41]. Briefly, the Achromobacter phage genome sequences were uploaded to the server as a FASTA file, compared

with other prokaryotic dsDNA phage genome sequences on the server, and output as a circular dendrogram.

Host range evaluation

Phage host range was evaluated by spot titer. A bacteria lawn was established using 450 µL of 0.2 OD600 bacteria mixed with 6.75 mL of molten 0.3% LB top agar and poured onto 1.5% LB plates (150 mm). Two microliters of each serial dilution were spotted and dried in a hood. Plates were incubated for 24 h at 37 °C. After incubation, phage titer and spot turbidity were recorded. Efficiency of plating (EOP) was determined by dividing the titer of the phage on the host by the titer determined on the reference host. For most of the phages, the reference host was *Achromobacter* spp. AX19. However, not all phages were capable of infecting AX19 and required different hosts that were used for calculating EOP, including phages Malbec (AX11), Mochifu (AX24), Prosecco (AX14), Marine1 (AX48), Sirah (AX02), Fargo (AX11), and Kiitash (AX24).

Phage-antibiotic synergy in liquid media

A 1:1000 subculture was started from an overnight culture of bacteria in LB media. OD600 was monitored until 0.1 was reached. An antibiotic was added to a 96-well plate in 30 µL of sterile water, to achieve concentrations ranging from 64 mcg/mL to 0.016 mcg/mL, and no antibiotic. Phages were added in 30 µL of LB media to achieve MOIs of 0, 0.1, 1, 10 or 100. One hundred and forty microliters of bacteria were added to each well in the plate. Each condition was tested in triplicate. Negative controls as well as no treatment controls were included in triplicate. The plates were incubated for 24 h at 37 °C with shaking in a LogPhase600 reader, and OD600 was measured every 15 min with growth curves generated in GraphPad Prism (version 10.6.1). OD600 at 24 h was used to calculate the percentage growth for each condition relative to the no treatment controls. The OD600 of each replicate of the treatment condition was divided by the average of the no treatment wells and multiplied by 100. The average of the triplicates was used to obtain the mean relative percentage growth for each treatment. These values were displayed as heatmaps using R (version 4.5.2). Pairwise differences in percentage growth between treatment groups were assessed using an independent two-sample t-test, which was implemented using the compare_means function implemented in the R package ggpubr (version 0.6.0), p-values were adjusted for multiple comparisons using the Holm method. Statistical significance was displayed in the figures. Significance thresholds were determined as follows: $p \le 0.05$ (*), $p \le 0.01$ (**), $p \le 0.001$ (***), while $p > 0.05$ not significant (ns).

All strains were evaluated for phage-antibiotics-synergy between meropenem and phages BuckLyse, Kaach or Taco independently using the liquid media method described above. Three antibiotic concentrations were used: 4 µg/mL, 0.5 mcg/mL, and 1/16 µg/mL. MOIs of 1,10, or 100 were used. Synergy was determined by using the end point OD600 measurements as percentage inhibition in the highest single agent model.

Data availability

All phage genomes have been deposited in NCBI Genbank (Table 1) with the following accession numbers: with the following accession codes: PV441885, PV340829, PV340830, PV340831, PV340832, PV340833, PV340834, PV340835, PV340836, PV324754, PV340837, PV340838, PV340846, PV340845, PV340844, PV340843, PV324754, PV340842, PV340841, PV544237, PV943645, PV340839, PV340840, PX354141, PZ013370, PZ01337.

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References

1. Sharma, S. et al. Emerging challenges in antimicrobial resistance: implications for pathogenic microorganisms, novel antibiotics, and their impact on sustainability. *Front. Microbiol.* **15**, 1403168 https://doi.org/10.3389/fmicb.2024.1403168 (2024).
2. Geremia, N. et al. Rare or unusual non-fermenting gram-negative bacteria: therapeutic approach and antibiotic treatment options. *Antibiotics* **14**, https://doi.org/10.3390/antibiotics14030306 (2025).
3. Darby, E. M. et al. Molecular mechanisms of antibiotic resistance revisited. *Nat. Rev. Microbiol.* **21**, 280–295 https://doi.org/10.1038/s41579-022-00820-y (2023).
4. Isler, B., Kidd, T. J., Stewart, A. G., Harris, P. & Paterson, D. L. Achromobacter infections and treatment options. *Antimicrob Agents Chemother* **64**, https://doi.org/10.1128/AAC.01025-20 (2020).
5. Bhowmik, N. & Stubbendieck, R. M. Achromobacter spp.: emerging pathogens in the cystic fibrosis lung. *PLoS Pathog.* **21**, e1013067 https://doi.org/10.1371/journal.ppat.1013067 (2025).
6. Lambiase, A. et al. Achromobacter xylosoxidans respiratory tract infection in cystic fibrosis patients. *Eur. J. Clin. Microbiol. Infect. Dis.* **30**, 973–980, https://doi.org/10.1007/s10096-011-1182-5 (2011).
7. Kerem, E. et al. Clinical outcomes associated with Achromobacter species infection in people with cystic fibrosis. *J. Cyst. Fibros.* **22**, 334–343 https://doi.org/10.1016/j.jcf.2022.11.001 (2023).
8. Acharya, P. et al. Achromobacter xylosoxidans isolates exhibit genome diversity, variable virulence, high levels of antibiotic resistance, and potential intrahost evolution. *mSphere*, e0087025 https://doi.org/10.1128/msphere.00870-25 (2026).
9. Antoun, N., Marchandin, H., Houdouin, V. & Mayer Lacrosniere, S. High diversity of Achromobacter sp. isolation from children with CF, a retrospective case-control study. *Heliyon* **12**, https://doi.org/10.1016/j.heliyon.2026.e44516 (2026).
10. Veschetti, L. et al. Achromobacter spp. prevalence and adaptation in cystic fibrosis lung infection. *Microbiol. Res.* **263**, 127140 https://doi.org/10.1016/j.micres.2022.127140 (2022).
11. Cobian Guemes, A. G. et al. Compounding Achromobacter phages for therapeutic applications. *Viruses* **15**, https://doi.org/10.3390/v15081665 (2023).
12. Bayless, D. R. et al. Clinical and microbiologic features of Achromobacter species: a 10-year, multicenter experience. *J. Clin. Microbiol.* **63**, e0072425. https://doi.org/10.1128/jcm.00724-25 (2025).
13. Bradley, J. S. et al. Bacteriophage therapy of multidrug-resistant Achromobacter in an 11-year-old boy with cystic fibrosis assessed by metagenome analysis. *Pediatr. Infect. Dis. J.* **42**, 754–759 https://doi.org/10.1097/INF.0000000000004000 (2023).
14. Segall, A. M., Roach, D. R. & Strathdee, S. A. Stronger together? Perspectives on phage-antibiotic synergy in clinical applications of phage therapy. *Curr. Opin. Microbiol.* **51**, 46–50 https://doi.org/10.1016/j.mib.2019.03.005 (2019).
15. Gomila, M. et al. Genotypic and phenotypic applications for the differentiation and species-level identification of Achromobacter for clinical diagnoses. *PLoS One* **9**, e114356 https://doi.org/10.1371/journal.pone.0114356 (2014).
16. Garrigos, T. et al. Development of a database for the rapid and accurate routine identification of Achromobacter species by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS). *Clin. Microbiol. Infect.* **27**, 126 e121–126 e125 https://doi.org/10.1016/j.cmi.2020.03.031 (2021).
17. Yoon, S. H., Ha, S. M., Lim, J., Kwon, S. & Chun, J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van. Leeuwenhoek* **110**, 1281–1286 https://doi.org/10.1007/s10482-017-0844-4 (2017).
18. Kaliniene, L. et al. Analysis of a novel bacteriophage vB_AchrS_AchV4 highlights the diversity of Achromobacter Viruses. *Viruses* **13**, https://doi.org/10.3390/v13030374 (2021).
19. Dreiseikelmann, B. et al. Characterization and genome comparisons of three Achromobacter phages of the family Siphoviridae. *Arch. Virol.* **162**, 2191–2201 https://doi.org/10.1007/s00705-017-3347-8 (2017).
20. Panteleev, V., Kulbachinskiy, A. & Gelfenbein, D. Evaluating phage lytic activity: from plaque assays to single-cell technologies. *Front.*

Microbiol. **16**, 1659093 https://doi.org/10.3389/fmicb.2025.1659093 (2025).
21. Knoll, R. L. et al. CFTR modulator therapy drives microbiome restructuring through improved host physiology in cystic fibrosis: the IMMProveCF phase IV trial. *Nat. Commun.* **16**, 10111 https://doi.org/10.1038/s41467-025-64218-z (2025).
22. Filipic, B. et al. Uncovering differences in virulence markers associated with achromobacter species of CF and Non-CF Origin. *Front. Cell Infect. Microbiol.* **7**, 224 https://doi.org/10.3389/fcimb.2017.00224 (2017).
23. Winzig, F. G. S. et al. Inhaled bacteriophage therapy for multi-drug resistant Achromobacter. *Yale J. Biol. Med.* **Dec 22**, 413–427 (2022).
24. Lebeaux, D. *et al*. A Case of Phage Therapy against Pandrug-Resistant Achromobacter xylosoxidans in a 12-Year-Old Lung-Transplanted Cystic Fibrosis Patient. *Viruses* **13**, https://doi.org/10.3390/v13010060 (2021).
25. Birkholz, E. A., Armbruster, E. G. & Pogliano, J. The biology of nucleus-forming jumbo phages. *Annu. Rev. Genet.* **59**, 51–68 https://doi.org/10.1146/annurev-genet-111523-102019 (2025).
26. Khong, E. et al. A simple solid media assay for detection of synergy between bacteriophages and antibiotics. *bioRxiv* https://doi.org/10.1101/2023.08.23.554535 (2023).
27. Ghatbale, P. et al. In-vitro resensitization of multidrug resistant clinical isolates of Enterococcus faecium and faecalis through phage-antibiotic synergy. *Antimicrob. Agents Chemother.* **69**, 00740–00724 https://doi.org/10.1128/aac.00740-24 (2024).
28. Khong, E. et al. A simple solid media assay for detection of synergy between bacteriophages and antibiotics. *Microbiol. Spectr.* **12**, e0322123 https://doi.org/10.1128/spectrum.03221-23 (2024).
29. Nikolic, I. et al. An optimized checkerboard method for phage-antibiotic synergy detection. *Viruses* **14**, https://doi.org/10.3390/v14071542 (2022).
30. Kunz Coyne, A. J. et al. Phage cocktails with daptomycin and ampicillin eradicates biofilm-embedded multidrug-resistant Enterococcus faecium with preserved phage susceptibility. *Antibiotics* **11**, https://doi.org/10.3390/antibiotics11091175 (2022).
31. Cantallops, I. et al. Phage-meropenem synergy against OXA-48-producing Klebsiella pneumoniae clinical isolates. *NPJ Antimicrob. Resist* **4**, 12 https://doi.org/10.1038/s44259-026-00186-8 (2026).
32. Luo, J. et al. Synergy of lytic phage pB23 and meropenem combination against carbapenem-resistant Acinetobacter baumannii. *Antimicrobial Agents Chemother.* https://doi.org/10.1128/aac.00448-24 (2024).
33. Chen, B. et al. Alginate microbeads and hydrogels delivering meropenem and bacteriophages to treat Pseudomonas aeruginosa fracture-related infections. *J. Control Release* **364**, 159–173 https://doi.org/10.1016/j.jconrel.2023.10.029 (2023).
34. Stuart, E. C. & Meyer, J. R. Sequential phage delivery can outperform cocktails by delaying cross-resistance evolution. *Viruses* **18**, https://doi.org/10.3390/v18040404 (2026).
35. Papp, J. R. et al. Accuracy and reproducibility of the Etest to detect drug-resistant Neisseria gonorrhoeae to contemporary treatment. *J. Med. Microbiol.* **67**, 68–73 https://doi.org/10.1099/jmm.0.000651 (2018).
36. Rafailidis, P. I. & Kofteridis, D. Proposed amendments regarding the definitions of multidrug-resistant and extensively drug-resistant bacteria. *Expert Rev. Anti Infect. Ther.* **20**, 139–146 https://doi.org/10.1080/14787210.2021.1945922 (2022).
37. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. *Clinical and Laboratory Standards Institute* (2025).
38. Olson, R. D. et al. Introducing the Bacterial and Viral Bioinformatics Resource Center (BV-BRC): a resource combining PATRIC, IRD and ViPR. *Nucleic Acids Res.* **51**, D678–D689 https://doi.org/10.1093/nar/gkac1003 (2023).
39. Darling, A. E., Mau, B. & Perna, N. T. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* **5**, e11147 https://doi.org/10.1371/journal.pone.0011147 (2010).
40. McNair, K., Zhou, C., Dinsdale, E. A., Souza, B. & Edwards, R. A. PHANOTATE: a novel approach to gene identification in phage genomes. *Bioinformatics* **35**, 4537–4542 https://doi.org/10.1093/bioinformatics/btz265 (2019).
41. Nishimura, Y. et al. ViPTree: the viral proteomic tree server. *Bioinformatics* **33**, 2379–2380 https://doi.org/10.1093/bioinformatics/btx157 (2017).

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Author contributions

Conceived and designed the project: A.G.C.G. and D.T.P. Performed experiments: M.O., N.T., N.K., M.T., Y.H., P.R., J.L., A.B., A.S., V.H., G.K., Z.P., J.O., M.B., A.A., V.C., B.S.B., and A.G.C.G. Analyzed data: M.O., A.G.C.G., and D.T.P. Wrote and edited the manuscript: J.B., D.C., J.P., J.B., A.G.C.G., and D.T.P. Provided materials for the study: R.K. All authors have reviewed the manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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