

Review

Phage–Antibiotic Combinations Against Mature Multidrug-Resistant Biofilms: A Critical Narrative Review of Synergy Determinants, Treatment Sequencing, and Translation

Touati Abdelaziz ^{1,*}, Kirat Hassina ²  and Bellil Zahra ³ 

¹ Laboratory of Microbial Ecology, Faculté des Sciences de la Nature et de la Vie (FSNV), University of Bejaia, Bejaia 06000, Algeria

² Laboratoire de Recherche des Interactions, Biodiversité, Ecosystèmes et Biotechnologie, Faculté des Sciences, Department of Natural and Life Sciences, University 20 August 1955—Skikda, Skikda 21000, Algeria; hasnakrt17@gmail.com

³ Centre de Recherche en Technologie Agro-Alimentaire, Route de Targa Ouzemour, Campus Universitaire, Bejaia 06000, Algeria

* Correspondence: abdelaziz.touati@univ-bejaia.dz

Abstract

Background: Phage–antibiotic combinations (PACs) are increasingly proposed for difficult biofilm infections, but planktonic synergy does not reliably predict activity in mature multidrug-resistant (MDR) biofilms. **Objective:** To identify the biological, pharmacological, evolutionary, methodological, and translational determinants that govern whether phage–antibiotic synergy (PAS) persists in mature MDR biofilms. **Methods:** This critical narrative review used a structured evidence map updated through 21 August 2026. Fifty-four primary studies met the final operational core criteria after endpoint-eligibility and publication-status verification and were evaluated for biofilm maturity, phage and antibiotic exposure, treatment sequence, resistance evolution, assay choice, and translational level. **Main Results:** Twenty of 54 studies (37.0%) included at least one qualifying biofilm/PAC condition aged 48 h or longer, and 14 directly compared treatment order. Interaction direction depended on phage identity, bacterial strain, matrix, absolute phage exposure, antibiotic concentration, timing, and endpoint; the schedule maximizing immediate killing did not necessarily best suppress resistance. Nine core studies reached animal models and four human cases met the defined translational criteria, but uncontrolled cases support feasibility rather than comparative efficacy. Negative, antagonistic, and indeterminate outcomes were retained, and CFU, biomass, metabolic, and lysis-based assays could classify the same regimen differently. **Conclusions:** PAS should be treated as a context-dependent phenotype requiring direct mature-biofilm validation, factorial dose-sequence testing, longitudinal resistance assessment, and site-matched translational confirmation.

Keywords: bacteriophage; phage–antibiotic synergy; multidrug resistance; mature biofilm; treatment sequence; resistance evolution; MBEC; translational standardization



Academic Editor: Niels Frimodt-Møller

Received: 30 August 2026

Revised: 16 September 2026

Accepted: 21 September 2026

Published: 24 September 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction: From Phage–Antibiotic Synergy (PAS) to the Biofilm Translation Problem

Phage–antibiotic combinations (PACs) are attractive because they can impose mechanistically distinct pressures on bacteria while potentially reducing the dose needed from either component. In established biofilms, however, antibiotic resistance is superimposed

on phenotypic tolerance, spatially heterogeneous physiology and restricted access to bacterial receptors, so a combination that is synergistic in broth may be indifferent or antagonistic in a mature community [1,2]. The systematic review of in vitro phage-biofilm experiments by Amorim Perestrelo de Meneses et al. found that most experiments still relied on 24 h microplate biofilms and indirect endpoints, confirming that the mature-biofilm translation problem is not a marginal methodological concern but a structural feature of the literature [3]. The major biofilm-specific determinants considered in this review are summarized in Figure 1.

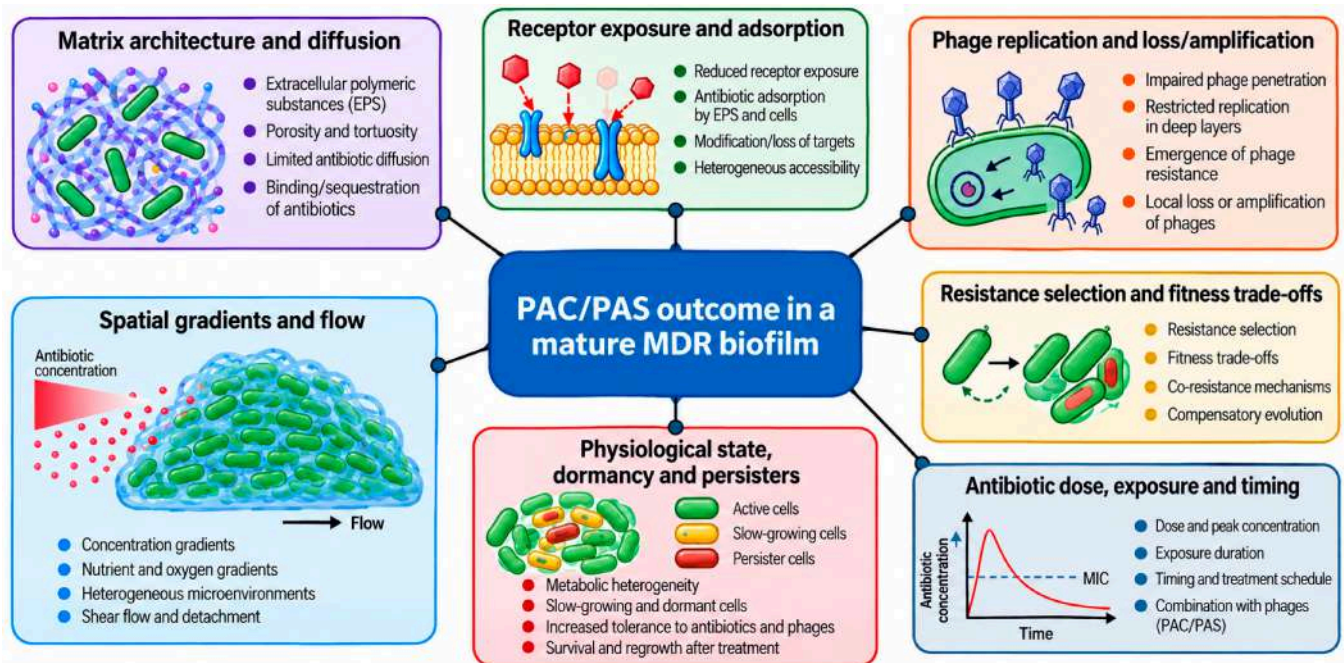


Figure 1. Biofilm-specific determinants of PAC/PAS outcome. Matrix, receptor access, physiology, spatial gradients, antibiotic action, phage replication and resistance selection interact to produce the observed phenotype. PAC, phage–antibiotic combination; PAS, phage–antibiotic synergy.

The term synergy is often used as though it were an intrinsic property of a phage–antibiotic pair. The evidence instead supports at least four distinguishable dimensions: planktonic synergy, biofilm synergy, evolutionary synergy and translational synergy. Planktonic synergy is an interaction measured in suspended bacteria. Biofilm synergy requires additional killing in a preformed community with a viable endpoint. Evolutionary synergy means that the combination suppresses resistance or selects a therapeutically useful trade-off. Translational synergy means that benefit persists under realistic surfaces, fluids, communities, pharmacokinetics, host immunity, and clinical co-interventions [4–7]. A regimen can satisfy one dimension and fail another, so each stage requires explicit revalidation. These four dimensions are summarized in Figure 2.

For terminology used throughout this review, PAC denotes any regimen in which a whole lytic phage and an antibacterial antibiotic are evaluated together, irrespective of interaction class. PAS is reserved for a favorable interaction that meets the original study’s stated synergy criterion; because the included literature uses heterogeneous definitions, no universal numerical cutoff is imposed retrospectively. Additivity denotes an effect consistent with the study’s stated no-interaction reference model; an effect below a synergy threshold is not automatically additive. Antagonism denotes an effect below that reference expectation or the relevant comparator under the study’s stated criterion. Translational synergy is reserved for benefit that persists under clinically realistic material, fluid, PK/PD, immune, and co-intervention conditions.

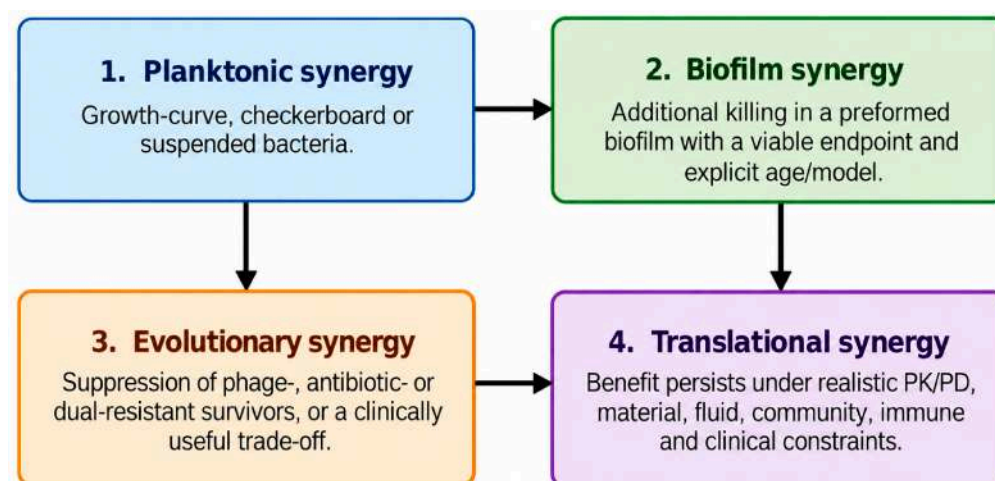


Figure 2. Four dimensions of synergy. A positive interaction must be re-established as the system progresses from planktonic screening to mature biofilm killing, resistance control and translational performance.

This review asks which biological, pharmacological, evolutionary and methodological factors determine whether a PAC/PAS signal survives the transition from simplified screening to a clinically relevant multidrug-resistant (MDR) biofilm. The synthesis is organized around five linked problems: discordance between planktonic and biofilm assays; biofilm maturity and community complexity; phage dose, antibiotic class and administration sequence; resistance evolution and fitness trade-offs; and the lack of standardized testing and translational validation. It does not attempt to rank a universal best phage or antibiotic. Instead, it develops a decision framework for selecting, testing and reporting combinations in the specific biofilm context in which they are intended to be used.

2. Methods

2.1. Review Design, Search Window and Information Sources

This article is a critical narrative review with a structured, question-led evidence map; it is not a systematic review. The evidentiary window covered publications from 1 January 2015 through 21 August 2026. The original search protocol began at 1 January 2017; after targeted backward and forward citation review identified a potentially relevant 2016 clinical-isolate biofilm study [8], the lower boundary was retrospectively extended to 2015. This amendment expanded the search window; final endpoint reconciliation later classified [8] as supporting rather than core but was not a PRISMA-style exhaustive rerun of all 2015–2016 records. Search concepts combined bacteriophages, antibiotics, combination effects, biofilms and antimicrobial resistance, including XDR and PDR phenotypes. The PubMed/MEDLINE search concepts are summarized as follows (display notation, not a verbatim executable search): (bacteriophage or phage or “Phage Therapy”) AND (antibiotic or antimicrobial) AND (synerg or combin or sequential or simultaneous) AND biofilm* AND (resistant or resistance or multidrug or MDR or XDR or PDR). Targeted searches addressed treatment timing and sequence, mature and polymicrobial biofilms, MBIC, MBEC, time-kill methods, resistant survivors, evolutionary trade-offs, animal models, drug-delivery systems and human phage–antibiotic use.

PubMed/MEDLINE served as the principal bibliographic database. To reduce single-database dependence, supplementary discovery during revision used targeted scholarly web searches, journal and publisher platforms, DOI metadata, and backward and forward citation searching. These supplementary searches were used to identify and verify supporting evidence. Newly identified studies were evaluated against the same operational

eligibility criteria and non-core sources were recorded in Supplementary Table S3. The review was not designed or reported as a PRISMA-style systematic review. Accordingly, record-level retrieval, duplicate removal, title/abstract screening, full-text exclusion counts and a PRISMA flow diagram were not produced. The displayed PubMed/MEDLINE expression is a conceptual description rather than a reconstructed executable search log; the original dated query log was not retained. The manuscript therefore reports the final body of directly relevant evidence included in the structured synthesis without claiming exhaustive capture of every potentially eligible record.

Every bibliographic record used in the final synthesis was checked against PubMed, the journal publisher, DOI metadata or an equivalent authoritative record. Original study results were extracted only when the report provided enough detail to identify the biofilm model, intervention and outcome. Three peer-reviewed systematic reviews were used for contextual triangulation and redundancy checking, but their summarized studies were not added to the primary denominator [3,9,10]. Mechanistic, method-comparison, pharmacodynamic, delivery, ex vivo, human, regulatory and implementation sources that did not meet the core definition were retained as supporting evidence in Supplementary Table S3 and excluded from all core n/N calculations. Final eligibility reconciliation moved Nouraldin et al. [8] outside the core because its reported MBEC and PAC biofilm outcome were defined by fixation and crystal violet staining without a viability-related endpoint; the same biomass-only rule applies to Young et al. [11] and Bhutada et al. [12]. Cano et al. [13] was retained only as a Level 9 translational case because the complete regimen was tested against a patient-specific preformed biofilm and linked to the clinical case; its safranin assay is treated as structural biomass evidence, not viable eradication. A final publisher-status check also identified De Soir et al. [14] as retracted; it was removed from all quantitative and narrative efficacy synthesis and is retained only in the exclusion ledger. Two additional primary-source checks changed the final denominator. Moulton-Brown and Friman [15] was reclassified as supporting evolutionary evidence because phage and gentamicin were present from initial bacterial inoculation while bead biofilms developed under selection, so the PAC was not applied to a preformed biofilm. Akturk et al. [16] was reclassified as supporting three-dimensional/sequence evidence because the lung epithelial model was treated after only 2 h of bacterial colonization, which does not meet the operational established/preformed-biofilm boundary used for the experimental core. These decisions yield a 54-study core evidence map.

2.2. Eligibility and Maturity Boundary

Eligible primary studies used one or more whole lytic bacteriophages together with an antibacterial antibiotic against an established or preformed bacterial biofilm. For in vitro, ex vivo and animal biofilm effect studies, core inclusion required a viability-related bacterial outcome (for example, CFU/time-kill, direct viable burden, viable regrowth/eradication, or a directly linked viability measure); biomass-only staining could support structural effects but was insufficient by itself. Human reports could enter Level 9 when the complete phage–antibiotic regimen was directly tested against a patient-specific preformed biofilm and linked to the reported clinical case. In this human translational exception, a biomass-only assay was retained as structural evidence and was not interpreted as viable-cell eradication. Infection models without direct characterization of an established biofilm, including simulated endocardial vegetation or endocarditis models used primarily for PK/PD or translational context, were kept as supporting rather than core evidence. Clinically relevant MDR, XDR or otherwise difficult-to-treat pathogens were prioritized, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, coagulase-negative staphylococci such as *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Klebsiella* spp., *Enterococcus* spp. and *Escherichia coli*.

Phage-only and antibiotic-only papers were excluded from the combination-effect synthesis. Planktonic-only PAS studies were excluded from the primary denominator, although paired planktonic data within an included biofilm study were retained to analyze discordance. Studies in which the combination was used only to prevent biofilm formation, while no preformed biofilm received the combination, were excluded from the mature-biofilm denominator and could be retained as supporting evidence. Method-validation studies whose primary aim was assay comparison, and clinical reports in which phage and antibiotics were co-administered for a biofilm-associated infection without regimen-specific combination-level biofilm evidence, were likewise kept outside the denominator when informative [7,11,17–23]. Phage-derived enzymes without whole phage, non-bacterial biofilms and temperate-phage applications without a justified therapeutic context were also excluded. Bacterial-fungal biofilms were eligible only when the antibacterial phage–antibiotic component was evaluated against the bacterial member and community context was central to interpretation [24–26]. Retracted publications were excluded from all evidence synthesis; reference [14] is retained only to document this publication-status exclusion.

2.3. Evidence Selection, Extraction and Coding

The final evidence map comprised 54 primary studies that met the reconciled operational eligibility criteria. Selection was based on direct relevance to the review question and sufficient information to characterize the biofilm model, intervention and outcome. The study-level matrix extracted publication year, pathogen and resistance phenotype, infection context, model and surface, pretreatment biofilm age, and community composition. It also recorded phage identity and strategy, antibiotic identities and classes, treatment order, direct sequence comparison, repeated dosing, and assay/interaction definition. Viable and structural endpoints, explicit resistance/evolution assessment, survivor genomics, advanced-model use, animal validation, human evidence, and one highest translational level were coded. Supplementary Table S1A provides the core study characteristics; Supplementary Table S1B,C provide treatment-design details and complete highest-level assignments, and Supplementary Table S4A,B provide the reproducible study-level coding ledgers used for the final recount.

Each study was assigned to the highest level reached on a nine-level translational ladder. Levels 1–3 represented planktonic screening, young static biofilm, and mature monospecies biofilm. Levels 4–6 represented clinically relevant surface/flow or dynamic models, polymicrobial biofilms, and three-dimensional tissue, mucus, or implant models. Levels 7–9 represented ex vivo biological material, animal infection, and human clinical evidence. Level 1 was necessarily zero in the primary corpus because planktonic-only studies were excluded. A study could contribute to multiple overlapping pathogen, antibiotic, sequence and assay categories, but only one highest translational level.

A descriptive reporting-completeness appraisal was used instead of a validated risk-of-bias score because the corpus mixed static assays, PK/PD reactors, delivery platforms, ex vivo systems, animal experiments and uncontrolled human cases. Pretreatment age was coded as ≥ 48 h when at least one qualifying PAC biofilm condition explicitly reached that age, < 48 h when the qualifying conditions were clearly younger, and not assignable when a comparable pretreatment age could not be established. On this rule, 20/54 (37.0%) studies included a qualifying ≥ 48 h condition, 32/54 (59.3%) were clearly < 48 h, and 2/54 (3.7%) were not assignable. A direct viable-count or burden endpoint was present in 45/54 (83.3%). Explicit resistance/evolution assessment was coded in 18/54 (33.3%), while combination-relevant survivor genomics was explicitly reported in 1/54 (1.9%) [5]. The

final flags and reference IDs are provided in Supplementary Table S4A,B, so each aggregate can be reconstructed from study-level coding.

2.4. Synthesis Strategy

Counts and percentages are reported as n/N (%) with an explicit denominator. Overlapping categories, such as antibiotic class or assay type, can therefore sum to more than 100%. Quantitative effects were retained at study level when the underlying definitions were clear, but no pooled effect was calculated. Biofilm age, starting density, phage concentration, antibiotic exposure, treatment duration, sequence, outcome scale and synergy definition differed too substantially to support a defensible common effect measure. The synthesis consequently emphasizes direction, determinants, reproducibility across model levels and reasons for discordance rather than a pooled average.

Causal language was restricted to systems that directly manipulated the relevant determinant. Associations observed across studies are described as patterns, and mechanistic interpretations are labeled as such when they integrate several experiments. Human reports satisfying the defined regimen-specific Level 9 biofilm rule are treated as Level 9 evidence for feasibility and ecological behavior, not as comparative efficacy estimates; biofilm-associated clinical PAC reports without direct complete-regimen biofilm testing are retained as supporting translational evidence. This separation prevents uncontrolled clinical improvement from being counted as proof of pharmacological synergy.

AI-assisted revision and audit: During revision, ChatGPT (OpenAI, San Francisco, CA, USA; GPT-5.6 Sol, accessed 13 September 2026) was used to assist with language editing, cross-document consistency checking, structured coding/tabulation, and targeted bibliographic/source verification. AI output was not treated as primary evidence; study eligibility, extracted data, quantitative coding, interpretation, and final wording were checked against the cited source material and remain the responsibility of the authors.

3. Current Evidence Landscape of Phage–Antibiotic Therapy in MDR Biofilms

The evidence base accelerated after 2021: 41/54 (75.9%) core studies were published from 2022 through 2026, including eight studies available in 2026 by the final search date (Supplementary Table S1A). The literature remained pathogen-concentrated. *P. aeruginosa* appeared in 19/54 (35.2%) studies, *S. aureus* in 17/54 (31.5%), *A. baumannii* in 9/54 (16.7%), *Klebsiella* spp. in 6/54 (11.1%), *Enterococcus* spp. in 5/54 (9.3%), *E. coli* in 3/54 (5.6%) and *S. epidermidis* in 2/54 (3.7%). These are overlapping pathogen counts because eight studies contained dual- or poly-species communities.

Single phages were the sole strategy in 37/54 (68.5%) studies, cocktails in 11/54 (20.4%), and both single-phage and cocktail conditions in 6/54 (11.1%). After completion of the study-level drug identities, beta-lactams were represented in 30/54 (55.6%) studies, fluoroquinolones in 22/54 (40.7%), glycopeptides or lipoglycopeptides in 12/54 (22.2%), aminoglycosides in 15/54 (27.8%) and lipopeptides in 10/54 (18.5%). The distribution reflects organism-specific clinical practice: antipseudomonal beta-lactams and fluoroquinolones dominated Gram-negative systems, whereas daptomycin, ceftaroline, vancomycin and dalbavancin were prominent in staphylococcal and enterococcal models [1,27–30]. The exact agents and class flags used for the recount are provided in Supplementary Tables S1A and S4B.

Model maturity and complexity were uneven. Twenty of 54 studies (37.0%) included at least one qualifying biofilm/PAC condition aged 48 h or longer, 32/54 (59.3%) used clearly younger conditions, and 2/54 (3.7%) lacked a comparable assignable pretreatment age (Supplementary Table S4A). Mono-species models comprised 46/54 (85.2%), while dual- or poly-species models comprised 8/54 (14.8%). Advanced core models included

Dacron vascular graft, titanium orthopedic surfaces, flow cells, implant materials, endotracheal tubes, artificial sputum, synovial fluid, urine, plasma-blood, artificial dermis, pig skin, animal infection and human chronic infection [26,31–35]. A three-dimensional lung epithelial colonization model [16] remains informative supporting evidence but is outside the experimental core because PAC treatment began after only 2 h of bacterial colonization.

Direct viable-count or burden endpoints were available in 45/54 (83.3%), biomass in 28/54 (51.9%), metabolic or regrowth endpoints in 17/54 (31.5%), microscopy or bioluminescence imaging in 17/54 (31.5%), and MBIC/MBEC-type endpoints in 11/54 (20.4%). Only a minority used an explicit mathematical or log-reduction interaction rule directly comparable across treatment arms, while many others used the word synergy for any statistically greater combination effect. This definitional spread supports structured mapping but not meta-analysis.

The dominant finding was conditional rather than universal benefit [36–41]. Multi-log killing occurred in selected systems, yet weaker effects or failure were linked to resistant host range, mature age, dynamic growth, matrix phenotype, low phage dose, high antibiotic exposure, mixed communities or unfavorable order [4,5,25,29,32,42,43]. The UJB10 study further showed that antibiotic class interactions can change with time, while supporting multi-strain pharmacodynamic data showed strong ciprofloxacin antagonism but more favorable or strain-variable interactions for other drugs [44,45]. The corpus therefore maps determinants and failure modes rather than universally effective pairs; Table 1 summarizes the resulting validation logic.

Table 1. Evidence-based determinants of phage–antibiotic combination performance in mature biofilms and the corresponding validation requirement.

Determinant	Critical Evidence Pattern	Required Validation
Biological state and maturity	Planktonic interaction does not reliably predict killing in established or mature biofilms; age, matrix, physiology and flow can change the direction or magnitude of the interaction [1,2].	Retest the same isolate in paired planktonic, young-biofilm and clinically matched mature-biofilm models.
Phage exposure	The same nominal MOI can represent different accessible exposures, and several systems showed a phage-dose window rather than a universal optimum [5,46,47].	Report absolute administered PFU/mL, the MOI calculation and recovered PFU over time; map log-spaced doses.
Antibiotic exposure	Drug class provides a mechanistic hypothesis, not a fixed interaction: concentration, time, strain and phage identity can produce synergy, additivity or antagonism [44,45].	Report mg/L and exposure relative to MIC/MBIC/MBEC; use a concentration-by-time matrix.
Sequence and repetition	Fourteen core studies directly compared order, but no schedule dominated across systems; the schedule maximizing immediate killing can differ from that suppressing resistance [48,49].	Compare phage-first, simultaneous and antibiotic-first schedules and include an explicitly repeated-dose arm.
Community and matrix	Polymicrobial composition, surface and biological fluid altered treatment ranking and could leave non-targeted community members uncontrolled [25,26].	Enumerate each species separately and reproduce the intended material, fluid, oxygenation and flow conditions.
Resistance trajectory	Resistance suppression and antibiotic resensitization were regimen-specific; immediate killing did not establish durable evolutionary control [5,32].	Measure phage-, antibiotic- and dual-resistant survivors longitudinally and sequence representative independent lineages.

Table 1. Cont.

Determinant	Critical Evidence Pattern	Required Validation
Assay endpoint	CFU, biomass, metabolic, microscopy and lysis-based methods can classify the same regimen differently [18].	Use viable burden as the primary bactericidal endpoint, with structural/spatial and regrowth or resistance follow-up.
Translation	Nine animal studies and four core human reports meeting the defined Level 9 translational criteria show feasibility but not comparative efficacy; PK/PD, immunity, delivery and clinical co-interventions remain major confounders.	Confirm site-matched exposure and delivery, measure immunity and resistance, and separate regimen effects from surgery/source control.

4. Why Planktonic Synergy Does Not Necessarily Predict Biofilm Synergy

The most direct evidence of planktonic-to-biofilm discordance comes from paired experiments. In methicillin-resistant *S. aureus* (MRSA), several PACs that were active in planktonic time-kill failed to retain the same magnitude or bactericidal classification in 24 h bead biofilms, and only the phage-daptomycin-ceftaroline regimen was consistently bactericidal across both states [1]. In seven-day staphylococcal biofilms on titanium, planktonic antagonism had some value for rejecting poor combinations, but planktonic synergy did not reliably identify the combinations that would be synergistic in the mature biofilm [2]. UJB10 provided a complementary time-resolved example in *P. aeruginosa*: amikacin and meropenem could slow phage-mediated biofilm clearance at early time points even though the antagonism was no longer evident at 24 h, while cefepime produced a durable favorable interaction [44]. Across cystic fibrosis isolates and culture systems, interactions between a phage cocktail and ceftazidime or tobramycin likewise ranged from synergy/additivity to inhibition depending on strain and model [50]. Supporting quantitative pharmacodynamic work across nine *P. aeruginosa* strains further found strong ciprofloxacin antagonism, broadly increased antibiotic sensitivity with aztreonam, colistin or meropenem, and divergent strain-specific effects with amikacin and ceftazidime [45]. These studies show that the biofilm is not simply a higher-density version of the planktonic assay and that interaction direction may depend simultaneously on state, strain, concentration and time.

Matrix structure changes both delivery and infection. Phages must reach a susceptible receptor, adsorb, inject, replicate and disseminate through a matrix whose chemical composition can trap, delay or redirect particles. Sb-1 and vB_KpUKJ_2 were associated with loss of extracellular matrix components, and that disruption coincided with enhanced combination killing in their respective systems [5,51]. Conversely, mucoid alginate production protected *P. aeruginosa* flow-cell biofilms from repeated phage exposure, while a separate phage-first ciprofloxacin regimen in PAO1 produced an approximately 6-log reduction in viable cells [32]. The expanded corpus adds direct phenotype-level evidence: in PAO1 and PA14 biofilms, PAC activity varied with exopolysaccharide phenotype; ciprofloxacin often enhanced phage activity, but individual-phage performance, triple-cocktail efficacy and regrowth differed between PAO1 and PA14 [43]. Matrix degradation can therefore be a determinant of benefit, but it cannot be assumed from plaque halos or genome annotation without biofilm-level confirmation.

Receptor accessibility and host range remain equally important. Endotracheal-tube biofilms formed by phage-susceptible *P. aeruginosa* isolates showed approximately 5-log reductions with combined therapy and approximately 6-log reductions with sequential treatment after 48–72 h, whereas resistant strains showed no consistent antibiofilm activity [52]. PEV20-ciprofloxacin performance varied across cystic fibrosis and wound isolates;

performance tracked with the phage-resistance profile of the target isolate [53]. Receptor-complementary cocktail design broadened coverage across 153 clinical *P. aeruginosa* isolates, and complementary-phage cocktails also suppressed resistance and retained activity in mixed and in vivo systems. However, the corresponding biofilm assay exposed bacteria to phage during biofilm formation rather than treating an established biofilm. It therefore supports host-range design rather than mature-biofilm efficacy [17]. A broad-host-range phiA85 study similarly supports host-range and phage-antibiotic pairing development, but its combination biofilm outcome emphasized formation inhibition rather than treatment of a preformed mature biofilm [54]. Because phage activity can differ between planktonic and biofilm states, host-range qualification should be repeated under biofilm conditions [43].

Physiological heterogeneity creates a second discordance. Antibiotics generally depend on the activity of their molecular target, whereas productive phage infection requires sufficient host metabolism for genome replication and virion assembly. High antibiotic exposures can reduce bacterial density or suppress phage replication, whereas selected cell-wall-targeting antibiotics increased phage adsorption or proliferation in the pB3074 system [55,56]. Persister-rich subpopulations can also behave differently: Sb-1 showed direct killing at high titre and lytic activity after lower-titre phage-treated persisters were transferred to fresh medium [51]. The interaction is consequently governed by timing and local physiology, not only by two independent killing rates.

Spatial gradients and hydrodynamics further separate biofilm from broth. Repeated phage exposure in a flow cell could select resistant biomass and increase total biovolume, an outcome that would be missed by a short planktonic optical-density curve [32]. Dynamic polyether ether ketone (PEEK) biofilms were more resilient than static biofilms grown on the same implant material, and a human-simulated dynamic PK/PD reactor showed that regimens selected from static biofilm time-kill did not all retain bactericidal activity over 96 h [4,57]. Flow can remove free phage, replenish antibiotic, redistribute nutrients and select surface phenotypes, so a static microplate may overestimate persistence of both agents or underestimate repeated delivery, depending on the clinical site.

A practical implication is that planktonic screens are best used as exclusion and design tools rather than as efficacy surrogates. They can identify nonpermissive phage-host pairs, gross antagonism, candidate concentration windows and antibiotic-induced changes in phage productivity. The candidate must then be retested in an explicitly aged biofilm using the intended surface or biological matrix, a direct viable endpoint, phage titres during treatment and resistance monitoring. The transition should be treated as a new experiment, not a confirmatory repetition.

5. Biofilm Maturity and Community Complexity as Determinants of PAS

Biofilm age was one of the most inconsistently standardized variables. Using the prespecified final coding rule, 20/54 studies (37.0%) included at least one qualifying biofilm/PAC condition aged ≥ 48 h, whereas 32/54 (59.3%) were clearly < 48 h and 2/54 (3.7%) could not be assigned a comparable pretreatment age (Supplementary Table S4A). The ≥ 48 h group includes studies with explicit 48–72 h conditions and the small number of substantially older biofilms or established day-7 implant infection models; mixed-age studies were coded positive only when a qualifying ≥ 48 h PAC condition was explicitly reported. This imbalance matters because mature communities generally have greater biomass, steeper nutrient and oxygen gradients, more differentiated matrix, and a larger fraction of slowly growing or dormant cells. The clearest within-study comparison found that one-week three-dimensional single-, dual- and poly-species biofilms were approximately one $\log_{10}$ more recalcitrant than matched 72 h communities after a single 24 h

phage-ciprofloxacin exposure [58]. A seven-day titanium model showed that planktonic testing predicted antagonism but not synergy in the mature-biofilm model [2].

Maturity does not make combination treatment futile, but it changes what must be optimized. In a humanized MRSA reactor, 40 h conditioned biofilms received treatment for 168 h; selected phage-daptomycin-ceftaroline regimens sustained roughly four-log killing under maintained exposure [28]. In a 72 h multidrug-resistant *E. coli* biofilm, phage-first treatment followed by amoxicillin-clavulanate after 7 h produced the strongest reduction across CFU, biomass and microscopy readouts [59]. In contrast, short single exposures in older three-dimensional communities left substantial residual burden even when the interaction was classified as synergistic [58]. Eradication, suppression and a statistically significant extra reduction are not equivalent endpoints; mature-biofilm studies should state which is being claimed.

Surface and flow are components of maturity rather than optional decoration. Dynamic PEEK biofilms were more resilient than static biofilms grown on the same implant material, while endotracheal-tube and titanium models provided device-specific tests on clinically relevant materials [2,52,57]. A 72 h Dacron graft assay linked device-biofilm testing to compassionate human treatment [31]. In 48 h *S. epidermidis* biofilms, higher phage inputs reduced bulk biofilm signal, but co-administration with therapeutic-level vancomycin did not resolve a phage-dose advantage and was limited by high assay variability [11]. A chronic *S. epidermidis* mouse PJI model used biofilm-coated titanium implants but unexpectedly low infection persistence prevented a confident treatment-efficacy conclusion [35]. These results argue for a clinically matched surface, delivery format and shear condition whenever the intended indication is device-associated infection, airway mucus, urinary flow or a perfused wound.

Community complexity was even less common: dual- or poly-species core models accounted for only 8/54 (14.8%). In *P. aeruginosa*-*S. aureus* communities, phage-first treatment remained effective, but dual-species biofilms required a higher gentamicin concentration to approach mono-species killing [60]. In bacterial-fungal communities, ciprofloxacin-phage performance was growth-model dependent; phage-antibacterial combinations active against *P. aeruginosa* alone produced no measurable overall-biomass reduction in 24 h dual-species biofilms containing *Candida albicans* [24,25]. Artificial-sputum core evidence showed unequal species responses [34]. Supporting three-dimensional lung epithelial evidence likewise showed strong sequence- and species-dependent effects after 2 h of bacterial colonization [16], but this model was not counted in the preformed-biofilm core.

Polymicrobial studies show that community composition can change treatment response and that total-community readouts may mask species persistence or replacement [25,58,61]. The 2026 three-dimensional hetero-cocktail study measured both total reduction and species composition, showing that treatment altered the proportions of species remaining in the biofilm [58]. Supporting clinical evidence makes the same problem visible at the bedside: in a polymicrobial bone-allograft biofilm infection, anti-*S. aureus* phage therapy with antibiotics produced initial improvement but recurrence followed when the other bacterial members were not covered by the phage regimen [20]. Biomass alone is particularly hazardous in polymicrobial systems because it cannot identify which organism remains.

Biological fluids add another layer. In a human-urine model, individual *A. baumannii* phages showed lower or unchanged antibiofilm activity versus tryptic soy broth, and phage-antibiotic interactions were antibiotic dependent; a later *S. aureus*-*Candida* study found that treatment rankings differed among urine, heat-inactivated plasma-blood and *Galleria* infection; the triple regimen was ineffective in the human-urine model [26,62]. Synovial-fluid aggregates, by contrast, retained a strong phage-first vancomycin effect

against MRSA [33]. The intended fluid should therefore be part of regimen selection, not reserved for late confirmation.

A minimum maturity comparison should include a young screening biofilm and an older model selected for the clinical site, with identical isolate, inoculum, surface, dose, sequence and endpoint. For polymicrobial indications, each species should be enumerated separately and the untreated community trajectory reported. This paired design makes age and ecology explicit determinants rather than hidden sources of between-study heterogeneity.

6. Treatment Design: Dose, Antibiotic Class, and Sequence of Administration

Phage dose is reported both as absolute concentration and as multiplicity of infection, but the two are not interchangeable in an established biofilm. MOI assumes a known, uniformly accessible bacterial denominator at the moment of dosing, whereas mature biofilms contain spatially segregated cells, inaccessible cells and a rapidly changing susceptible fraction. Absolute PFU/mL therefore provides the reproducible administered exposure, while MOI is best retained as a secondary descriptor calculated from a clearly defined pretreatment viable count. The dose-dependence observed with vB_KpUKJ_2-ceftazidime, YC#06-antibiotic mixtures, pB23-meropenem and early clinical-isolate *P. aeruginosa* phage-antibiotic testing shows that apparently similar MOIs can sit on different sides of an amplification or clearance threshold [5,8,46,47]. Supporting *S. epidermidis* experiments likewise found substantially greater persistence at low phage inputs in a 48 h biofilm, reinforcing the need to report absolute PFU/mL rather than MOI alone [11].

Higher phage input is not uniformly superior; dose-response behavior is phage- and model-dependent. In the YC#06 system, a moderate phage dose produced the maximum PAS effect [46]. In the *K. quasipneumoniae* model, 10^8 PFU/mL combined with $0.25 \times$ MIC ceftazidime produced rapid and sustained biofilm killing and was the most effective mature-biofilm condition tested [5]. In the carbapenem-resistant *A. baumannii* system, synergy remained stable over an intermediate MOI range, indicating a usable exposure window rather than a single exact ratio [47]. Future studies should therefore map at least three log-spaced phage concentrations and measure PFU recovery within the biofilm over time.

Antibiotic concentration has a similarly non-linear role. Sub-MIC or low-multiple MIC exposures often produced substantial additional benefit, but the effect was concentration- and drug-dependent rather than uniformly monotonic. Low antibiotic concentrations enhanced adjunct phage activity in staphylococcal biofilms, and sub-MIC ciprofloxacin, imipenem, vancomycin, ceftazidime or amoxicillin-clavulanate supported strong combinations in several species [5,32,59,63–65]. In resistant *E. coli* biofilms, staggered phage-first exposure reduced minimum biofilm bactericidal concentration (MBBC) values by up to 512-fold and markedly lowered MBECs for selected agents [66]. Conversely, high antibiotic exposure could suppress phage production or create order-dependent antagonism [42,55]. Concentration should therefore be expressed relative to the measured MIC and, when available, MBIC or MBEC, while also reporting the absolute mg/L exposure.

Antibiotic mechanism helps explain, but does not guarantee, the interaction. Beta-lactams were the most common class in the corpus and, in several *Klebsiella* and *Acinetobacter* systems, were associated with enhanced combined killing and, in some cases, increased phage proliferation or restored antibiotic susceptibility [5,47]. *E. faecium* biofilms broadened the cell-wall-active evidence through daptomycin-ampicillin and related beta-lactam-phage regimens that produced bactericidal activity while stabilizing phage or antibiotic susceptibility [27,67]. UJB10 sharpened the class argument by showing favorable cefepime activity, early time-dependent antagonism with selected aminoglycoside or carbapenem

conditions, and reduced MBEC₅₀ values for several antibiotics [44]. Supporting nine-strain pharmacodynamic data showed that the direction of interaction could remain strongly drug- and strain-dependent even within one phage system [45]. Negative evidence is equally informative: in *S. epidermidis* biofilms, AICAT plus vancomycin was additive rather than synergistic, with no antagonism but no significant improvement over phage alone in the 24 h biofilm condition [29]. Mechanistic class hypotheses should therefore guide a dose-time matrix, not replace one.

Treatment order was directly compared in 14 core studies, while phage-first exposure was explicitly tested in 17/54 (31.5%) and antibiotic-first exposure in 8/54 (14.8%); categories overlap because some studies tested multiple schedules (Supplementary Tables S1B and S4B). Phage-first schedules were favorable in several systems: a 72 h *E. coli* model was strongest when phage preceded amoxicillin-clavulanate by 7 h, and staggered phage-first exposure markedly lowered biofilm-active antibiotic concentrations in resistant *E. coli* [59,66]. In MDR *A. baumannii* biofilms, phage-first and simultaneous treatment produced the largest immediate viable-count reductions, whereas antibiotic-first was weaker [48]. A 24-isolate *A. baumannii* study directly compared sequential and simultaneous phage-colistin administration: sequential treatment was more effective in the 24 h comparison, whereas by 48 h both combination schedules generally outperformed the corresponding single agents [49]. These findings show that treatment order is an experimental variable rather than a fixed property of a phage–antibiotic pair.

Phage-first is not a universal rule, and the schedule that maximizes short-term killing need not optimize resistance control. In the *A. baumannii* sequence study, phage-first and simultaneous schedules were strongest for immediate biofilm killing, but only simultaneous treatment retained phage susceptibility through 48 h [48]. Simultaneous treatment was effective in many MRSA, *Pseudomonas*, *Acinetobacter* and *Klebsiella* systems [1,4,5,47]. Supporting daptomycin-nonsusceptible MRSA work outside the core denominator further showed that simultaneous or antibiotic-first schedules could outperform phage-first in selected ex vivo endocardial models and that the ranking changed with isolate and antibiotic concentration [68]. The correct conclusion is therefore not that phage should always come first, but that order is a manipulable pharmacological variable with separable bactericidal and evolutionary outcomes.

Table S1B identifies 10/54 core studies (18.5%) with repeated administration of at least one treatment component, including a repeated phage-only arm [32]; this is not a count of repeated full-PAC experiments. Longitudinal measurement alone was not counted. Three phage-gentamicin doses separated by eight hours outperformed a single dose in an artificial-dermis dual-species wound model [69]. Seven-day humanized reactors maintained repeated pharmacological pressure and exposed regrowth and resistance [28]. In chronic *P. aeruginosa* hip PJI, Pa53 was repeatedly administered locally with systemic meropenem after patient-specific biofilm testing [70]. Up to three daily PAC exposures were used in the seven-day titanium model, whereas the *S. epidermidis* PJI mouse model combined three phage administrations with twice-daily vancomycin and documented neutralizing antibodies [2,35]. Repeated phage alone can also select resistant biomass, as the flow-cell experiment demonstrated [32]. Repetition should therefore be tested with the full combination and longitudinal PFU/CFU sampling.

A practical factorial design crosses at least three absolute phage concentrations, three antibiotic exposures, three schedules (phage-first, antibiotic-first and simultaneous), and one repeated-dose arm in the intended mature biofilm. The primary comparison should be viable killing relative to the most active monotherapy at matched exposure, with phage titre and resistant survivors measured at several time points. Immediate bacterial reduction and resistance suppression should be treated as distinct co-primary design questions

because the same schedule can optimize one and worsen the other [48]. This design is more informative than optimizing one component while holding an arbitrary schedule constant. The biological windows created by these dosing orders are summarized in Figure 3.

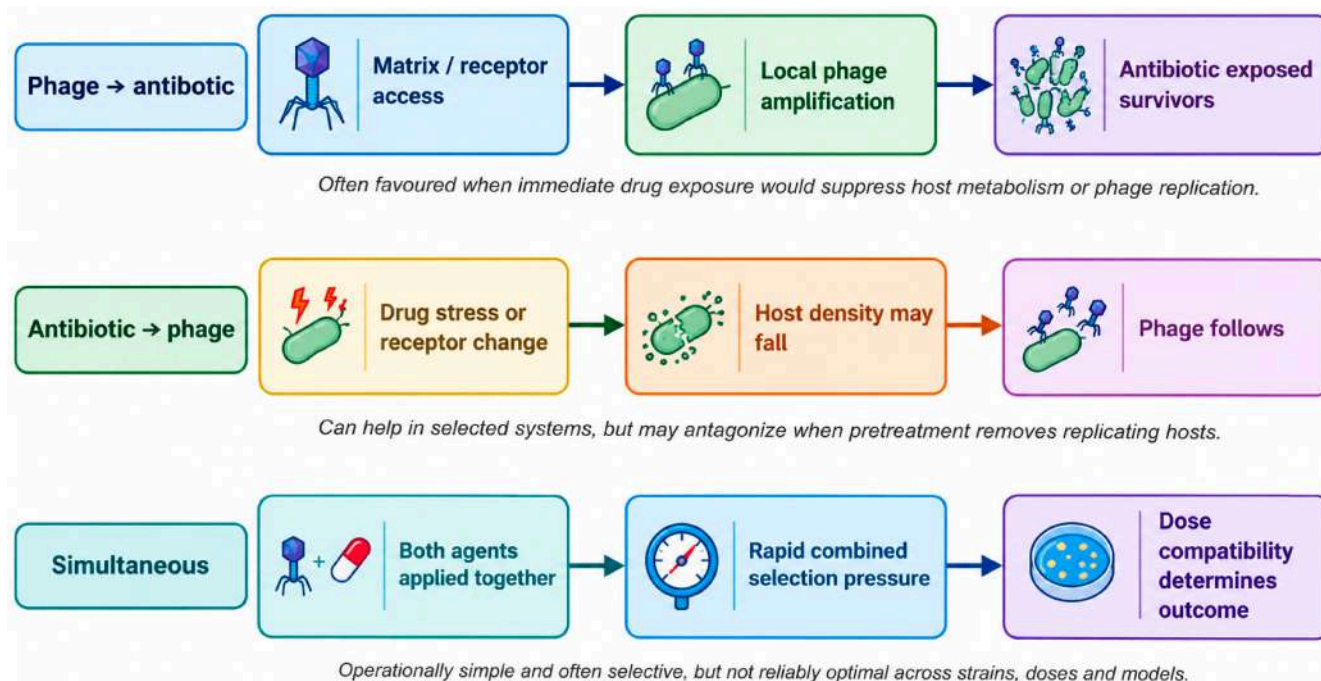


Figure 3. Treatment-sequence model. Phage-first, antibiotic-first and simultaneous schedules create different biological windows; the preferred schedule must be identified in the intended model and concentration range.

7. Resistance Evolution and Evolutionary Trade-Offs During Combination Therapy

An explicit treatment-related resistance or evolutionary-susceptibility assessment was reported in 18/54 (33.3%) core studies under the final strict coding rule (Supplementary Table S4B). This rule required direct post-exposure resistance/susceptibility, resistant-outgrowth, or evolutionary trade-off evidence; baseline MDR status alone was not counted. The depth ranged from survivor spot testing to longitudinal susceptibility analysis, and only one core study explicitly coupled combination-relevant survivors to genomic analysis [5]. Most of the corpus therefore still cannot distinguish transient killing from durable control. Resistance should be measured separately for phage, antibiotic and the combination because the relevant failure modes are not interchangeable.

Several studies showed suppression of phage-resistant outgrowth by a compatible antibiotic. In MRSA bead biofilms, phage resistance appeared under phage monotherapy but was avoided in active phage–antibiotic regimens [1]. In MDR *E. faecium*, daptomycin-beta-lactam-phage combinations stabilized phage and/or daptomycin susceptibility across selected strains [27,67]. Dynamic *P. aeruginosa* experiments identified phages that limited regrowth and resistance under human-simulated antibiotic exposure [4]. In mature *K. quasipneumoniae* biofilms, the optimized ceftazidime-phage combination produced no detectable resistant colonies even though phage monotherapy readily selected them [5]. Conversely, PA14 biofilms exposed to some multi-phage conditions showed regrowth, suggesting possible, strain-specific phage resistance [43]. These are evolutionary outcomes that cannot be inferred from the immediate viable-count difference alone.

Suppression is not inevitable. Supporting evolutionary evidence from a 15-day spatial experiment found that generalized resistance evolved rapidly and progressively con-

strained combination efficacy [15]; because phage and gentamicin were present from initial bacterial inoculation while bead biofilms developed, this study is not counted in the preformed-biofilm core. Repeated phage monotherapy in a core flow-cell biofilm study yielded resistant and reduced-sensitivity survivors while increasing biovolume, showing that a treatment can reduce susceptible cells while worsening the structural burden [32]. The *A. baumannii* sequence experiment adds a particularly important counterexample: phage-first and simultaneous treatment produced the largest biofilm reductions, but phage-first did not suppress phage resistance, whereas simultaneous treatment retained phage susceptibility through 48 h [48]. Combination studies that terminate at 6 or 24 h may therefore miss the clinically important phase in which resistant survivors expand.

The most informative studies connected resistance to a mechanistic trade-off. The foundational OMKO1 study showed that selection for phage resistance through the OprM component of MexAB/MexXY efflux systems restored susceptibility to several antibiotic classes in MDR *P. aeruginosa* [71]. The later aortic-graft case linked that mechanism to 72 h Dacron biofilm testing and adjunctive clinical treatment, although the uncontrolled case cannot establish causal efficacy [31]. Whole-genome sequencing of phage-resistant *K. quasipneumoniae* survivors identified changes including *fhuA*, *purA* and *rpoC*, and several mutants formed less biofilm than the parental strain [5]. In a human vascular-graft infection, in vitro testing showed phage-driven sensitization to levofloxacin and piperacillin/tazobactam and identified MexAB-OprM as a receptor for one therapeutic phage; post-treatment isolate profiling was unavailable to confirm in vivo resistance reversion [6]. A carbapenem-resistant *A. baumannii* study likewise found that pB23-resistant isolates regained meropenem susceptibility [47], while HS106-gentamicin retained activity against phage-resistant and dual-resistant *K. pneumoniae* mutants [72]. Supporting *A. baumannii* studies extend this trade-off spectrum: HZY2308-resistant AB48-R shifted from resistance to susceptibility for amikacin and tobramycin and toward intermediate susceptibility for cefepime and gentamicin, although ciprofloxacin susceptibility worsened and the receptor mechanism remained unresolved [73]. Phage Indie similarly restored ceftazidime susceptibility and prolonged killing in MDR *A. baumannii*, whereas its combination with piperacillin-tazobactam was antagonistic, emphasizing that resensitization is drug-specific rather than a generic consequence of phage exposure [74]. A 2026 hypermutator XDR *P. aeruginosa* hardware infection further shows why longitudinal genomics matters clinically: *mutS*-associated hypermutation accompanied rapidly evolving antibiotic resistance before adjunctive intravenous phage plus cefiderocol was used [22].

Trade-offs can also be ecological or immunological rather than genetic. In a longitudinal cystic fibrosis case, phages and antibiotics acted in different temporal niches; anti-phage immunoglobulin M emergence tracked attenuation of the dominant phage after approximately one week, and resistant variants showed limited expansion relative to susceptible populations [7]. A longitudinal *E. faecium* bacteremia report outside the core biofilm denominator similarly associated rising neutralizing activity with renewed bacterial burden despite earlier clinical improvement during adjunctive phage use [21]. In the *S. epidermidis* mouse PJI study, neutralizing antibodies also emerged during repeated dosing even though no treatment-emergent phage or vancomycin resistance was detected [35]. These observations cannot be summarized by a checkerboard score and show why host immunity must be integrated into resistance and PK/PD interpretation.

Future experiments should plate survivors on phage, antibiotic and combined selective conditions; quantify resistance frequencies at multiple times; retest stability after drug-free passage; measure growth, biofilm formation and virulence-related phenotypes; and sequence representative lineages. A combination should not be called resistance-suppressing merely because no colony was observed at one endpoint. The detection limit, plated population, number of independent cultures and duration of follow-up must be reported.

8. How Should PAS Against Biofilms Be Measured? Methodological Discordance and Standardization

Because PAS detection methods are established, this review does not reiterate general assay principles. The critical issue is endpoint discordance. Among the 54 core studies, direct viable-count or burden endpoints were used in 45/54 (83.3%) and biomass in 28/54 (51.9%). Metabolic or regrowth measures were used in 17/54 (31.5%), microscopy or bioluminescence in 17/54 (31.5%), and MBIC/MBEC-type endpoints in 11/54 (20.4%). These non-equivalent endpoints can assign different interaction categories to the same regimen and therefore limit cross-study comparability.

Accordingly, PAC is used throughout the synthesis for the treatment combination itself, whereas PAS is used only when the source study classifies the interaction as synergistic using an explicit criterion.

The most informative evidence comes from direct method comparisons. In the 2026 CApEsid study, CFU classified all four tested strains as additive, whereas extracellular DNA (eDNA) and extracellular adenosine triphosphate (ATP) classified all four as synergistic [18]. FICI can be useful for screening concentration regions, but its planktonic MIC basis and the changing concentration of a replicating phage make it insufficient as the sole definition of mature-biofilm synergy. Mature-biofilm studies should therefore define synergy prospectively using a viable endpoint at matched time and exposure and report the exact threshold used [1,4,5,18,28,75,76].

Secondary endpoints should explain, rather than replace, viable killing. Biomass can capture matrix removal or detachment, microscopy can localize structural change, and metabolic or regrowth assays can reveal residual activity; none alone establishes bactericidal eradication. Discordant endpoints should be reported explicitly rather than resolved by selecting the most favorable result. A practical evidence package is therefore viable burden as the primary endpoint, one orthogonal structural or spatial measure, and longitudinal regrowth or resistance follow-up [11,18,25,30,58,59,77].

The standardization problem is therefore not the absence of PAS assays but inconsistent application and reporting. The broader phage-biofilm literature is dominated by young static models [3], and the present PAC/PAS corpus likewise remains heterogeneous in age, interaction definition, and endpoint selection. Standardization should prioritize prespecified synergy criteria, direct mature-biofilm validation, transparent reporting of administered and recovered phage exposure, and cross-endpoint concordance. The minimum reporting elements are listed below.

Proposed Minimum Reporting Standard

- Identify the bacterial strain, source, resistance phenotype and relevant genotype; report whether the phage is strictly lytic and genomically screened for lysogeny, toxin and resistance genes.
- State biofilm age, pretreatment viable burden, community composition, surface/material, medium or biological fluid, temperature, oxygenation and static/flow conditions.
- Report the administered phage concentration in PFU/mL and the calculation used for MOI; measure PFU during treatment when mechanistic amplification is claimed.
- Report antibiotic concentration in mg/L and relative to MIC, MBIC or MBEC measured for the same strain and method.
- Specify phage-first, antibiotic-first or simultaneous dosing, the interval between components, treatment duration, wash steps and whether doses were repeated.
- Include untreated, phage-only, antibiotic-only, vehicle and, where relevant, inactive-phage or nonhost controls at matched sampling times.

- Use a direct viable endpoint, preferably time-resolved CFU with validated biofilm recovery and phage neutralization; give the limit of detection.
- Add at least one orthogonal endpoint for matrix, architecture, metabolic regrowth or host tissue effect, and explain how discordant results were interpreted.
- Define synergy prospectively relative to the most active monotherapy and specify the time point, baseline and threshold; do not use the word synergy for any statistically significant difference.
- Quantify phage-, antibiotic- and dual-resistant survivors over time; report plating denominator, detection limit and phenotype stability after drug-free passage.
- Sequence representative survivors when resistance suppression, receptor trade-offs or antibiotic resensitization are central claims.
- Report biological replicates, technical replicates, randomization or blinding where feasible, statistical model, raw study-level values and all tested dose-sequence conditions, including antagonistic results.

9. From In Vitro Biofilms to Clinical Translation

The translational ladder remains broad but shallow. Fifteen studies reached only young static biofilm Level 2, 11 reached mature mono-species Level 3, six reached surface/flow Level 4, and four reached polymicrobial Level 5. Three studies reached three-dimensional tissue/mucus/implant Level 6, two reached ex vivo Level 7, nine reached animal Level 8, and four reached human Level 9 (Figure 4). Assigning the highest attained level prevents an animal study from inflating lower rungs, but it does not imply equal quality within a level. Supplementary Table S1C now provides the individual highest-level assignment for all 54 core studies, and Supplementary Table S4B reproduces the same level code alongside the treatment and evolutionary flags.

Dynamic and humanized models provided the clearest bridge from screening to exposure realism. Four-day *P. aeruginosa* PK/PD and seven-day MRSA reactor studies simulated changing antibiotic concentrations and repeatedly measured bacterial and phage populations [4,28]. Supporting evidence outside the core denominator strengthened this bridge through fibrin-clot and simulated endocardial models, humanized ex vivo PAC systems, and quantitative multi-strain pharmacodynamic mapping [45,76,78–80]. These supporting studies were not added to the primary denominator because they did not directly test a qualifying preformed biofilm under the operational boundary.

Ex vivo and animal studies confirmed that biological context can preserve, weaken or reverse an in vitro interaction. pB3074 and pB23 combinations improved killing in pig skin, while YC#06, vB8388, phiLCL12 and pB23 regimens produced benefit in zebrafish or *Galleria* infection models [46,47,56,65,81]. The expanded corpus adds two useful counterpoints. In *E. coli*, phiWL-3 plus fosfomycin improved *Galleria* survival for the reference strain but not for the ciprofloxacin/ceftriaxone-resistant clinical isolate despite strong in vitro antibiofilm effects [66]. HS106-gentamicin improved zebrafish survival in MDR *K. pneumoniae*, whereas the *S. epidermidis* mouse PJI study could not support a confident efficacy conclusion because unexpectedly low infection persistence compressed the model's dynamic range [35,72]. Animal survival is valuable, but it should not replace direct sampling of bacterial burden, phage titre, antibiotic exposure and resistance at the infected site.

PAS also has a plausible role in animal production, particularly where MDR pathogens form biofilms on farm surfaces or cause infections that drive antimicrobial use. Recent poultry studies provide proof-of-concept. Bhutada et al. reported surface-dependent phage–antibiotic effects on crystal violet biomass in MDR avian pathogenic *Escherichia coli* biofilms on polypropylene, stainless steel, and polyvinyl chloride, although some ciprofloxacin combinations were antagonistic [12]. Luo et al. demonstrated in vitro synergy

with doxycycline or florfenicol; the chick experiment tested PTolC-69 plus doxycycline and showed reduced lung and spleen burdens [82]. Translation to production settings will require broad field-isolate coverage, practical delivery in relevant farm matrices, stability and dosing studies, resistance and microbiome surveillance, and controlled field trials that measure both animal-health outcomes and reductions in antibiotic use. PAS should therefore be developed as a targeted adjunct to, rather than a replacement for, hygiene, vaccination, biosecurity, and antimicrobial stewardship.

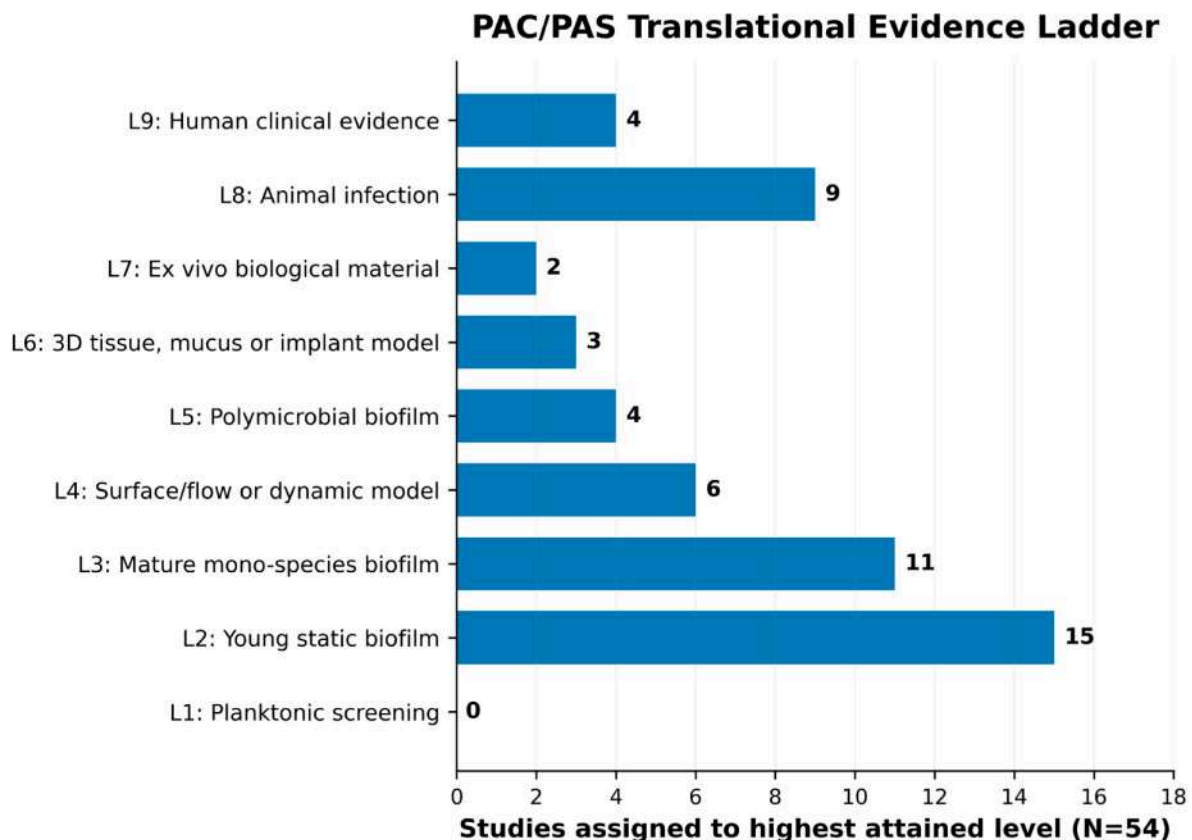


Figure 4. PAC/PAS translational evidence ladder (N = 54). Each core study was assigned to the highest level reached. Level 1 is zero because planktonic-only studies were excluded from the primary synthesis. The ladder represents increasing biological realism, not a validated hierarchy of study quality. PAC, phage–antibiotic combination; PAS, phage–antibiotic synergy.

Core human evidence consisted of four deeply characterized cases rather than controlled trials. A personalized regimen for limb-threatening *K. pneumoniae* prosthetic-knee infection combined isolate-specific combination biofilm testing with clinical phage use and limb-sparing management [13]. In chronic *P. aeruginosa* aortic-graft infection, 72 h Dacron biofilm testing excluded antagonism before local OMKO1-ceftazidime application [31]. In chronic hip PJI, patient-specific 24 h biofilm testing showed synergistic Pa53-meropenem eradication at sub-eradicating exposures before repeated local phage administration with meropenem; absence of relapse at two years remains confounded by surgery and antibiotic therapy [70]. A refractory *P. aeruginosa* mediastinitis and vascular-graft infection was treated with a bespoke regimen selected partly for an efflux-pump trade-off, with clinical and radiologic improvement and no recurrent bacteremia at one year [6]. None of these uncontrolled observations provides a comparative efficacy estimate.

Additional clinical reports were incorporated as supporting translational evidence without changing the core denominator because they did not provide qualifying direct complete-regimen biofilm testing. The longitudinal cystic fibrosis study by Luong et al.

contributed unusually detailed population genomics, phage kinetics and antibody data, but it evaluated ecological cooperation during treatment rather than a complete PAC regimen against a preformed patient-specific biofilm [7]. Other supporting reports include repeated local phage administration with systemic antibiotics for MDR *P. aeruginosa* PJI/osteomyelitis [19], a polymicrobial bone-allograft biofilm case in which incomplete phage coverage preceded recurrence [20], intravenous phage plus cefiderocol for a hypermutator XDR *P. aeruginosa* hardware infection [22], and multisite local, intravenous and nebulized phage delivery with ceftolozane/tazobactam for relapsing MDR *P. aeruginosa* frontal osteitis [23]. A systematic review of hip and knee PJI identified 18 clinical studies involving 53 patients; phage therapy was consistently adjunctive to antibiotics, while the evidence remained heterogeneous and methodologically weak [10]. These reports broaden feasibility, delivery and safety information but should not be interpreted as comparative PAC efficacy estimates.

The largest translational uncertainty is joint phage–antibiotic PK/PD in a biofilm compartment. Antibiotic exposure can often be simulated from known pharmacokinetics, whereas phage concentration depends on administration route, distribution, adsorption, replication, washout and immune neutralization. A high starting titre in a well does not prove that the same local exposure is achievable in lung mucus, bone, synovial fluid or a vascular graft. Conversely, local phage amplification may partly compensate for low delivered dose only if susceptible, metabolically permissive bacteria remain. The longitudinal cystic fibrosis and *Enterococcus* reports and the *S. epidermidis* PJI model show that neutralizing antibodies can emerge during therapy, while biological-fluid studies show that local matrices can change phage activity [7,21,26,35,62]. Both PFU and host response must therefore be measured during translation.

A translational package should include activity across a representative isolate panel, an explicitly mature biofilm on the intended material or fluid, and factorial dose-sequence optimization. It should also include repeated exposure with resistance follow-up, human-simulated antibiotic pharmacokinetics (PK) with realistic phage delivery, at least one site-matched animal or ex vivo confirmation, and a clinical monitoring plan for CFU, PFU, susceptibility, neutralizing antibodies, and recurrence. Human treatment should be linked prospectively to the laboratory regimen rather than supported by a retrospective demonstration that some combination was active.

Regulatory, Manufacturing, and Implementation Considerations

Regulatory readiness has clearer but still evolving anchors. Recent regulatory reviews emphasize defined phage and bacterial banks, identity and potency, purity and microbial safety, impurity and endotoxin control, validated manufacturing processes, and stability specifications for phage therapy medicinal products [83,84]. The regulatory review by Fuerst-Wilmes et al. describes the European Pharmacopoeia general chapter on phage therapy medicinal products as a framework for harmonized quality expectations, but adaptable or personalized products still raise the practical question of how phage composition can be changed rapidly while remaining within a validated quality and authorization framework [83].

Manufacturing and implementation constraints are equally important. Reproducible propagation, purification of bacterial contaminants and endotoxin, lot-to-lot activity, validated potency assays, product stability, GMP-compatible production capacity, and the turnaround time required for strain-matched products can all limit deployment [83,84]. Clinical implementation additionally requires timely phage–host matching, standardized phage–susceptibility and PAS testing, qualified phage banks, biofilm-relevant assays, PK/PD support, trained laboratory personnel, and reporting workflows fast enough to

influence antibiotic decisions [85]. A scalable PAC/PAS pathway therefore has to connect manufacturing quality systems with diagnostic selection, antibiotic stewardship, pharmacology, and prospective microbiological and clinical monitoring; biological synergy alone is insufficient for routine adoption.

10. Limitations of the Review

This review is narrative- and question-led rather than exhaustive or PRISMA-systematic. PubMed/MEDLINE was the principal database, and the retrospective extension of the search window to include 2015–2016 records was targeted rather than a complete rerun. Supplementary discovery during revision broadened source identification, but a formal multi-database systematic search was not performed and the original executable query log was not retained; relevant studies may therefore have been missed. The final 54-study denominator and its percentages describe the mapped corpus, not the prevalence of methods or effects in all published PAC/PAS research. Eligibility and publication-status checks were reconciled consistently before the final recount: biomass-only experimental studies are supporting rather than core, the patient-specific Level 9 structural-biofilm exception is explicitly labeled, studies in which combination exposure begins before a qualifying established/preformed biofilm are supporting rather than core, and one retracted publication [14] is excluded from all synthesis estimates. Because the corpus remains heterogeneous in models, endpoints and interaction definitions, the quantitative summaries are descriptive rather than meta-analytic effect estimates.

Interpretation is also constrained by heterogeneous biofilm ages, surfaces, inocula, phage doses, antibiotic exposures, sequence definitions, endpoints and synergy criteria; incomplete reporting prevented uniform coding for every variable and precluded meta-analysis. The mixed corpus did not support a single validated risk-of-bias instrument, advanced models remained sparse, and the four core human cases were uncontrolled and confounded by surgery, source control, concurrent antibiotics, host immunity and natural disease course. These limitations support cautious structured mapping rather than pooled efficacy claims.

11. Research Priorities

The evidence supports PAC/PAS as a rational strategy for mature MDR biofilms, but not as a universal class effect. Strong killing occurred in multiple organisms and advanced models, yet benefit repeatedly depended on strain-level phage susceptibility, matrix and receptor access, bacterial physiology, antibiotic mechanism and concentration, treatment sequence, dose repetition and model ecology [4,32,42–44,58]. Quantitative multi-strain pharmacodynamics further argues against treating antibiotic class as a universal predictor because interaction direction can vary substantially among strains [45]. Receptor-complementary cocktail design can broaden host range, but prevention-model and planktonic findings must still be revalidated in preformed mature biofilms [17,54]. Method-comparison data add a second requirement: robustness should be demonstrated across orthogonal endpoints rather than defined by the most favorable assay [18]. The field should therefore move from asking whether phages and antibiotics can be synergistic to asking which measurable conditions make a particular regimen robust.

Three cross-cutting priorities should be treated as non-negotiable for the next phase of the field: standardized mature-biofilm models with prespecified age, burden, matrix or surface, exposure, and endpoints; longitudinal resistance monitoring across repeated exposure and regrowth; and multicenter translational validation using shared protocols and representative isolate panels. These priorities are needed to distinguish robust regimen

effects from laboratory-specific signals and to support reproducible progression toward clinical or production use.

The most urgent experiments are paired, factorial and translational rather than simply larger. Each candidate should be tested against the same isolate in planktonic culture, a young biofilm and an older clinically matched biofilm; only then can discordance be quantified instead of inferred. Dose-sequence matrices should preserve absolute PFU/mL and mg/L data, while repeated dosing and longitudinal CFU/PFU sampling should expose regrowth. Mature mono-species work should be followed by the relevant surface, fluid and community, not by an unrelated animal model chosen only for convenience.

Resistance must become a co-primary outcome. The strongest mechanistic evidence came from experiments that combined viable killing with survivor phenotyping, genomics or clinically useful resensitization [5,6,27,67,71]. Supporting *A. baumannii* work shows both clinically useful resensitization and drug-specific trade-offs after phage selection [73,74], while a hypermutator clinical case illustrates how rapidly antibiotic resistance can evolve in a retained-hardware infection before adjunctive phage use [22]. Sequence experiments further show that the schedule producing the largest immediate reduction may not produce the best evolutionary outcome [48]. By contrast, a short endpoint with no survivor testing cannot establish durability, and enterobacterial repetitive intergenic consensus polymerase chain reaction fingerprint change should not be treated as equivalent to survivor whole-genome sequencing [59]. At minimum, future studies should quantify phage, antibiotic and dual resistance, and test whether selected variants pay costs in growth, biofilm formation, virulence or susceptibility to other drugs.

The following program would resolve the major unanswered questions:

- Build multicenter isolate panels that include the intended MDR lineage, phage-resistant subpopulations, receptor diversity and clinically relevant matrix phenotypes such as mucoidy or capsule variation; use receptor-complementary cocktails as a testable design strategy rather than assuming that more phages are intrinsically better.
- Use paired 24 h, 72 h and 7-day biofilms on the intended material or in the intended biological fluid to quantify the age penalty for each regimen.
- Run full concentration-by-sequence experiments with phage-first, antibiotic-first, simultaneous and repeated-dose arms; measure both administered and recovered PFU.
- Adopt CFU time-kill as the primary endpoint and require an orthogonal structural/spatial endpoint plus regrowth and resistance follow-up; when feasible, use a prespecified multiparametric workflow so that endpoint discordance is reported rather than averaged away.
- Sequence independent survivors from phage, antibiotic and combination arms and link mutations to adsorption, antibiotic susceptibility, biofilm fitness and virulence.
- Advance only regimens that retain benefit in human-simulated antibiotic PK/PD and realistic phage delivery, then confirm them in a site-matched ex vivo or animal model.
- For compassionate or trial use, predefine laboratory-clinical concordance measures, serial isolate testing, phage titres, antibiotic concentrations, neutralizing-antibody measurements, adverse events and recurrence.

A concise translational workflow is: match, model, optimize, stress-test and monitor. Match means confirming productive infection and genomic safety in the patient or target isolate. Model means recreating the mature biofilm age, material, fluid and community. Optimize means jointly selecting phage concentration, antibiotic exposure, sequence and repetition. Stress-test means challenging the regimen with resistant survivors, realistic PK/PD and an advanced model. Monitor means measuring bacterial burden, phage exposure, resistance, immunity and recurrence during clinical use.

12. Conclusions

The central finding of this review is that PAS in MDR biofilms is a context-dependent phenotype rather than a fixed property of a phage–antibiotic pair. Across the mapped evidence, outcomes depended on biofilm maturity and matrix, strain-level phage susceptibility, absolute phage and antibiotic exposure, treatment order, repeated dosing, endpoint selection, and resistance evolution. Planktonic synergy was therefore insufficient to predict mature-biofilm activity, and the schedule producing the greatest immediate killing did not necessarily best suppress resistance. Evidence beyond static in vitro systems remained limited: nine core studies reached animal models and four core human reports meeting the defined Level 9 translational criteria supported feasibility, but not comparative efficacy.

Future research should prioritize paired planktonic-to-mature-biofilm validation, factorial dose-sequence experiments, longitudinal CFU/PFU and resistance tracking, survivor genomics, and orthogonal assay confirmation. Candidate regimens should advance only when activity persists in clinically or production-relevant surfaces, fluids, and communities and under realistic PK/PD, delivery, and host-immunity constraints. The most useful translational framework is therefore to match the phage to the target isolate, model the intended biofilm, optimize dose and sequence, stress-test resistance and exposure, and monitor microbiological and clinical outcomes. This staged approach is more defensible than assuming a universal phage-first, simultaneous, or cocktail rule.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/microbiolres17100191/s1>: Table S1A. Characteristics of the 54 core PAC/PAS biofilm studies; Table S1B. Treatment design and sequence coding for studies that directly compared order, explicitly used repeated administration, or provided informative dose–response evidence; Table S1C. Highest attained translational level for all 54 core studies; Table S2. Assay methods and definitions of synergy for PAC/PAS biofilm experiments; Table S3. Supporting, contextual and excluded evidence incorporated through 21 August 2026, including biomass-only studies, non-preformed-biofilm combination models [15,16], and the retracted reference [14], all excluded from core n/N calculations; Table S4A. Reproducible study-level descriptive and endpoint coding; Table S4B. Reproducible treatment, antibiotic class, resistance/genomics and translational-level coding.

Author Contributions: Methodology, B.Z. and K.H.; formal analysis, T.A., B.Z. and K.H.; investigation, K.H.; writing—original draft, T.A. and K.H.; writing—review and editing, T.A. All authors have read and agreed to the published version of the manuscript.

Funding: The research received no external funding.

Institutional Review Board Statement: Not applicable. This article is a critical narrative review and reports no new study involving humans or animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: The complete 54-study core evidence matrix is presented in Supplementary Table S1A. Supplementary Table S1B provides selected treatment-design details; Table S1C provides the highest translational level for every core study; Table S2 summarizes assay interpretation; Table S3 documents all non-core supporting/contextual sources and explicit exclusions, including references [14–16]; Table S4A provides the reproducible study-level coding for year, pathogen, community, phage strategy, pretreatment-age category and endpoint classes; and Table S4B provides the sequence, repeated-dosing, antibiotic class, resistance/genomics and translational-level flags. All final n/N values reported in the manuscript can therefore be traced to the supplementary coding tables.

Acknowledgments: During the preparation and revision of this manuscript, the authors used ChatGPT (OpenAI, San Francisco, CA, USA; GPT-5.6 Sol, accessed 13 September 2026) for language editing, cross-document consistency checks, structured coding and tabulation, targeted bibliographic/source

verification, and AI-assisted generation, redesign, and graphical optimization of selected schematic figures and illustrative elements. The underlying scientific concepts, data, figure structure, labels, and interpretations were provided and verified by the authors. All AI-assisted outputs were critically reviewed and edited by the authors, who take full responsibility for the accuracy, integrity, and content of this publication.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

3D, three-dimensional; ATCC, American Type Culture Collection; ATP, adenosine triphosphate; CApEsid, multiparametric clinical biofilm pipeline; CDC, Centers for Disease Control and Prevention; CF, cystic fibrosis; CFU, colony-forming units; DAP, daptomycin; DOI, digital object identifier; eDNA, extracellular DNA; ERIC-PCR, enterobacterial repetitive intergenic consensus polymerase chain reaction; ESBL, extended-spectrum beta-lactamase; ESKAPE, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.; FICI, fractional inhibitory concentration index; MBBC, minimum biofilm bactericidal concentration; MBEC, minimum biofilm eradication concentration; MBIC, minimum biofilm inhibitory concentration; MDR, multidrug-resistant; MIC, minimum inhibitory concentration; MOI, multiplicity of infection; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; OD, optical density; PAC, phage-antibiotic combination; PAS, phage-antibiotic synergy; PDR, pandrug-resistant; PEEK, polyether ether ketone; PFU, plaque-forming units; PJI, periprosthetic joint infection; PK, pharmacokinetics; PK/PD, pharmacokinetics/pharmacodynamics; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; STEC, Shiga toxin-producing *Escherichia coli*; VRE, vancomycin-resistant enterococci; XDR, extensively drug-resistant.

References

1. Kebriaei, R.; Lev, K.L.; Shah, R.M.; Stamper, K.C.; Holger, D.J.; Morrisette, T.; Kunz Coyne, A.J.; Lehman, S.M.; Rybak, M.J. Eradication of biofilm-mediated methicillin-resistant *Staphylococcus aureus* infections in vitro: Bacteriophage-antibiotic combination. *Microbiol. Spectr.* **2022**, *10*, e00411-22. [[CrossRef](#)] [[PubMed](#)]
2. Verheul, M.; Schonkeren-Ravensbergen, E.; Bus, E.M.; de Boer, M.G.J.; Nelissen, R.G.H.H.; Pijls, B.G.; Nibbering, P.H. Efficacy prediction of bacteriophage-antibiotic combinations against *Staphylococcus aureus* biofilms using planktonic bacteria. *Front. Microbiol.* **2026**, *16*, 1706083. [[CrossRef](#)] [[PubMed](#)]
3. Amorim Perestrelo de Meneses, L.; Brandão, A.C.; Coenye, T.; Braga, A.C.; Pires, D.P.; Azeredo, J. A systematic review of the use of bacteriophages for in vitro biofilm control. *Eur. J. Clin. Microbiol. Infect. Dis.* **2023**, *42*, 919–928. [[CrossRef](#)] [[PubMed](#)]
4. Holger, D.J.; El Ghali, A.; Bhutani, N.; Lev, K.L.; Stamper, K.; Kebriaei, R.; Kunz Coyne, A.J.; Morrisette, T.; Shah, R.; Alexander, J.; et al. Phage-antibiotic combinations against multidrug-resistant *Pseudomonas aeruginosa* in in vitro static and dynamic biofilm models. *Antimicrob. Agents Chemother.* **2023**, *67*, e00578-23. [[CrossRef](#)] [[PubMed](#)]
5. Tchatchiashvili, T.; Marquet, M.; Gabashvili, E.; Mirza, K.A.; Lohde, M.; Brandt, C.; Ehrlich, R.; Pletz, M.W.; Makarewicz, O. Phage-antibiotic synergy restores beta-lactam efficacy in MDR *Klebsiella quasipneumoniae* biofilms and suppresses resistance. *J. Biomed. Sci.* **2026**, *33*, 25. [[CrossRef](#)] [[PubMed](#)]
6. Chung, S.J.; Liu, Y.; Thong, S.; Zhong, Y.; Chong, Z.S.; Lim, Z.; Tan, S.; Yeo, J.H.; Koh, M.G.; Chua, N.G.S.; et al. Timely bespoke phage-antibiotic combination to treat refractory *Pseudomonas aeruginosa* mediastinitis and vascular graft infection. *Nat. Commun.* **2026**, *17*, 1385. [[CrossRef](#)] [[PubMed](#)]
7. Luong, T.; Kharrat, L.; Champagne-Jorgensen, K.; Melendez, J.A.; Pride, D.; Conrad, D.J.; Roach, D.R. Ecological partitioning enables phage-antibiotic cooperation in a human *Pseudomonas* infection. *Nat. Commun.* **2026**, *17*, 2615. [[CrossRef](#)] [[PubMed](#)]
8. Nouraldin, A.A.M.; Baddour, M.M.; Harfoush, R.A.H.; Essa, S.A.M. Bacteriophage-antibiotic synergism to control planktonic and biofilm producing clinical isolates of *Pseudomonas aeruginosa*. *Alex. J. Med.* **2016**, *52*, 99–105. [[CrossRef](#)]
9. Mobarezi, Z.; Esfandiari, A.H.; Abolbashari, S.; Meshkat, Z. Efficacy of phage therapy in controlling staphylococcal biofilms: A systematic review. *Eur. J. Med. Res.* **2025**, *30*, 605. [[CrossRef](#)] [[PubMed](#)]
10. Migliorini, F.; Schäfer, L.; Vaishya, R.; Eschweiler, J.; Oliva, F.; Driessen, A.; Pipino, G.; Maffulli, N. Bacteriophages in hip and knee periprosthetic joint infections: A promising tool in the era of antibiotic resistance. *Med. Sci.* **2026**, *14*, 9. [[CrossRef](#)] [[PubMed](#)]

11. Young, J.; Shariyate, M.J.; Hedayatzadeh Razavi, A.; Nazarian, A.; Rodriguez, E.K. Bacteriophage dosing and its effect on bacterial growth suppression in a *Staphylococcus epidermidis* model: An in vitro study. *PHAGE Ther. Appl. Res.* **2024**, *5*, 223–229. [[CrossRef](#)] [[PubMed](#)]
12. Bhutada, P.; Joshi, N.; Saroj, S.D.; Koratkar, S. Dynamics of multidrug-resistant avian pathogenic *E. coli* biofilm formation on various surfaces and its dispersion with phage antibiotic synergism. *BMC Microbiol.* **2026**, *26*, 483. [[CrossRef](#)] [[PubMed](#)]
13. Cano, E.J.; Cafilisch, K.M.; Bollyky, P.L.; Van Belleghem, J.D.; Patel, R.; Fackler, J.; Brownstein, M.J.; Horne, B.; Biswas, B.; Henry, M.; et al. Phage therapy for limb-threatening prosthetic knee *Klebsiella pneumoniae* infection: Case report and in vitro characterization of anti-biofilm activity. *Clin. Infect. Dis.* **2021**, *73*, e144–e151. [[CrossRef](#)] [[PubMed](#)]
14. De Soir, S.; Paré, H.; Kamarudin, N.H.N.; Wagemans, J.; Lavigne, R.; Braem, A.; Merabishvili, M.; De Vos, D.; Pirnay, J.-P.; Van Bambeke, F. Exploiting phage-antibiotic synergies to disrupt *Pseudomonas aeruginosa* PAO1 biofilms in the context of orthopedic infections. *Microbiol. Spectr.* **2024**, *12*, e03219–23. [[CrossRef](#)] [[PubMed](#)]
15. Moulton-Brown, C.E.; Friman, V.-P. Rapid evolution of generalized resistance mechanisms can constrain the efficacy of phage-antibiotic treatments. *Evol. Appl.* **2018**, *11*, 1630–1641. [[CrossRef](#)] [[PubMed](#)]
16. Akturk, E.; Pinto, G.; Ostyn, L.; Crabbé, A.; Melo, L.D.R.; Azeredo, J.; Coenye, T. Combination of phages and antibiotics with enhanced killing efficacy against dual-species bacterial communities in a three-dimensional lung epithelial model. *Biofilm* **2025**, *9*, 100245. [[CrossRef](#)] [[PubMed](#)]
17. Kim, M.K.; Chen, Q.; Echterhof, A.; Pennetzdorfer, N.; McBride, R.C.; Banaei, N.; Burgener, E.B.; Milla, C.E.; Bollyky, P.L. A blueprint for broadly effective bacteriophage-antibiotic cocktails against bacterial infections. *Nat. Commun.* **2024**, *15*, 9987. [[CrossRef](#)] [[PubMed](#)]
18. Rimón, A.; Braunstein, R.; Yerushalmy, O.; Katz, N.; Yosef, L.; Gvili, Y.; Copenhagen-Glazer, S.; Hazan, R. CAPesid biOfilm: A suggested pipeline for clinical phage microbiology for biofilm infections based on comparative method study. *Sci. Rep.* **2026**, *16*, 23242. [[CrossRef](#)] [[PubMed](#)]
19. Tkhalishvili, T.; Winkler, T.; Müller, M.; Perka, C.; Trampuz, A. Bacteriophages as adjuvant to antibiotics for the treatment of periprosthetic joint infection caused by multidrug-resistant *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **2020**, *64*, e00924–19. [[CrossRef](#)] [[PubMed](#)]
20. Van Nieuwenhuysse, B.; Galant, C.; Brichard, B.; Docquier, P.-L.; Djebara, S.; Pirnay, J.-P.; Van der Linden, D.; Merabishvili, M.; Chatzis, O. A case of in situ phage therapy against *Staphylococcus aureus* in a bone allograft polymicrobial biofilm infection: Outcomes and phage-antibiotic interactions. *Viruses* **2021**, *13*, 1898. [[CrossRef](#)] [[PubMed](#)]
21. Stellfox, M.E.; Fernandes, C.; Shields, R.K.; Haidar, G.; Hughes Kramer, K.; Dembinski, E.; Mangalea, M.R.; Arya, G.; Canfield, G.S.; Duerkop, B.A.; et al. Bacteriophage and antibiotic combination therapy for recurrent *Enterococcus faecium* bacteremia. *mBio* **2024**, *15*, e03396–23. [[CrossRef](#)] [[PubMed](#)]
22. Ngauy, V.; Bevans, S.; Kido, D.; Ibrahimovic, R.; Kamau, E.; Caldwell, A.; Pangilinan, R.; Levit, R.; Lebreton, F.; Filippov, A.A.; et al. Clearance of hypermutator XDR *Pseudomonas osteomyelitis* and hardware infection with adjunctive bacteriophage therapy. *ASM Case Rep.* **2026**, *2*, e00108–25. [[CrossRef](#)] [[PubMed](#)]
23. Denis, A.; Ferry, T.; Jegoux, F.; Menez, T.; Briot, T.; Gomes Von Borowski, R.; Fevre, C.; Le Corre, A.; Revest, M.; Luque Paz, D. Adjuvant multisite bacteriophage cocktail administration as salvage treatment in a patient with a multidrug-resistant *Pseudomonas aeruginosa* frontal relapsing complex osteitis. *Int. J. Infect. Dis.* **2026**, *169*, 108756. [[CrossRef](#)] [[PubMed](#)]
24. Roszak, M.; Dołęgowska, B.; Cecerska-Heryć, E.; Serwin, N.; Jabłońska, J.; Grygorcewicz, B. Bacteriophage-ciprofloxacin combination effectiveness depends on *Staphylococcus aureus*-*Candida albicans* dual-species communities' growth model. *Microb. Drug Resist.* **2022**, *28*, 613–622. [[CrossRef](#)] [[PubMed](#)]
25. Manohar, P.; Loh, B.; Nachimuthu, R.; Leptihn, S. Phage-antibiotic combinations to control *Pseudomonas aeruginosa*-*Candida* two-species biofilms. *Sci. Rep.* **2024**, *14*, 9354. [[CrossRef](#)] [[PubMed](#)]
26. Gliźniewicz, M.; Dołęgowska, B.; Dubrowska, K.; Honselmann Genannt Humme, J.; Gawęda, M.; Grygorcewicz, B. Model-dependent efficacy of sequential phage-antibiotic-antifungal therapy against dual-species biofilms. *Biofilm* **2026**, *11*, 100365. [[CrossRef](#)] [[PubMed](#)]
27. Kunz Coyne, A.J.; Stamper, K.; Kebriaei, R.; Holger, D.J.; El Ghali, A.; Morrisette, T.; Biswas, B.; Wilson, M.; Deschenes, M.V.; Canfield, G.S.; et al. Phage cocktails with daptomycin and ampicillin eradicates biofilm-embedded multidrug-resistant *Enterococcus faecium* with preserved phage susceptibility. *Antibiotics* **2022**, *11*, 1175. [[CrossRef](#)] [[PubMed](#)]
28. Kunz Coyne, A.J.; Stamper, K.; Bleick, C.; Kebriaei, R.; Lehman, S.M.; Rybak, M.J. Synergistic bactericidal effects of phage-enhanced antibiotic therapy against MRSA biofilms. *Microbiol. Spectr.* **2024**, *12*, e03212–23. [[CrossRef](#)] [[PubMed](#)]
29. Duarte, A.C.; Fernández, L.; Rodríguez, A.; García, P. A new bacteriophage infecting *Staphylococcus epidermidis* with potential for removing biofilms by combination with chimeric lysin CHAPSH3b and vancomycin. *mSphere* **2025**, *10*, e01014–24. [[CrossRef](#)] [[PubMed](#)]

30. Fanaei Pirlar, R.; Irigoyen-von-Sierakowski, A.; Wagemans, J.; Ning, Y.; Lavigne, R.; Trampuz, A.; Karbysheva, S. Novel lytic phages improve the antibiofilm activity of dalbavancin, daptomycin, and fosfomycin against vancomycin-resistant enterococci. *Microbiol. Spectr.* **2026**, *14*, e01818-25. [[CrossRef](#)] [[PubMed](#)]
31. Chan, B.K.; Turner, P.E.; Kim, S.; Mojibian, H.R.; Elefteriades, J.A.; Narayan, D. Phage treatment of an aortic graft infected with *Pseudomonas aeruginosa*. *Evol. Med. Public Health* **2018**, *2018*, 60–66. [[CrossRef](#)] [[PubMed](#)]
32. Henriksen, K.; Rørbo, N.; Rybtke, M.L.; Martinet, M.G.; Tolker-Nielsen, T.; Høiby, N.; Middelboe, M.; Ciofu, O. *Pseudomonas aeruginosa* flow-cell biofilms are enhanced by repeated phage treatments but can be eradicated by phage-ciprofloxacin combination. *Pathog. Dis.* **2019**, *77*, ftz011. [[CrossRef](#)] [[PubMed](#)]
33. Taha, M.; Arnaud, T.; Lightly, T.J.; Peters, D.; Wang, L.; Chen, W.; Cook, B.W.M.; Theriault, S.S.; Abdelbary, H. Combining bacteriophage and vancomycin is efficacious against MRSA biofilm-like aggregates formed in synovial fluid. *Front. Med.* **2023**, *10*, 1134912. [[CrossRef](#)] [[PubMed](#)]
34. Wang, Z.; De Soir, S.; Glorieux, A.; Merabishvili, M.; Knoop, C.; De Vos, D.; Pirnay, J.-P.; Van Bambeke, F. Bacteriophages as potential antibiotic potentiators in cystic fibrosis: A new model to study the combination of antibiotics with a bacteriophage cocktail targeting dual species biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Int. J. Antimicrob. Agents* **2024**, *64*, 107276. [[CrossRef](#)] [[PubMed](#)]
35. Štilec, V.; Marušić, M.; Motaln, H.; Gregorič, K.; Draksler, U.; Čater, M.; Izlakar, J.; Janež, N.; Trebše, R.; Horvat, S.; et al. Vancomycin and phage COP-80B combination therapy for *Staphylococcus epidermidis* periprosthetic joint infections: A preclinical mouse study. *Eur. J. Clin. Microbiol. Infect. Dis.* **2026**, *45*, 2859–2873. [[CrossRef](#)] [[PubMed](#)]
36. Shlezinger, M.; Copenhagen-Glazer, S.; Gelman, D.; Beyth, N.; Hazan, R. Eradication of vancomycin-resistant enterococci by combining phage and vancomycin. *Viruses* **2019**, *11*, 954. [[CrossRef](#)] [[PubMed](#)]
37. Necel, A.; Bloch, S.; Topka-Bielecka, G.; Janiszewska, A.; Łukasiak, A.; Nejman-Faleńczyk, B.; Węgrzyn, G. Synergistic effects of bacteriophage vB_Eco4-M7 and selected antibiotics on the biofilm formed by Shiga toxin-producing *Escherichia coli*. *Antibiotics* **2022**, *11*, 712. [[CrossRef](#)] [[PubMed](#)]
38. Soontarach, R.; Nwabor, O.F.; Voravuthikunchai, S.P. Interaction of lytic phage T1245 with antibiotics for enhancement of antibacterial and anti-biofilm efficacy against multidrug-resistant *Acinetobacter baumannii*. *Biofouling* **2022**, *38*, 994–1005. [[CrossRef](#)] [[PubMed](#)]
39. Kebriaei, R.; Lehman, S.M.; Shah, R.M.; Stamper, K.C.; Kunz Coyne, A.J.; Holger, D.; El Ghali, A.; Rybak, M.J. Optimization of phage-antibiotic combinations against *Staphylococcus aureus* biofilms. *Microbiol. Spectr.* **2023**, *11*, e04918-22. [[CrossRef](#)] [[PubMed](#)]
40. Fanaei Pirlar, R.; Halili, N.; Travník, T.; Trampuz, A.; Karbysheva, S. In vitro activity of cefiderocol against Gram-negative aerobic bacilli in planktonic and biofilm form-alone and in combination with bacteriophages. *Sci. Rep.* **2025**, *15*, 17105. [[CrossRef](#)] [[PubMed](#)]
41. Ilyina, V.; Gatina, A.; Trizna, E.; Siniagina, M.; Yadykova, L.; Ivannikova, A.; Ozhegov, G.; Zhuravleva, D.; Fedorova, M.; Gorshkova, A.; et al. New bacteriophage *Pseudomonas* phage Ka2 from a tributary stream of Lake Baikal. *Viruses* **2025**, *17*, 189. [[CrossRef](#)] [[PubMed](#)]
42. Kumaran, D.; Taha, M.; Yi, Q.; Ramirez-Arcos, S.; Diallo, J.-S.; Carli, A.; Abdelbary, H. Does treatment order matter? Investigating the ability of bacteriophage to augment antibiotic activity against *Staphylococcus aureus* biofilms. *Front. Microbiol.* **2018**, *9*, 127. [[CrossRef](#)] [[PubMed](#)]
43. Van Helden, S.R.; Bleick, C.R.; Holger, D.J.; El Ghali, A.; Alexander, J.; Kaye, K.S.; Marshall, S.H.; Rojas, L.J.; Bonomo, R.A.; Lehman, S.M.; et al. Impact of *Pseudomonas aeruginosa* biofilm exopolysaccharide composition on bacteriophage and bacteriophage-antibiotic combination activity. *Antimicrob. Agents Chemother.* **2026**, *70*, e00925-25. [[CrossRef](#)] [[PubMed](#)]
44. Nicholls, P.; Clark, J.R.; Gu Liu, C.; Terwilliger, A.; Maresso, A.W. Class-driven synergy and antagonism between a *Pseudomonas* phage and antibiotics. *Infect. Immun.* **2023**, *91*, e00065-23. [[CrossRef](#)] [[PubMed](#)]
45. Baars, L.R.; Gu, M.; Guo, T.; van Hasselt, J.G.C. Strain-dependent variability in pharmacodynamic interactions for phage-antibiotic combinations in *Pseudomonas aeruginosa*. *Int. J. Antimicrob. Agents* **2026**, *67*, 107895. [[CrossRef](#)] [[PubMed](#)]
46. Luo, J.; Xie, L.; Liu, M.; Li, Q.; Wang, P.; Luo, C. Bactericidal synergism between phage YC#06 and antibiotics: A combination strategy to target multidrug-resistant *Acinetobacter baumannii* in vitro and in vivo. *Microbiol. Spectr.* **2022**, *10*, e00096-22. [[CrossRef](#)] [[PubMed](#)]
47. Luo, J.; Liu, M.; Ai, W.; Zheng, X.; Liu, S.; Huang, K.; Zhang, C.; Li, Q.; Luo, C. Synergy of lytic phage pB23 and meropenem combination against carbapenem-resistant *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **2024**, *68*, e00448-24. [[CrossRef](#)] [[PubMed](#)]
48. Mukhopadhyay, S.; Zhang, P.; To, K.K.W.; Liu, Y.; Bai, C.; Leung, S.S.Y. Sequential treatment effects on phage-antibiotic synergistic application against multi-drug-resistant *Acinetobacter baumannii*. *Int. J. Antimicrob. Agents* **2023**, *62*, 106951. [[CrossRef](#)] [[PubMed](#)]
49. Erol, H.B.; Kaskatepe, B.; Yildiz, S.; Altanlar, N. The effect of phage-antibiotic combination strategy on multidrug-resistant *Acinetobacter baumannii* biofilms. *J. Microbiol. Methods* **2023**, *210*, 106752. [[CrossRef](#)] [[PubMed](#)]

50. Martin, I.; Morales, S.; Alton, E.W.F.W.; Davies, J.C. Lytic bacteriophage is a promising adjunct to common antibiotics across cystic fibrosis clinical strains and culture models of *Pseudomonas aeruginosa* infection. *Antibiotics* **2023**, *12*, 593. [[CrossRef](#)] [[PubMed](#)]
51. Tkhilaishvili, T.; Lombardi, L.; Klatt, A.-B.; Trampuz, A.; Di Luca, M. Bacteriophage Sb-1 enhances antibiotic activity against biofilm, degrades exopolysaccharide matrix and targets persisters of *Staphylococcus aureus*. *Int. J. Antimicrob. Agents* **2018**, *52*, 842–853. [[CrossRef](#)] [[PubMed](#)]
52. de Oliveira, V.C.; Soler-Comas, A.; Rocha, A.C.S.D.; Silva-Lovato, C.H.; Watanabe, E.; Torres, A.; Fernández-Barat, L. The synergistic effect between phages and ceftolozane/tazobactam in *Pseudomonas aeruginosa* endotracheal tube biofilm. *Emerg. Microbes Infect.* **2024**, *13*, 2420737. [[CrossRef](#)] [[PubMed](#)]
53. Chang, R.Y.K.; Das, T.; Manos, J.; Kutter, E.; Morales, S.; Chan, H.-K. Bacteriophage PEV20 and ciprofloxacin combination treatment enhances removal of *Pseudomonas aeruginosa* biofilm isolated from cystic fibrosis and wound patients. *AAPS J.* **2019**, *21*, 49. [[CrossRef](#)] [[PubMed](#)]
54. Li, Z.; Fan, Z.; Fu, T.; Chen, Y.; Gan, L.; Du, B.; Cui, X.; Xue, G.; Feng, Y.; Zhao, H.; et al. A novel broad host range phage phiA85 displays a synergistic effect with antibiotics targeting carbapenem-resistant *Klebsiella pneumoniae*. *Microbiol. Spectr.* **2025**, *13*, e02019-25. [[CrossRef](#)] [[PubMed](#)]
55. Chaudhry, W.N.; Concepción-Acevedo, J.; Park, T.; Andleeb, S.; Bull, J.J.; Levin, B.R. Synergy and order effects of antibiotics and phages in killing *Pseudomonas aeruginosa* biofilms. *PLoS ONE* **2017**, *12*, e0168615. [[CrossRef](#)] [[PubMed](#)]
56. Luo, J.; Xie, L.; Yang, M.; Liu, M.; Li, Q.; Wang, P.; Fan, J.; Jin, J.; Luo, C. Synergistic antibacterial effect of phage pB3074 in combination with antibiotics targeting cell wall against multidrug-resistant *Acinetobacter baumannii* in vitro and ex vivo. *Microbiol. Spectr.* **2023**, *11*, e00341-23. [[CrossRef](#)] [[PubMed](#)]
57. Joo, H.; Wu, S.M.; Soni, I.; Wang-Crocker, C.; Matern, T.; Beck, J.P.; Loc-Carrillo, C. Phage and antibiotic combinations reduce *Staphylococcus aureus* in static and dynamic biofilms grown on an implant material. *Viruses* **2023**, *15*, 460. [[CrossRef](#)] [[PubMed](#)]
58. Glonti, T.; Aktan, M.K.; Cochez, C.; Zayed, N.; Braem, A.; Teughels, W.; Pirnay, J.-P. Single treatment of mature 3D single-, dual- and poly-species biofilms using a combination therapy of phage or phage-hetero-cocktails and ciprofloxacin. *Antibiotics* **2026**, *15*, 537. [[CrossRef](#)] [[PubMed](#)]
59. Vishwakarma, R.K.; Yadav, B.S.; Gautam, P.; Sahu, M.; Singh, A.; Kumar, A.; Nath, G. In-vitro analysis of biofilm formation and synergistic antibiotic-phage therapy for amoxicillin-resistant *Escherichia coli*. *Arch. Microbiol.* **2025**, *207*, 336. [[CrossRef](#)] [[PubMed](#)]
60. Akturk, E.; Oliveira, H.; Santos, S.B.; Costa, S.; Kuyumcu, S.; Melo, L.D.R.; Azeredo, J. Synergistic action of phage and antibiotics: Parameters to enhance the killing efficacy against mono and dual-species biofilms. *Antibiotics* **2019**, *8*, 103. [[CrossRef](#)] [[PubMed](#)]
61. Gliźniewicz, M.; Dołęgowska, B.; Augustyniak, A.; Rakoczy, R.; Kędzierski, T.; Mijowska, E.; Grygorcewicz, B. Effects of sequential administration of phage cocktail, ciprofloxacin, and caspofungin on *Staphylococcus aureus* and *Candida albicans* dual-species biofilms. *Microbes Infect.* **2025**, *27*, 105531. [[CrossRef](#)] [[PubMed](#)]
62. Grygorcewicz, B.; Wojciuk, B.; Roszak, M.; Łubowska, N.; Błażejczak, P.; Jursa-Kulesza, J.; Rakoczy, R.; Masiuk, H.; Dołęgowska, B. Environmental phage-based cocktail and antibiotic combination effects on *Acinetobacter baumannii* biofilm in a human urine model. *Microb. Drug Resist.* **2021**, *27*, 25–35. [[CrossRef](#)] [[PubMed](#)]
63. Dickey, J.; Perrot, V. Adjunct phage treatment enhances the effectiveness of low antibiotic concentration against *Staphylococcus aureus* biofilms in vitro. *PLoS ONE* **2019**, *14*, e0209390. [[CrossRef](#)] [[PubMed](#)]
64. Sahu, M.; Vishwakarma, R.K.; Karn, S.L.; Nath, G. Synergistic efficacy of phages along with vancomycin for eradication of vancomycin-resistant *Enterococcus faecalis* biofilms. *World J. Virol.* **2025**, *14*, 95826. [[CrossRef](#)] [[PubMed](#)]
65. Lin, L.-C.; Tsai, Y.-C.; Lin, N.-T. Phage-antibiotic synergy enhances biofilm eradication and survival in a zebrafish model of *Pseudomonas aeruginosa* infection. *Int. J. Mol. Sci.* **2025**, *26*, 5337. [[CrossRef](#)] [[PubMed](#)]
66. Wang, L.; Tkhilaishvili, T.; Bernal Andres, B.; Trampuz, A.; Gonzalez Moreno, M. Bacteriophage-antibiotic combinations against ciprofloxacin/ceftriaxone-resistant *Escherichia coli* in vitro and in an experimental *Galleria mellonella* model. *Int. J. Antimicrob. Agents* **2020**, *56*, 106200. [[CrossRef](#)] [[PubMed](#)]
67. Lev, K.; Kunz Coyne, A.J.; Kebriaei, R.; Morrisette, T.; Stamper, K.; Holger, D.J.; Canfield, G.S.; Duerkop, B.A.; Arias, C.A.; Rybak, M.J. Evaluation of bacteriophage-antibiotic combination therapy for biofilm-embedded MDR *Enterococcus faecium*. *Antibiotics* **2022**, *11*, 392. [[CrossRef](#)] [[PubMed](#)]
68. Bleick, C.R.; Van Helden, S.R.; Berti, A.D.; Richa, R.; Lehman, S.M.; Bayer, A.S.; Rybak, M.J. Optimizing phage-antibiotic combinations: Impact of administration order against daptomycin non-susceptible (DNS) MRSA clinical isolates. *Antimicrob. Agents Chemother.* **2025**, *69*, e00699-25. [[CrossRef](#)] [[PubMed](#)]
69. Akturk, E.; Melo, L.D.R.; Oliveira, H.; Crabbé, A.; Coenye, T.; Azeredo, J. Combining phages and antibiotic to enhance antibiofilm efficacy against an in vitro dual species wound biofilm. *Biofilm* **2023**, *6*, 100147. [[CrossRef](#)] [[PubMed](#)]
70. Cesta, N.; Pini, M.; Mulas, T.; Materazzi, A.; Ippolito, E.; Wagemans, J.; Kutateladze, M.; Fontana, C.; Sarmati, L.; Tavanti, A.; et al. Application of phage therapy in a case of a chronic hip-prosthetic joint infection due to *Pseudomonas aeruginosa*: An Italian real-life experience and in vitro analysis. *Open Forum Infect. Dis.* **2023**, *10*, ofad051. [[CrossRef](#)] [[PubMed](#)]

71. Chan, B.K.; Siström, M.; Wertz, J.E.; Kortright, K.E.; Narayan, D.; Turner, P.E. Phage selection restores antibiotic sensitivity in MDR *Pseudomonas aeruginosa*. *Sci. Rep.* **2016**, *6*, 26717. [[CrossRef](#)] [[PubMed](#)]
72. Wang, S.; Fan, W.; Jin, R.; Lan, W.; Zhao, Y.; Sun, X. Bactericidal synergism between phage and antibiotics: A combination strategy to target multidrug-resistant *Klebsiella pneumoniae* in vitro and in vivo. *Eur. J. Pharm. Biopharm.* **2025**, *213*, 114759. [[CrossRef](#)] [[PubMed](#)]
73. Wang, R.; You, X.; Liu, X.; Fei, B.; Li, Y.; Wang, D.; Zhu, R.; Li, Y. Characterization of phage HZY2308 against *Acinetobacter baumannii* and identification of phage-resistant bacteria. *Viol. J.* **2024**, *21*, 283. [[CrossRef](#)] [[PubMed](#)]
74. Orozco-Ochoa, A.K.; González-Gómez, J.P.; Quiñones, B.; Castro-del Campo, N.; Valdez-Torres, J.B.; Chaidez-Quiroz, C. Bacteriophage Indie resensitizes multidrug-resistant *Acinetobacter baumannii* to antibiotics in vitro. *Sci. Rep.* **2025**, *15*, 11578. [[CrossRef](#)] [[PubMed](#)]
75. Vashisth, M.; Yashveer, S.; Jaglan, A.B.; Virmani, N.; Bera, B.C.; Vaid, R.K.; Anand, T. Synergy of a virulent phage (phiAB182) with antibiotics leading to successful elimination of biofilms formed by MDR *Acinetobacter baumannii*. *Can. J. Microbiol.* **2022**, *68*, 731–746. [[CrossRef](#)] [[PubMed](#)]
76. Kunz Coyne, A.J.; Bleick, C.; Stamper, K.; Kebriaei, R.; Bayer, A.S.; Lehman, S.M.; Rybak, M.J. Phage-antibiotic synergy against daptomycin-nonsusceptible MRSA in an ex vivo simulated endocardial pharmacokinetic/pharmacodynamic model. *Antimicrob. Agents Chemother.* **2024**, *68*, e01388-23. [[CrossRef](#)] [[PubMed](#)]
77. Wang, L.; Tkhalishvili, T.; Trampuz, A. Adjunctive use of phage Sb-1 in antibiotics enhances inhibitory biofilm growth activity versus rifampin-resistant *Staphylococcus aureus* strains. *Antibiotics* **2020**, *9*, 749. [[CrossRef](#)] [[PubMed](#)]
78. Oechslin, F.; Piccardi, P.; Mancini, S.; Gabard, J.; Moreillon, P.; Entenza, J.M.; Resch, G.; Que, Y.-A. Synergistic interaction between phage therapy and antibiotics clears *Pseudomonas aeruginosa* infection in endocarditis and reduces virulence. *J. Infect. Dis.* **2017**, *215*, 703–712. [[CrossRef](#)] [[PubMed](#)]
79. El Ghali, A.; Stamper, K.; Kunz Coyne, A.J.; Holger, D.; Kebriaei, R.; Alexander, J.; Lehman, S.M.; Rybak, M.J. Ciprofloxacin in combination with bacteriophage cocktails against multi-drug resistant *Pseudomonas aeruginosa* in ex vivo simulated endocardial vegetation models. *Antimicrob. Agents Chemother.* **2023**, *67*, e00728-23. [[CrossRef](#)] [[PubMed](#)]
80. Kunz Coyne, A.J.; Stamper, K.; El Ghali, A.; Kebriaei, R.; Biswas, B.; Wilson, M.; Deschenes, M.V.; Tran, T.T.; Arias, C.A.; Rybak, M.J. Phage-antibiotic cocktail rescues daptomycin and phage susceptibility against daptomycin-nonsusceptible *Enterococcus faecium* in a simulated endocardial vegetation ex vivo model. *Microbiol. Spectr.* **2023**, *11*, e00340-23. [[CrossRef](#)] [[PubMed](#)]
81. Zhao, Y.; Feng, L.; Zhou, B.; Zhang, X.; Yao, Z.; Wang, L.; Wang, Z.; Zhou, T.; Chen, L. A newly isolated bacteriophage vB8388 and its synergistic effect with aminoglycosides against multi-drug resistant *Klebsiella oxytoca* strain FK-8388. *Microb. Pathog.* **2023**, *174*, 105906. [[CrossRef](#)] [[PubMed](#)]
82. Luo, X.; Wu, Y.; Ming, Y.; Wang, J.; Ma, Z.; Zhang, A.; Hu, S. Targeted isolation of TolC-dependent phages reveals dual strategies for combating multidrug-resistant avian *Escherichia coli*: From evolutionary trade-offs to antibiotic synergy. *Front. Microbiol.* **2026**, *17*, 1868543. [[CrossRef](#)] [[PubMed](#)]
83. Fuerst-Wilmes, M.; Respondek, V.; Schramm, M.; Lilienthal, N.; Buss, K.; Duechting, A. Regulation of phage therapy medicinal products: Developments, challenges, and opportunities. *Front. Cell. Infect. Microbiol.* **2025**, *15*, 1631359. [[CrossRef](#)] [[PubMed](#)]
84. Fukaya-Shiba, A.; Ogata, A.; Kuribayashi, R.; Sakurai, A.; Suzuki, K.; Takadama, S.; Nishimura, J.; Uchiyama, J.; Ohge, H.; Takeuchi, T.; et al. Regulatory considerations for developing phage therapy medicinal products for the treatment of antimicrobial resistant bacterial infections. *Front. Pharmacol.* **2025**, *16*, 1713471. [[CrossRef](#)] [[PubMed](#)]
85. Kline, A.; Cobián Güemes, A.G.; Yore, J.; Ghose, C.; Van Tyne, D.; Whiteson, K.; Pride, D.T. Current Clinical Laboratory Challenges to Widespread Adoption of Phage Therapy in the United States. *Antibiotics* **2025**, *14*, 553. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.