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PHAGE THERAPY AGAINST MULTIDRUG-RESISTANT PSEUDOMONAS AERUGINOSA IN A MURINE WOUND MODEL

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Abstract

Multidrug-resistant Pseudomonas aeruginosa is a leading cause of difficult wound and burn infections, and bacteriophages have returned as a possible treatment. This review summarises published literature on phage therapy against the organism, with emphasis on murine wound and burn models. It describes wound infection biology, phage action and product quality, the design and findings of animal studies, combinations with antibiotics and the emergence of phage resistance, and the clinical experience gathered so far. It reports no new experimental data. Animal studies are encouraging but rely on early treatment and variable reporting, while controlled human evidence is sparse, and formulation stability and regulation remain unresolved obstacles.

1. INTRODUCTION

Pseudomonas aeruginosa is a patient's problem long before it is a laboratory one. It colonises burns, pressure sores and surgical wounds, forms biofilms that shield it from antibiotics, and carries an unusually wide set of resistance mechanisms, from efflux pumps and reduced porin permeability to inducible β-lactamases [1, 2]. Carbapenem-resistant isolates sit in the highest priority group of the World Health Organization list of bacteria for which new treatments are urgently needed [3]. When a wound infection reaches that

point the usual options are toxic, such as colistin, or absent. That gap has revived interest in bacteriophages, the viruses that infect bacteria, which were used therapeutically in the early twentieth century and then largely set aside in Western medicine [4, 5].

The murine wound or burn model has become the standard preclinical test bed for this idea. A small, defined injury, a measured bacterial inoculum and a phage preparation applied at a chosen time and route make it possible to ask simple questions about survival, bacterial load and healing [6, 7]. The results are easy to over-read. Mice do not have the immune environment, wound chronicity or microbiome of a person with a diabetic foot ulcer, and the dosing logic transfers imperfectly. This review therefore treats the animal literature as evidence about mechanism and feasibility, not as a forecast of clinical success. Animal studies are nonetheless the only place where phage, bacteria, wound tissue and host defences can be examined together under controlled conditions, which is why they remain central.

This article is a review of published work on phage therapy against multidrug-resistant *P. aeruginosa* with emphasis on murine wound and burn models. It reports no new experiments. The sections below outline the biology of wound infection and phage action, the design and findings of published murine studies, the effect of combining phages with antibiotics and the emergence of phage resistance, and the clinical experience that has followed. Limitations of the evidence, including the small number of controlled human studies, are discussed at the end.

2. WOUND INFECTION AND PHAGE BIOLOGY

Burn wounds are almost ideal for *P. aeruginosa*. Dead tissue is a nutrient-rich, poorly perfused substrate, local and systemic immune function is depressed after thermal injury, and systemic antibiotics reach the eschar poorly [8, 9]. Colonisation is common within days, and invasive infection can progress to sepsis. In chronic wounds the organism lives mainly in biofilms, communities embedded in an extracellular matrix that tolerate antibiotic concentrations many times higher than those that kill free-living cells [10]. Any new therapy for wounds has to work on that biofilm lifestyle and not only on planktonic bacteria in broth.

Lytic phages attach to a specific receptor on the bacterial surface, inject their genome, replicate inside the cell and burst it open, releasing new particles. Because each round of infection multiplies the phage, dosing behaves unlike a conventional drug: at the infection site the concentration can rise as long as susceptible bacteria remain, a property sometimes called self-amplification, and Marza and colleagues detected phage multiplication in the tissues of burn patients [4, 11]. Phages are highly specific, usually acting on some strains of one species, and this spares the normal flora but means that a preparation must be matched to the isolate or be a mixture covering many.

Delivery of phage to a wound is as much a pharmaceutical question as a microbiological one. A liquid applied to a dry, crusted burn may run off or be absorbed by the dressing, while a viscous gel holds the preparation against the tissue but may slow diffusion to the bacteria beneath the eschar [12, 13]. Alginate, hydrogel and nanofibre carriers have each been proposed, mainly in laboratory or small animal work, and they differ in how well they keep phage infective on the shelf. Because the field has borrowed freely from antibiotic wound dressings, the compatibility of phage with silver, chlorhexidine and other topical agents is a practical concern that few papers address [13, 14].

Some phages carry enzymes that degrade the biofilm matrix, and in vitro work has shown that phage mixtures can reduce *P. aeruginosa* biofilm formation on catheter material [13]. Whether this carries over to wounds is less clear, since the matrix of a wound biofilm incorporates host proteins and debris that are absent from laboratory models. Several murine studies assess bacterial load in homogenised tissue rather than biofilm structure, so the biofilm benefit is inferred more often than measured [6, 15].

Host defences interact with phage in ways that were underestimated for years. Phage

particles are cleared from the circulation by the spleen and liver within hours, and neutralising antibodies can appear after repeated dosing, though these effects are less relevant for a topical product than for an injected one [4, 16]. Locally, neutrophils and macrophages engulf phages and can also attack bacteria already weakened by phage lysis, a cooperation highlighted by work in mouse lung infection [17]. Burn injury changes this cellular environment, which is one reason results from healthy animals should not be assumed to transfer without comment [8, 9].

Preparation quality is rarely emphasised in animal papers but matters a great deal. Raw lysates contain bacterial endotoxin, which can itself provoke inflammation in a wound, and purification steps affect phage titre and stability [12, 18]. The Belgian group that prepared a defined cocktail for burn wound trials described a quality-controlled, small-scale process, an important step towards a pharmaceutical-grade product [18]. Phage particles lose infectivity with heat, acid and some formulation excipients, so storage and the vehicle used for topical application, such as saline, hydrogel or dressing, are real variables [12].

3. MURINE WOUND AND BURN MODELS

Early experimental work established the principle. Soothill showed that a small number of phage particles could rescue mice infected with *P. aeruginosa* and *Acinetobacter*, and later that phage applied to guinea pig skin grafts prevented their destruction by the organism [7, 19]. These papers are short, but they set the pattern of the later literature: infect, treat with phage, and compare with untreated or antibiotic-treated controls. The comparison with antibiotics is a recurring theme, and phage is rarely tested on an infection that antibiotics cannot treat, which would be the real clinical need.

The most cited study in this area is the mouse burn model of McVay and colleagues, in which a full-thickness burn was colonised with *P. aeruginosa* and a phage cocktail was given by intraperitoneal, subcutaneous or intramuscular routes [6]. Survival improved substantially compared with untreated animals, although the size of the effect depended on the route and on the delay between infection and treatment. The authors also noted that the benefit was greatest when treatment began soon after infection. That finding is consistent with the amplification logic described above and a reminder that the model rewards early treatment.

Several groups have since tested topical or local application, which is closer to how a wound product would be used. Kumari and colleagues compared a single phage against antimicrobial agents in a mouse burn wound infection caused by *Klebsiella pneumoniae*, and Chadha and colleagues compared a single phage with a cocktail in a BALB/c burn wound infection, reporting an advantage for the mixture [15, 20]. Neither is a *Pseudomonas* study, but they use the same burn framework and are widely cited alongside the *Pseudomonas* work, so they are included here as parallel evidence on dosing and the single-versus-cocktail question.

Choice of strain affects interpretation. Many animal studies use a laboratory reference strain such as PAO1 or a well-characterised clinical isolate, and the multidrug-resistant phenotype is not always the focus [6, 21]. This matters because phage susceptibility and antibiotic resistance are largely independent, so a resistant isolate is not intrinsically harder or easier for phage to kill. Resistance mechanisms such as efflux can, however, interact with phage receptors, as the work of Chan and colleagues showed [22]. Testing on well-defined resistant clinical isolates would give the field a clearer answer than the extrapolation now used [1, 3].

Other infection sites show the same tendencies. Intranasal phage treated and prevented acute *P. aeruginosa* lung infection in mice, and phage given to mice with gut-derived sepsis improved survival [21, 23]. These models are not wound models, yet they help separate what is specific to wounds from what is general to phage pharmacology: bacterial killing in vivo, a modest immune contribution and a short window in which the phage is most effective. They also reinforce that results depend on the strain and phage pair, with some combinations working

strikingly well and others barely at all.

Animal welfare and design issues also shape the literature. Burn and wound models involve pain and require analgesia and humane end-points, which in turn influence what can be measured and for how long [9]. Group sizes in several older papers are small, randomisation and blinding are seldom described, and results are sometimes reported for survival alone. Modern reporting guidance for animal research would improve the reliability of the field, particularly where outcomes such as wound area are scored by eye [15, 20].

Endpoints vary widely between laboratories. Survival is the clearest but is a crude measure for a wound that rarely kills a human patient. Bacterial counts in tissue, wound area, histology and cytokine levels all appear, often in different combinations from paper to paper [6, 9, 15]. This diversity complicates comparison. A common core set of outcomes, together with a statement of the phage dose per area of wound and the timing from infection, would make the literature much easier to synthesise. A summary of reported models and findings is given in table 1 and fig. 1 shows the distribution of the evidence by type.

Table 1: Selected published evidence on phage therapy for Pseudomonas aeruginosa infection

Model or setting	Reported finding	Ref.
Mouse burn wound P. aeruginosa cocktail	Survival improved; effect depended on route and timing of treatment	[6, 12]
Mouse burn wound K. pneumoniae, single phage	Compared favourably with antimicrobial agents in the same model	[20]
Mouse burn wound Single phage versus cocktail	Cocktail reported to be more effective than single phage	[15]
Guinea pig skin graft Phage applied locally	Graft protected from destruction by the organism	[19]
Mouse lung infection Intranasal phage	Treated established infection and prevented colonisation	[21]
Mouse gut-derived sepsis Injected or oral phage	Survival improved relative to untreated controls	[23]
Rat endocarditis Phage plus ciprofloxacin	Greater clearance than either alone; virulence reduced	[24]
Mouse respiratory infection Phage and neutrophils	Immune cells needed alongside phage for success	[17]
Wax moth larvae Sequential phages	Sequential use limited resistance better than simultaneous use	[25]
Chronic otitis, human Randomised, double-blind	Clinical improvement reported with phage preparation	[26]
Burn wounds, human PhagoBurn trial	Slower bacterial reduction than standard care; low phage titre in product	[27]
Venous leg ulcers, human Phase I safety	No safety signal on topical phage application	[14]

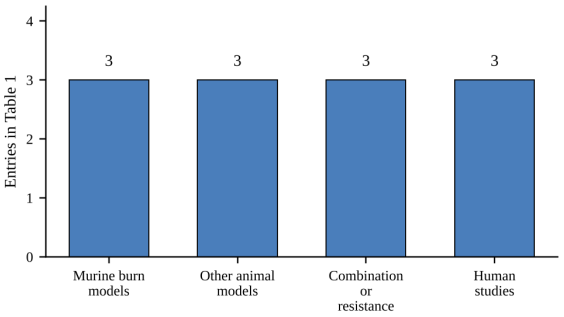


Fig. 1: Number of entries in table 1 by type of evidence

Dose reporting is a particular weakness. Papers state the titre in plaque-forming units but often leave the ratio of phage to bacteria, the volume and the surface area treated unclear, so that the dose intensity at the wound cannot be reconstructed [6, 12]. Without that, a negative result cannot be told apart from underdosing. Reporting a simple table of dose, volume, wound area and timing would resolve much of this uncertainty and cost authors very little effort.

4. COMBINATIONS AND RESISTANCE

Bacteria evolve resistance to phages, as they do to antibiotics, usually by altering or losing the receptor to which the phage binds [28]. In animal studies this appears as regrowth after an initial fall in counts, and in the wax moth larva model Hall and colleagues found that sequential application of different phages controlled populations better than a single or simultaneous application [25]. Cocktails with different receptor specificities therefore make good sense, although the number of components that can be combined is limited

by manufacturing and regulatory burden [29, 30]. Such resistance is predictable, and plans for it should be part of any protocol [25, 28].

Resistance to phage is not always a disadvantage for the host. Chan and colleagues described a phage that used the outer membrane component of a multidrug efflux pump as its receptor, so that bacteria which escaped the phage often lost efflux function and regained antibiotic susceptibility [22]. This trade-off is a neat example of evolutionary steering, although it has been shown in laboratory work and not in a wound. It supports the wider argument that phages and antibiotics can complement each other, an argument set out clearly by Torres-Barceló and Hochberg [31].

Synergy with antibiotics has been shown in other animal models. In a rat endocarditis model, a phage combined with ciprofloxacin cleared *P. aeruginosa* more effectively than either alone and reduced virulence of the surviving bacteria [24]. In mouse lung infection, the host immune response, especially neutrophils, was found to work with phage, and immunocompromised animals benefited less [17]. The second finding is a caution for burn patients, whose neutrophil function is impaired, and for the many phage studies that use immunocompetent animals [9, 17].

Case reports of compassionate use illustrate what combination treatment may look like in practice. Schooley and colleagues treated a patient with a disseminated, resistant *Acinetobacter baumannii* infection using a personalised cocktail with antibiotics and reported clinical recovery, while stressing that the case could not show efficacy by itself [32]. Such reports are valuable for feasibility, timing and safety, including the need to screen for endotoxin and to monitor bacterial susceptibility during therapy, yet they cannot replace controlled studies, and they are subject to the usual bias towards reporting successes [29, 32, 33].

5. CLINICAL EVIDENCE AND TRANSLATION

Human experience is older than the animal literature suggests but of uneven quality. Polish and Georgian clinics treated large numbers of patients with phages for decades, and reviews of that work describe favourable outcomes, though mostly uncontrolled [5, 16]. Controlled trials are few. Wright and colleagues reported a double-blind trial of a *Pseudomonas* phage preparation in chronic otitis with a clinical benefit [26], and Rhoads and colleagues found no safety signal when phages were applied to venous leg ulcers [14]. Rose and colleagues described the difficult first steps of experimental phage treatment of burn wounds in Belgium [33]. Taken as a whole, this history supplies hints about safety and plausibility but little firm evidence of efficacy against resistant isolates [5, 16].

The most relevant trial is PhagoBurn, a randomised controlled phase 1/2 study of a twelve-phage cocktail against *P. aeruginosa* burn infections [27]. It was stopped early. The phage group reduced bacterial load more slowly than the standard silver sulfadiazine dressing, and analysis showed that the phage titre in the applied preparation had fallen far below the intended level during storage [27]. Safety was acceptable, and the trial taught the field more about formulation stability than about efficacy. It is a vivid example of why the product, and not only the idea, has to be validated before a clinical test.

Regulation remains a barrier. Conventional drug approval assumes a fixed, characterised product tested in large trials, while phage therapy often needs a bespoke or frequently updated preparation [29, 30]. Pirnay and colleagues contrasted a ready-made, standardised model with a tailor-made one and argued for a hybrid, and Cooper and colleagues have proposed adapting existing approval pathways [29, 30]. Brüssow summarised the missing pieces in plain terms: good manufacturing, defined doses, controlled trials and a clear regulatory home [34]. Progress on any one of these depends on progress on the others.

Safety has been reassuring so far, with limits. The venous leg ulcer trial, PhagoBurn and the otitis study reported no serious adverse effects attributed to phage [14, 26, 27]. Phages are abundant in the human body and environment, and their narrow host range limits damage to commensal bacteria. The

main theoretical concerns are release of endotoxin when bacteria lyse, transfer of toxin or resistance genes by temperate phages, and immune responses to repeated dosing, which is why lytic phages with sequenced, screened genomes are preferred [4, 18, 34]. Long-term follow-up data remain scarce.

Pharmaceutical issues receive less attention than they deserve. Delivery routes, formulation, concentration and timing all influence outcome, and Ryan and colleagues showed how thoroughly these variables can alter results across published studies [12]. For wounds, a dressing that releases phage over days, a hydrogel that keeps it viable or a spray that spreads it across an irregular surface are all plausible, but few have been tested with the rigour applied to antibiotic formulations. Stability data under real storage conditions, as PhagoBurn showed, should be a starting requirement [12, 27].

Economic considerations also influence translation. Producing a phage product to pharmaceutical standards demands purification, titre testing, sterility control and cold storage, and a cocktail that must be updated as resistance emerges adds yet more cost [18, 30]. Against that, development costs for phages may be lower than for new small molecules, and wound care is a field in which topical products are accepted. The trade-off between a stable, standardised product and a tailored, rapidly adjustable one remains the central commercial question [29, 34].

6. LIMITATIONS AND FUTURE DIRECTIONS

The evidence base has clear weaknesses. Murine studies are mostly short, use young immunocompetent animals, favour early treatment and often target laboratory strains rather than multidrug-resistant clinical isolates [6, 15, 20]. Dose and route reporting is inconsistent, publication bias probably favours positive results, and few studies test phage against infections that antibiotics fail to cure [9, 12]. Human data are limited to small trials, case series and historical reports. A wound model that includes biofilm, diabetes or delayed healing would answer questions that the burn model cannot [8, 10]. Finally, publication of negative or neutral animal results would help calibrate expectations, since a literature of successes alone tends to overstate what phage can do [12, 27].

The next steps are fairly clear. Standard model protocols with shared outcomes would let results be pooled, and stability-tested, quality-controlled preparations should be used in animals before human trials [12, 18]. Combination studies with antibiotics, and studies that follow the evolution of resistance during treatment, deserve priority [22, 28, 31]. Clinical programmes will need regulatory frameworks suited to biologics that change with the pathogen [30, 34]. Until then, phage therapy for resistant *Pseudomonas* wound infection remains a promising approach with encouraging animal data and an unproven clinical record [27, 33].

7. CONCLUSION

Published work supports the idea that lytic phages can control Pseudomonas aeruginosa in experimental wounds. Mouse burn and related models show improved survival and lower bacterial counts, especially when treatment starts early and a cocktail is used [6, 15, 20]. Evidence from other organ systems and from laboratory studies suggests that combination with antibiotics and attention to receptor choice may limit resistance [22, 24, 25, 31]. Biofilm effects, though plausible, remain poorly demonstrated in wounds [10, 13].

The step from mouse to patient has proved harder than the animal data suggested. PhagoBurn showed that a product can fail through instability even when the concept is sound, and regulation has yet to settle how to assess a preparation that may need to change with the infecting strain [27, 29, 30]. Better standardised models, full reporting of dose and timing, and stability-tested formulations are the practical priorities, and they should be addressed before further large trials are attempted [12, 33, 34].

8. ABBREVIATIONS

PFU-Plaque-forming units, WHO-World Health Organization, MDR-Multidrug-resistant, PhagoBurn-Phage therapy trial in burn wounds, GMP-Good manufacturing practice, BALB/c-Inbred laboratory mouse strain,

i.p.-Intraperitoneal, CFU-Colony-forming units

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10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

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