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Phage-essential oil synergy as an evolutionary and biophysical strategy against antimicrobial resistance

D.X. Romero-Calle [a,b], F. Gordillo Altamirano [c,d,*], C.A. Carrasco Villanueva [b]

[a] Instituto de Investigaciones Industriales, Ingeniería Industrial, Facultad de Ingeniería, Universidad Mayor de San Andrés, La Paz, Bolivia
[b] Instituto de Investigación y Desarrollo de Procesos Químicos, Ingeniería Química, Facultad de Ingeniería, Universidad Mayor de San Andrés, La Paz, Bolivia
[c] Department of Infectious Diseases, The Alfred Hospital and Monash School of Translational Medicine, Monash University, Melbourne, Australia
[d] Centre to Impact AMR, Monash University, Melbourne, Australia

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ABSTRACT

The persistent challenge of global antimicrobial resistance means we can no longer rely solely on traditional small-molecule monotherapy. While bacteriophage therapy offers surgical precision, its clinical reach is often limited by biofilm-mediated protection, as well as the rapid evolution of resistance, and stability issues. At the same time, essential oils (EOs) show promising antibacterial activity, yet their clinical use remains stalled due to toxicity and volatility concerns. In this review, we summarize key biomedical concepts underpinning phage therapy and EOs and propose a shift in perspective: reframing EOs as biophysical adjuvants designed to reshape phage–bacteria interactions rather than treating them as standalone antimicrobials. We explore how EO-driven membrane disruption can prime bacteria for phage predation, enhancing phage adsorption and potentially steering populations toward more susceptible phenotypes. Moving beyond simple mixtures, we evaluate the mechanistic evidence for phage-EO synergy. We highlight the early stage of this field: current evidence is limited to *in vitro* studies, with a lack of preclinical and clinical validation, and significant barriers to clinical translation. However, by treating phage-EO combinations as rationally engineered systems, our review aims to map out a strategic path toward their responsible and meaningful development in the fight against resistant infections.

1. Introduction

Antimicrobial resistance (AMR) has intensified into a global health crisis. Although the evolution of resistance is a natural phenomenon, the misuse and overuse of antimicrobials in human medicine, livestock farming, aquaculture, and agriculture have accelerated its spread, reversing decades of therapeutic advances and compromising the effectiveness of conventional antibiotics (Chen et al., 2026). Multidrug-resistant pathogens cause significant increases in patient morbidity and mortality, while imposing substantial economic burdens on healthcare systems worldwide. The World Health Organization emphasizes that AMR threatens to reverse the progress of modern medicine and calls for critical global interventions, including the development of novel antimicrobial strategies (GBD 2021 Antimicrobial Resistance Collaborators, 2024; Salam et al., 2023).

The stagnation in new antibiotic development alongside the rapid emergence of resistant bacteria demand innovative solutions beyond traditional antibiotics. Bacteriophage therapy, using viruses that selectively infect and lyse bacteria, has gained renewed interest as a highly specific and self-amplifying antimicrobial approach (Gordillo Altamirano and Barr, 2019). Concurrently, essential oils (EOs), complex natural extracts with broad-spectrum antimicrobial properties, have demonstrated efficacy in inhibiting resistant bacterial growth and disrupting biofilms (El-Tarabily et al., 2021; Iskandar et al., 2025). Outstandingly, EOs may synergize with bacteriophages by increasing bacterial membrane permeability and interfering with phage-resistance mechanisms, thereby amplifying their combined efficacy.

This narrative review first summarizes the phage biology concepts that are essential to translational efforts, pinpoints some priority clinical problems that can benefit from advances in phage therapy, and highlights key challenges in clinical use. Next, we briefly characterize the properties of EOs from a chemical and pharmacological viewpoint. The backbone of this work, however, is the synthesis of current knowledge on phage and EO combination therapies as an emergent strategy against

* Correspondence to: Department of Infectious Diseases, School of Translational Medicine & The Alfred Hospital, Monash University, 89 Commercial Road, Melbourne, VIC 3004, Australia.
E-mail address: Fernando.gordilloaltamirano1@monash.edu (F. Gordillo Altamirano).

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AMR in human health. We evaluate mechanistic insights into their synergistic interaction, and critically review the antimicrobial efficacy data, while acknowledging the fact that most of the evidence still derives exclusively from *in vitro* studies. We finally discuss translational hurdles including the notable gap in *in vivo* and clinical evidence, safety, and regulatory barriers, and highlight prospective avenues for integrating these novel antimicrobials into clinical practice.

2. Bacteriophage therapy: Opportunities and constraints

2.1. Brief history of phage therapy

In 1896, Ernest Hankin observed bactericidal activity against *Vibrio cholerae* in the waters of the Ganges and Yamuna rivers (Kochhar, 2020). Frederick Twort, in 1915, and Félix d'Hérelle, in 1917, independently identified filterable agents capable of inhibiting bacterial growth, with the latter coining the term "bacteriophage" (Chanishvili, 2012; Summers, 2012). Early translational efforts followed rapidly, including significant use during World War II (Summers, 2012). The viral nature of phages was confirmed through electron microscopy when Ernst Ruska captured the first phage micrograph in 1940 (Ackermann, 2011), and the foundational work of Delbrück, Hershey, and Luria on phage structure, replication, and life cycle, recognized with the 1969 Nobel Prize in Medicine or Physiology (Strauss, 2017). The subsequent advent of antibiotics, with their broader spectrum and ease of production and use, displaced phage therapy for most of the 20th century (Hutchings et al., 2019). In the face of escalating antimicrobial resistance, however, phage therapy has begun a resurgence in recent decades (Gordillo Altamirano and Barr, 2019). Key milestones in this history are summarized in Table 1.

2.2. Mechanism of action: the bacteriophage life cycle

2.2.1. Bacterial recognition and adsorption

Phages initiate infection by binding to specific receptors on the bacterial surface through tail proteins, determining host specificity. For example, the long tail fibers of phage T4 recognize lipopolysaccharide (LPS) and OmpC on *E. coli*, providing the mechanical force required for stable attachment and subsequent DNA injection (Islam et al., 2019). Certain phage receptor-binding proteins possess depolymerase activity against capsular polysaccharides (CPS), with potential applications in biofilm disruption (Klumpp et al., 2023). Importantly, components of the bacterial envelope including CPS, LPS, teichoic acids, and peptidoglycan can simultaneously serve as initial recognition targets and mechanical barriers to deeper receptor access (McCalla et al., 2025; Ohshima et al., 1988), that could be breached with agents such as EOs.

Table 1
Milestones in the history of phages and phage therapy, amidst the AMR crisis.

Year	Event / Protagonist	Description	Key finding / Significance	Ref
1896	Ernest Hankin	Reported antibacterial agent passing through fine porcelain filter	First documented observation of phage-like activity; established biological rather than chemical nature of the agent	(Abedon et al., 2011b)
1915	Frederick Twort	Discovered small agent infecting and killing bacteria	First description of phage as transmissible lytic agent	
1917	Félix d'Hérelle	Identified bacteria-killing agents; coined "bacteriophage"	Established phage as a distinct biological entity; initiated first therapeutic applications	
1920s	Early phage therapy trials	Expansion against cholera, dysentery, plague, conjunctivitis, skin infections	Demonstrated broad antimicrobial potential but lacked standardisation	(Abedon et al., 2011a)
1928	Alexander Fleming	Discovery of penicillin	Initiated the antibiotic era that would displace phage therapy for decades	(Hutchings et al., 2019)
1930s	Critical review of phage therapy	Revealed lack of controls, narrow efficacy, low purity and stability	First systematic quality assessment; identified core translational barriers still relevant today	(Summers, 2012)
1939	Phage life cycle characterised	Description of the one-step kill curve	Established the quantitative framework for phage replication kinetics	(Keen, 2015)
1940	Ernst and Helmut Ruska	First electron micrograph of a phage	Visual confirmation of phage morphology; enabled structural classification	(Ackermann, 2011)
1942	Introduction of penicillin	Widespread use in clinical practice begins	First widely available antibiotic; transformed the management of bacterial infections	(Abraham et al., 1992)
1940s	Relegation of phage therapy	Largely abandoned in favour of antibiotics	Demonstrated how the superior convenience and spectrum of antibiotics displaced a promising biological approach	(Summers, 2012)
1940s–1970s	Golden age of antibiotics	Discovery of many new antimicrobial families	Established small-molecule antibiotics as standard of care; further reduced investment in phage research	(Hutchings et al., 2019)
1977	Frederik Sanger *et al.*	First phage DNA genome sequenced	Enabled genomic characterisation and safety screening of therapeutic phages	(Sanger et al., 1977)
1980s	Reconsideration of phage therapy	Evaluation as a treatment against antibiotic-resistant bacteria begins	Rising AMR drove renewed interest; first modern phage therapy publications	(Kropinski, 2006)
1990s	Preclinical expansion of phage therapy	Renewed phage therapy trials in animal models	Established preclinical proof-of-concept for modern phage therapy	(Wittebole et al., 2014)
2006	First commercial phage-based product	Released against *Listeria monocytogenes*	First regulatory approval of phage product; established food safety application pathway	(Carlton et al., 2005)
2015	PhagoBurn trial	First international phage therapy trial meeting modern standards: results published in 2019	Demonstrated GMP-compliant phage manufacturing feasibility; identified titre stability as critical failure point	(Jault et al., 2019)
2016	First IV phage therapy case in the US	Against *Acinetobacter baumannii* septicaemia	Established compassionate use pathway; demonstrated systemic phage therapy safety	(Schooley et al., 2017)
2018	Legal framework established in Belgium	Magistral Phage framework for tailor-made phage medicines	First national regulatory framework enabling individualised phage therapy outside clinical trials	(Pirnay et al., 2018)
2019	First clinical use of phage GMO	Against disseminated *Mycobacterium abscessus* infection	Demonstrated safety of engineered phages in humans; opened pathway for synthetic phage therapeutics	(Dedrick et al., 2019)
2021	Government funding expands	First NIH grants exclusively targeting phage therapy; USD$ 2.5 million to 12 institutes	Signalled mainstream scientific and governmental recognition of phage therapy	(Moon et al., 2025)
2025	AI-designed phage genome	First phage genome designed using AI, synthesised, with demonstrated activity	Demonstrated generative AI capacity for phage engineering; potential paradigm shift in phage precision medicine	(King et al., 2025)

2.2.2. Injection of genetic material

After adsorption, phages inject their genetic material across the bacterial membrane. T4 and other myoviruses use a contractile sheath mechanism: baseplate contact triggers conformational rearrangement, irreversible short tail fiber attachment, and sheath contraction, driving the tail tube through the outer membrane and periplasmic space (Hu et al., 2015; Leiman et al., 2004). Peptidoglycan-degrading enzymes facilitate passage to the cytoplasmic membrane, which fuses with the tail tube to complete the DNA conduit (Hu et al., 2015). In contrast, siphoviruses can transform their normally flexible tails into rigid channels, with DNA passage facilitated by charge distribution and a ratchet apparatus (Ares et al., 2014; Klumpp et al., 2023). Other phages may even use combinations of these mechanisms (Suhani et al., 2026). Understanding these injection processes is relevant for identifying potential adjuvants including EO constituents that may interact with phage structures or the bacterial surface, impacting infection efficiency.

2.2.3. Intracellular replication, transcription, and assembly

Upon entry, the phage genetic material redirects host machinery through a temporally controlled cascade of early (host takeover), middle (genome replication), and late (assembly and lysis) gene expression phases. Replication strategies vary by phage, each circumventing the end-replication problem inherent to 5′→3′ DNA synthesis (McAllister and Raskin, 1993; Putzeys et al., 2024; Tabib-Salazar et al., 2019; Weigel and Seitz, 2006; Yang et al., 2014). Transcriptional control is primarily mediated through RNA polymerase (RNAP) specificity: T4 hijacks host RNAP via phage-encoded σ factors or covalent modifications, while T7 encodes its own single-subunit RNAP for autonomous transcription (Hinton, 2025; Putzeys et al., 2024). Regulatory sophistication extends to small RNAs modulating mRNA stability, translation, and degradation, and physical compartmentalization of transcription and replication within the host cell (Tabib-Salazar et al., 2019). Finally, virion assembly proceeds through precise interactions between phage and host biomolecular components, yielding infectious progeny.

2.2.4. Release of new viral particles

The release of phage progeny involves a hierarchical lysis system (Young, 2014). Holins build up in the inner membrane and oligomerize to create big, nonspecific pores at a genetically predetermined time. These pores allow phage-encoded endolysins to access and degrade peptidoglycan, triggering osmotic lysis. Antiholins regulate this timing, optimizing replication before lysis onset (Tran et al., 2005; Ziedaite et al., 2005). Endolysins exhibit catalytic diversity, including amidase, glycosaminidase, or muramidase activity, each targeting specific peptidoglycan bonds, and their potent muralytic activity has positioned them as antimicrobial therapeutic candidates (Cui et al., 2025). In Gram-negative bacteria, spanins bridge the inner and outer membranes to complete cell disintegration and ensuring efficient phage release (Rajaure et al., 2015).

2.3. Lysogenic cycle: decision-making, phage-mediated horizontal gene transfer, and safety implications

From a simplistic perspective, the phage life cycle may be strictly lytic or include lysogenic and pseudolysogenic cycles. In lysogeny, the phage genome, then known as a prophage, replicates alongside the host DNA, either integrated into the host's chromosome or in a free, plasmid-like state, establishing stable cohabitation (Mantynen et al., 2021). The lysogenic-to-lytic switch is governed by competing regulatory proteins: CI repressor maintains lysogeny by silencing lytic genes, while Cro promotes lytic entry (Fortier and Sekulovic, 2013). Host stress signals, such as DNA damage, activate RecA, inducing CI autocleavage and prophage induction via the SOS response (Aertsen et al., 2004), while phage-encoded antirepressors can further destabilize CI complexes under environmental stress (Azulay et al., 2022; Rasmussen et al., 2020). Understanding the dynamics of this regulatory switch, and the risks of unintended gene transfer and lysogenic conversion associated with temperate phages, is essential in the development of phage therapeutics and associated synthetic biology applications (Durante-Rodriguez et al., 2016; Lewis and Adhya, 2024).

Transduction, or phage-mediated horizontal gene transfer occurs through three mechanisms (Fillol-Salom et al., 2019; Fillol-Salom et al., 2021): generalized transduction involves random host DNA packaging during lytic replication; specialized transduction, unique to temperate phages, results from imprecise prophage excision capturing flanking bacterial sequences; and lateral transduction, where an induced prophage starts DNA packaging while still integrated in the chromosome, results in phage–like particles with large regions of bacterial DNA that are released and transferred. The clinical implications are significant. Temperate phages capable of specialized and lateral transduction risk disseminating antimicrobial resistance and virulence determinants into the human microbiota. Therefore, strict selection of obligately lytic phages is a classic non-negotiable requirement for phage therapy. However, recent nuanced analyses suggest scenarios where the use of temperate phages could be considered (Cook and Hynes, 2025). Crucially, the ecological balance maintained by lysogeny, induction, and transduction represents a critical parameter to consider when designing combination phage treatments, including those with EOs. As we will further discuss, EOs can prompt bacterial stress responses that could theoretically influence prophage activation in polymicrobial infection contexts (Nazzaro et al., 2013; Sheng et al., 2016).

2.4. The co-evolutionary arms race: phage-host dynamics and therapeutic implications

Phage-host interactions are defined by continuous co-evolutionary pressures, relevant in the context of phage therapy. Bacteria can counter phage infection at every stage of the life cycle (Egido et al., 2022), notably through surface receptor mutation, masking or down-regulation to prevent adsorption (Egido et al., 2022; Gordillo Altamirano et al., 2021), and abortive infection systems that sacrifice infected cells to protect the population. Phages reciprocally evolve to overcome bacterial defense systems, often expanding host range through mutations in receptor-binding proteins (Zhang et al., 2025). This dynamic directly informs phage therapy design. Engineering receptor-binding proteins and leveraging bacterial immune systems can yield phages with improved host range and customized therapeutic characteristics, guiding cocktail composition and adjuvant selection including EO constituents that may modulate surface receptor accessibility (Jia et al., 2023).

2.5. Promising priority areas for clinical phage therapy use and development

Phage therapy is moving from theory to practical application, aiming to become a validated clinical tool against AMR. In this translational effort, it is expected that phage therapy will first be deployed in complex infections where conventional treatments have failed, including device-associated infections, osteoarticular infections, sepsis, and recurrent UTIs. In this context, groups of experts, such as the Antibacterial Resistance Leadership Group Phage Task Force, have formally endorsed the use of experimental phage therapy (Suh et al., 2022). In this section, we explore in more detail some of the clinical niches that could greatly benefit from the application of phage therapy.

2.5.1. Skin and soft tissue infections

Topical phage application has demonstrated a strong safety profile and promising efficacy in treating chronic wounds, including diabetic foot infections, venous leg ulcers, and burns (Zyman et al., 2022). These infections are frequently complicated by biofilm formation and poor vascularization. Preclinical data from rodent and porcine models suggest that phage therapy can be effective at preventing infection of skin

grafts in burned patients (Soothill, 1994), and treating chronic wounds in patients with diabetes (Mendes et al., 2013). A small (n = 42) phase I trial of a cocktail comprised of eight phages targeting *S. aureus*, *P. aeruginosa*, and *E. coli* strains, run in patients with venous leg ulcers, found no safety concerns, but the healing rates (up to 60% within a 24-week follow-up) were not higher than in a saline control group (Rhoads et al., 2009). In a case series (n = 107) that analyzed the use of a combined preparation of phages and antibiotics in a biodegradable polymer, venous leg ulcer healing was seen in 70% of patients, all of whom had previously failed conventional therapy (Markoishvili et al., 2002). In a more recent proof-of-concept study, strains of *S. aureus* and *P. aeruginosa* obtained from 87 patients with diabetic foot ulcers were treated *in vitro* with the phage cocktails AB-SA01 and AB-PA01. The cocktails demonstrated coverage against 88.7% of *S. aureus* and 92.7% of *P. aeruginosa* isolates, also achieving significant anti-biofilm activity (Kifelew et al., 2024). Phage therapy has also shown benefit as part of the treatment plan in patients with the chronic, immune-mediated inflammatory skin disease hidradenitis suppurativa (Bens et al., 2025). Finally, innovations like bacteriophage-loaded nanofibers as wound dressings are promising next-generation approaches that address stability and sustained-release requirements (Kielholz et al., 2023).

2.5.2. Respiratory tract infections

Inhaled phage therapy represents a promising approach for the treatment of chronic pulmonary infections, such as those frequently observed in cystic fibrosis (CF) patients colonized by multidrug-resistant *P. aeruginosa* and other pathogens. Administration of phages via nebulization or dry powder inhalation facilitates targeted delivery to the respiratory tract, enhancing phage deposition in lung tissue. Preclinical animal models have demonstrated significant reductions in bacterial burden and inflammation following inhaled phage treatment (Le Guellec et al., 2023). Initial human studies, including compassionate use cases, report that nebulized phage therapy is generally well-tolerated and capable of substantially reducing bacterial loads in sputum samples. A recent personalized phage therapy trial involving nine CF patients revealed a median reduction of 10^4 CFU/mL in *P. aeruginosa* burden after 1-to−2 weeks of inhaled phage treatment, matched by improved lung function as measured by forced expiratory volume (FEV1) (Chan et al., 2025). Interestingly, phage therapy also exerted selective pressure on bacterial populations, leading to evolutionary trade-offs that decreased antibiotic resistance or virulence. In other case studies, phage therapy has been successfully used to treat ventilator-associated pneumonia by *A. baumannii* and *K. pneumoniae* in an infant (Yan et al., 2025), and aspiration pneumonia by *A. baumannii* in a stroke patient (Liu et al., 2025), and often achieving synergy with antibiotics (Chan et al., 2025; Liu et al., 2025). However, limitations include highly variable individual responses, the technical challenge of maintaining phage viability during nebulization, and uneven distribution in the deep lung parenchyma. Validation in larger, controlled clinical trials is imperative to confirm efficacy, establish optimal dosing regimens, and evaluate long-term safety (Wang et al., 2021).

2.5.3. Urinary tract infections (UTIs)

Recurrent urinary tract infections caused by extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae*, *E. coli,* and other Gram-negatives, represent a critical frontier for phage therapy. A study tested the coverage of the commercially available cocktail Pyophage against uropathogens isolated from 118 patients undergoing planned transurethral resection of the prostate (TURP). The original cocktail coverage of 41% was increased to 75% after phage training by phage-bacteria coevolution (Ujmajuridze et al., 2018). When a cohort of patients (n = 9) was subsequently treated with the improved cocktail, a significant reduction in bacterial burden (1-to-5 log) was seen in two thirds of them (Ujmajuridze et al., 2018). Building upon these findings, in a three-arm randomized controlled trial of intravesical Pyophage in patients undergoing TURP (n = 97), it was shown that phages were non-inferior to standard-of-care antibiotics, but not superior to placebo bladder irrigation, in terms of efficacy or safety in treating UTIs (Leitner et al., 2021). Finally, a systematic review of 12 studies, comprising 89 participants, concluded that efficacy varied, going from complete infection resolution, particularly in high-risk patients, to partial or no improvement (Larcher et al., 2025). The approach remains promising, given that localized administration can achieve therapeutic thresholds in the bladder microenvironment, overcoming the dilutive effects of micturition and the sequestering nature of the urothelium, while also addressing the physical challenge of biofilm-associated persistence characteristic of ESBL-secreting reservoirs (Pirnay et al., 2022).

2.5.4. Gastrointestinal infections

The therapeutic potential of bacteriophages in the gastrointestinal tract is transitioning from simple pathogen eradication toward the precision editing of the human gut microbiome (Ding et al., 2025). Clinical prospects include treating chronic diseases linked to gut dysbiosis, such as inflammatory bowel disease (IBD). For example, a study of four geographically distinct IBD cohorts (n = 537) identified a clade of *K. pneumoniae* associated with disease exacerbation and severity, generated a five-phage cocktail targeting the clade, and used the cocktail in colitis-prone mice to successfully attenuate inflammation and disease severity, and in healthy volunteers as a proof-of-concept for the viability of the intervention (Federici et al., 2022). In an undergoing, phase I/II clinical trial, a similar approach using the phage preparation EcoActive to selectively eliminate adherent-invasive *Escherichia coli* (AIEC) in patients with Crohn's disease, will be tested (clinicaltrials.gov ID: NCT03808103). These targeted uses could be expanded to autoimmune diseases such as primary sclerosing cholangitis (Ichikawa et al., 2023), and metabolic diseases including non-alcoholic hepatic steatosis (Gan et al., 2023). Key limitations include gastric acid degradation of phage particles, highly variable intestinal transit times, and the complexity of the gut microenvironment which may sequester or neutralize phage activity.

2.5.5. Bioengineering approaches

Phage engineering has advanced from rudimentary gene editing to a sophisticated systems architecture. Recombineering (BRED) and retron-editing platforms enable the surgical disarming of lysogeny genes, expanding the pool of phages that can be used in therapy (Dedrick et al., 2019), while receptor-binding protein reconfiguration redirects host tropism and transforms phages into highly specific nanocarriers (Karimi et al., 2016). *In vitro* genome assembly platforms, such as Yeast Artificial Chromosome (YAC) assembly and Gibson or Golden Gate synthesis, have decoupled phage construction from the physiological constraints of the bacterial host (Kilcher and Loessner, 2019; Peng et al., 2025). Synthetic phage genomes can be assembled and rebooted via cell-free internal packaging (iPac) systems to generate Cas-capsids: delivery vehicles that selectively degrade bacterial virulence or resistance transcripts without inducing lysis (Alessa et al., 2025; Nozaki, 2022). Artificial Intelligence (AI) and machine learning tools can facilitate high-throughput screening, PK/PD modelling, and accurate prediction of the thermodynamic interaction between virions and bacteria under *in vivo* conditions (Wong et al., 2023). AI has even been used to synthesize complete functional phage genomes, mimicking natural speciation, and resulting in viable virions (King et al., 2025). The coming years will extend this qualitative leap, allowing for unprecedented optimization of lytic kinetics and viral fitness, overcoming biological limitations of wild-type phages, and opening the door for intelligent phage-based combinational therapies, such as those with EOs.

2.6. Key barriers to clinical translation of phage therapeutics

Thorough clinical trials with the same scope as those used to develop small-molecule antibiotics, but adequately adapted to the peculiarities

of phage therapy, must be funded. Strong, methodical pre-clinical research must serve as the foundation for these investigations. Some high-profile failures in early clinical trials of phage treatments have resulted from a lack of adequate understanding of important preclinical and pharmacological factors.

The PhagoBurn trial (Jault et al., 2019) highlighted significant translational hurdles. This phase 1/2 randomized controlled trial against *Pseudomonas aeruginosa* burn wound infections revealed the ineffectiveness of the PP1131 phage cocktail compared to the standard silver sulfadiazine treatment. The primary cause of this failure was an unforeseen and substantial decrease in phage titer following manufacturing, leading to patients receiving a dose four orders of magnitude lower than planned (1×10^2 PFU/mL instead of 1×10^6 PFU/mL). This ultimately resulted in a significantly extended median time for bacterial burden reduction in the phage-treated group (144 h versus 47 h in the control group), with additional research confirming that the bacteria remaining *in vivo* were resistant to these reduced phage concentrations.

These findings demonstrated two major issues to resolve before the clinical use of phage therapy can be fully realized: product stability and pharmacological optimization. Phage stability must be rigorously tested and maintained throughout the supply chain to ensure patients receive the requisite therapeutic dose, a critical pharmaceutical constraint (Jonczyk-Matysiak et al., 2019; Wdowiak et al., 2022). Furthermore, the observed low-dose resistance underscores the necessity for precise phage activity testing to confirm the susceptibility of the infecting strain, the determination of the optimal multiplicity of infection (MOI) to overcome high bacterial loads, and the possible interactions with antibiotics or other co-treatments (Abedon, 2016; Gordillo Altamirano et al., 2022).

Other critical issues that must be examined are the interaction of phages with the human immune system, and the existence and development of resistance against phages *in vivo*. A recent case study suggested phage therapeutic failure was driven by cross-reactive anti-prophage antibodies and bacterial heteroresistance (Gordillo Altamirano et al., 2026). The study demonstrated that pre-existing antibodies targeting conserved prophage-encoded structural components, often shared across different lytic therapeutic phages, could neutralize phage efficacy, increasing the minimum effective dose *in vivo*. Concurrently, heteroresistance, where a subpopulation of bacteria exhibited a higher level of phage resistance, facilitated rapid selection and regrowth, undermining the therapeutic objective (Gordillo Altamirano et al., 2026). Biofilm-associated infections, such as those produced by *Pseudomonas aeruginosa*, or those associated with medical devices, represent additional challenges. The dense exopolysaccharide matrix of these biofilms markedly impedes phage penetration and reduces therapeutic clearance of the bacterial population (Balducci et al., 2023; Meneses et al., 2025). These limitations could be mitigated through the smart use of adjuvants: molecules that, with or without inherent antibacterial activity, can support or enhance the antimicrobial effect of phages. We next present the case of EOs.

3. Essential oils (EOs) as antimicrobial adjuvants

3.1. Chemical diversity and mechanisms of action

EOs are chemically heterogeneous mixtures of volatile secondary metabolites obtained from plant material. EOs primarily comprise phenolic compounds (thymol, carvacrol), monoterpenoids (limonene, linalool), and bioactive aldehydes (cinnamaldehyde).

EOs exert a pleiotropic assault on microbial integrity (Aljaafari et al., 2021; Matte et al., 2023). First, EOs can cause lipophilic membrane disruption with subsequent cytoplasmic leakage. For instance, carvacrol, present in oregano and thyme, intercalates itself into phospholipid bilayers, increases membrane fluidity, decreases membrane potential, disrupts pH gradients, and causes ATP depletion (Ultee et al., 1999), effects primarily driven by its phenolic hydroxyl group (Ultee et al., 2002). These findings have been replicated for linalool from basil EO (Li et al., 2022). Second, EOs can attenuate virulence through diverse pathways. Cinnamaldehyde, derived from the cinnamon bark, has been shown to impair the production of long-chain acyl-homoserine lactones and pyocyanin, key modulators of the quorum sensing (QS) system of *P. aeruginosa* (Topa et al., 2020), also resulting in decreased biofilm production and swarming motility (Topa et al., 2018). Against *L. monocytogenes*, cinnamaldehyde disrupts the Agr QS system, resulting in reduced biofilm formation (Jiang et al., 2023). In another example, eugenol, from clove, can reverse multidrug resistance in *K. pneumoniae* and *E. coli* by downregulation of efflux pump genes (Dhara and Tripathi, 2020). Third, EOs can induce localized reactive oxygen species (ROS) cascades, thereby triggering irreversible oxidative damage to the microbial proteome and genome (Kong et al., 2022), effects seen with lavender (Yang et al., 2020) and tea tree (Brun et al., 2019) EOs against *K. pneumoniae, S. aureus* and *P. aeruginosa*.

These mechanisms of action have been robustly documented primarily *in vitro* and against an admittedly short list of key pathogens. Important challenges for high-impact translation are the diversity of biochemical interferences to EO activity *in vivo,* and the difficulty in achieving the stabilization of these volatile compounds under processing conditions (Fokas et al., 2025). Excitingly, there is evidence that the antimicrobial prowess of different EOs is not merely additive but often synergistic, and that minor constituents of an EO mixture can modulate its overall physicochemical properties (Belete et al., 2025; Caceres et al., 2020).

3.2. Pharmacokinetic considerations of EOs

An accurate assessment of EO efficacy requires a deep understanding of their pharmacokinetic profile, which often reveals a disconnect between *in vitro* potency and *in vivo* performance. The clinical utility of EOs is inherently limited by their high volatility, poor aqueous solubility, and susceptibility to environmental degradation (Ben Jemaa et al., 2017; Falleh, 2025). Nanotechnology can streamline EO transition from raw extracts to therapeutic agents, with advanced delivery systems such as liposomes, polymeric nanoparticles, or nanomicelles. These systems shield volatile constituents from oxidative inactivation, enhance their physicochemical stability, enable controlled release kinetics, and ultimately extend EO presence in complex tissue niches (Bilia et al., 2014).

For oral applications, active terpenoids face extensive enzymatic degradation and metabolic transformation during gastrointestinal (GI) transit (Garcia et al., 2020). Lipophilic constituents are absorbed via passive diffusion in the small intestine but undergo rapid first-pass hepatic glucuronidation and sulfation, paradoxically restricting systemic availability despite fast absorption. Hydrophilic glycosides, on the other hand, are physiologically inert until they get to the lower GI tract where the intestinal microbiota cleaves them (He et al., 2024). Because of this quick metabolic turnover, systemic concentrations often drop below the therapeutic threshold before EOs reach distant infection sites (Allaoua et al., 2018; Spisni et al., 2020).

The resulting hallmark of EO pharmacokinetics is short systemic persistence: carvacrol, thymol, and eugenol possess plasma half-lives of ~1.8–2.0 h, with near total elimination within 5–6 h (Michiels et al., 2008). This 'flash' kinetic profile is a challenge for maintaining a steady-state therapeutic window and further warrants the need for site-specific delivery strategies, such as pH-responsive microencapsulation, to protect the active fraction from premature metabolic clearance (Zhao et al., 2023). As adjuvants for phages, however, this limitation could be assuaged: if EO concentrations need only reach membrane-priming rather than independently bactericidal thresholds at the target site, the pharmacokinetic burden is substantially reduced.

3.3. Evaluating the safety of EOs

The application of EOs, even via the topical route, demands rigorous safety evaluation given their intrinsic cytotoxic and phototoxic potential (Oliveira Ribeiro et al., 2020; Sindle and Martin, 2021). Standard screening protocols include the 3T3 Neutral Red Uptake test (OECD TG 432) for phototoxicity prediction (Ceridono et al., 2012), followed by *ex vivo* penetration studies in models such as pig ear skin to establish clinical safety margins. One such study, for example, determined that 35% of administered tangerine EO reached the viable epidermis, yet remained below the fibroblast IC50-UV (concentration that reduces viability to 50% under UV irradiation, a proxy for phototoxicity), confirming formulation safety (Infante et al., 2022).

An important principle that can be leveraged to improve the safety of topical applications is the "competitive diffusion" effect. Here, the chemical composition of EOs can be modified through the addition of less permeable agents that saturate the intercellular lipid pathways. As a result, potentially harmful compounds can be sequestered within the superficial epidermal strata, stopping them from reaching deep viable skin layers (Mahrhauser et al., 2015). This effect was seen at play in a study of multicomponent synergistic frameworks, where the addition of mandarin orange EO, or terpenes including linalool from lavender and terpinen−4-ol from tea tree, increased the overall lipophilicity of mixtures, trapped the volatile compounds in the upper skin layers and produced a reservoir effect that limited flux into the viable epidermis (Infante et al., 2022). Furthermore, if thinking of EOs as antimicrobial adjuvants, their effect in improving skin barrier, hydration, and homeostatic restoration (Lopes et al., 2026), can arguably facilitate pathogen clearance by other agents, like phages.

For all routes of administration, however, translational viability is contingent upon the precise calibration of therapeutic potency against cellular biocompatibility. As discussed above, for pharmacokinetic constraints, the perhaps narrow therapeutic window of EOs is a strong argument for their use as antimicrobial adjuvants. By not expecting EOs to drive the entire antimicrobial effect, instead thinking of them as boosters or primers for the action of phages, we could use concentrations below their minimum inhibitory concentration (MIC), with more favorable safety profiles, that would nevertheless still require *in vivo* validation.

4. Phages and EOs combined: Rationale for combination therapy

4.1. Understanding combination therapy and synergy

Combination therapy, whether pairing phages with EOs, phages with antibiotics, two antibiotics, or chemoradiotherapy in oncology, aims to improve efficacy and durability of response by attacking the target system through multiple, mechanistically distinct pathways (ANON, 2017). Complex biological networks (tumors, bacterial communities, host–pathogen systems) possess redundancy and feedback loops that allow rapid escape from single-agent pressure, so multi-drug or multi-modality regimens are often required to prevent or delay resistance and to achieve deeper, more sustained control of disease (Woods and Read, 2023). In infectious diseases, combining antimicrobials can enhance bacterial killing, lower the required dose of each agent, and reduce the emergence of resistance, as recognized for phage–antibiotic and antibiotic–EO strategies (Supina and Dennis, 2025; Woods and Read, 2023).

From a pharmacological standpoint, synergy is defined as a combined effect that is greater than the expected additive effect. In contrast, additive interactions match the mathematically expected sum of individual effects, while antagonism occurs when the combination underperforms that expectation, for example, by mutual interference at the target or opposing signaling pathways (Rao et al., 2018). In the pursuit of designing rational phage–EO combinations, a proper quantification of their combined effect is crucial, so that genuinely synergistic regimens rather than merely additive, or even antagonistic ones, are selected for further mechanistic study and eventual real-world applications.

Combination therapy interactions can be readily measured *in vitro*. A keystone for this endeavor is the Fractional Inhibitory Concentration Index (FICI). The FICI compares the minimum inhibitory concentrations (MICs) of each agent alone versus in combination in a checkerboard microdilution assay. For a two-component phage–EO pair, the FICI is calculated as $FIC_A + FIC_B$, where FIC_A is the MIC of agent A in combination divided by its MIC when alone, and likewise for B. The resulting sum is then interpreted using conventional cut-offs, with FICI ≤ 0.5 typically indicating synergy, values around 1 showing additivity or no interaction, and FICI values ≥ 4 demonstrating antagonism (Hall et al., 1983). Other reference models include the Loewe additivity, Bliss independence, or the Chou–Talalay combination index (CI), the latter with thresholds of CI < 1 indicating synergism, CI $= 1$ additivity, and CI > 1 antagonism (Ma and Motsinger-Reif, 2019). Regardless of the measuring method, it is important to remember that phage–EO synergy may be conditionally fragile, and a function of at least three variables. First, the stoichiometric ratio, as excessive EO concentrations may inactivate phages (Chao et al., 2000). Second, the thermal kinetics of the experiment, as phage adsorption rates are temperature-dependent (Fernandez et al., 2025; Greenrod et al., 2025). Third, the receptor architecture, as the efficacy of phage-based treatment combinations is dictated by the nature and availability of specific bacterial receptors used by a given phage, which vary significantly across strains (Gordillo Altamirano and Barr, 2021).

After having reviewed the biological hallmarks of phage therapy and considered the biochemical properties of EOs in the previous sections of this work, we next explore the mechanisms through which phages and EOs can realistically achieve antimicrobial synergy. For this section, we performed a structured literature search across five databases (PubMed, Semantic Scholar, Dimensions, Web of Science, and Google Scholar), using terms combining "bacteriophage" with "essential oil," major EO constituents (carvacrol, thymol, eugenol, cinnamaldehyde), and synergy-related terms. The initial 103 results were then restricted to original peer-reviewed articles published in the last decade, in English or Spanish, excluding those that employed endolysins or studied non-human pathogens, and manually parsing them for relevance. Table 2 provides a structured synthesis of the studies that met the established criteria and formed the conceptual basis for our analyses, hypotheses, and frameworks detailed henceforth.

4.2. The biophysical adjuvant model (BAM) for phage–EO combination therapy

We propose a conceptual reframing of EOs in the context of phage therapy, shifting from their traditional role as standalone broad-spectrum antimicrobials toward a precisely defined function as biophysical adjuvants that reshape phage–bacteria interaction dynamics. Under the Biophysical Adjuvant Model (BAM), EO constituents are not evaluated primarily for their independent bactericidal activity but for three adjuvant functions: membrane priming, biofilm matrix disruption, and resistance landscape modulation, described in more detail below and summarized in Fig. 1. This model generates specific, testable predictions that distinguish it from the more general claim that phage and EOs "work better together": it predicts that sub-MIC EO concentrations will be more effective as phage adjuvants than bactericidal concentrations, that membrane-active EO constituents will show greater adjuvant potency than non-membrane-active ones, and that adjuvant efficacy will correlate with phage infection rate rather than with independent EO antibacterial activity. These predictions define a research agenda that the field can systematically pursue.

4.2.1. Membrane priming

This pathway for phage-EO synergy is anchored in the fundamental disruption of the bacterial envelope. As established by the classic model

Table 2
Primary evidence base for phage–EO synergy.

Pathogen(s)	Phage(s)	EOs	Interaction outcome	Phage stability in EO reported	Ref
Methicillin-resistant *S. aureus* (5 strains)	Phage K	σ-pinene 3-carene, limonene, β-pinene	Additive (variable)	Yes – adsorption and viability assays up to 120 h	(Ghosh et al., 2016)
E. coli ATCC 15597	MS2 + T7	Cinnamon, clove, oregano, thymol	Synergy, reduced emergence of phage-resistance	No	(Elafify et al., 2025)
Listeria monocytogenes	vB_LmoS-PLM9	Cassia, cinnamon	Combined efficiency higher than monotherapy, no formal analysis	Yes – no decrease in phage titer for up to 24 h	(Maung et al., 2024)
E. coli BALL 1119	phiLLS	Oregano (*L. graveolens*), 13 constituents*	Synergy against planktonic growth, variable against biofilms	Yes – no decrease in phage titer for up to 24 h	(Kao Godinez et al., 2025)
E. coli K12	4-phage cocktail: SUT_E420, 520, 1520 and 1620	Carvacrol	Combined efficiency higher than monotherapy, no formal analysis	Yes – no decrease in phage titer for up to 24 h at up to 6.25 μl/mL	(Kuek et al., 2023)
Salmonella spp. (8 strains)	SALMONELEX (commercial phage cocktail)	Thymol, carvacrol	Combined efficiency higher than monotherapy, no formal analysis	No	(Moon et al., 2020)
A. baumannii NPRCOE 160519	vWUPSU	Sacha inchi (*Plukenetia volubilis*) #	Synergy against planktonic growth, variable against biofilms	Yes – no decrease in phage titer for up to 24 h at ≤ 50% EO	(Wintachai and Voravuthikunchai, 2022)

#Not a volatile EO, but a fixed vegetable oil
* The only study to present a full EO chemotype through gas chromatography-mass spectrometry

of (Nikaido and Vaara, 1985), the outer membrane of Gram-negative bacteria serves as a highly selective molecular sieve; however, the lipophilic nature of EO terpenoids allows them to bypass these biophysical constraints. By partitioning into the lipopolysaccharide (LPS) leaflet, EOs induce a state of transient disordered fluidity that can be exploited by phages to increase adsorption rates, reach previously hidden receptors, or increasing the penetration of their genetic material. Carvacrol and thymol, dominant phenolic monoterpenes of oregano and thyme EOs, have demonstrated these properties (Ultee et al., 2002; Ultee et al., 1999), with a study even describing their effect as outer membrane "disintegration" (Helander et al., 1998). On the other hand, non-phenolic EO constituents such as α-pinene, 3-carene, limonene, and β-pinene, all from pine EO, have weaker membrane-disrupting activity and, against the Gram-positive microbe *S. aureus,* did not achieve synergy with phage K (Ghosh et al., 2016), supporting the BAM prediction that adjuvant potency correlates with membrane-activity profile. The unmasking of cryptic phage receptors by EOs can also occur through alternative pathways. For example, tea tree and thyme EOs, in combination with amikacin, suppressed the expression of capsule synthesis genes in *K. pneumoniae,* and molecular docking analysis identified terpinene−4-ol as a chief bioactive component (El-Demerdash et al., 2024). This de-capsulation effect on the pathogen would open the door to the action of non-capsule-dependent phages, shown to have broader host ranges, and slower emergence of phage-resistance (Lourenco et al., 2023).

4.2.2. Biofilm disruption

Biofilms represent a spatial sanctuary where the extracellular polymeric substance (EPS) acts as a diffusion filter against viral particles. In this context, EOs serve as matrix-degrading adjuvants (Matte et al., 2023). By disrupting hydrophobic interactions within the EPS, EOs can create micro-channels that enhance the convective transport of phages into hypoxic niches. This mechanical weakening of the matrix would ensure that phage titers remain high even in deep-seated infections. Perhaps the most interesting study into this mechanism was performed by (Kao Godinez et al., 2025) where oregano EO was first analyzed through gas chromatography-mass spectrometry (GC-MS) to determine that, at almost 59%, carvacrol was its most prevalent bioactive component. Then, this EO was paired with phage phiLLS, specific against *E. coli*, to treat mature biofilms of six common foodborne pathogens. Microscopic examination of the *E. coli* biofilm treated with the phage-EO combination identified pronounced morphological changes, with deep valleys, fragmented ridges, and overall disruption of the matrix architecture, although mathematical evaluation of the reductions in biofilm biomass and metabolic activity did not conclusively establish synergy. Unexpectedly, synergy was confirmed against biofilms of *S. aureus*, *L. monocytogenes*, and *Salmonella* spp., despite phage phiLLS being incapable of productive infection in these hosts. The findings pose the hypothesis that EO-mediated disruption of the extracellular matrix can expose sequestered bacteria to phage-encoded lytic enzymes and depolymerases, and even in the absence of direct phage replication, achieve synergistic antibiofilm effects (Kao Godinez et al., 2025). Beyond EOs strictly defined, a study looked at sacha inchi oil (*Plukenetia volubilis*), a fixed vegetable oil rather than a volatile EO, reporting antimicrobial enhancement of phage vWUPSU against MDR *A. baumannii* in planktonic cultures, and biofilms (Wintachai and Voravuthikunchai, 2022). Although sacha inchi oil lacks the volatile terpene-based composition characteristic of EOs, the findings suggest that the biophysical adjuvant mechanism may extend to other lipophilic plant-derived oils, a hypothesis warranting systematic investigation with chemically defined EO constituents.

4.2.3. Evolutionary trade-offs and resistance modulation

The most deliberate advantage of the phage-EO interplay would be the imposition of an evolutionary dead-end. Because EOs act through different mechanisms and with several targets in the cell, bacterial resistance against EOs is possible but rare (Becerril et al., 2012). When it occurs, it emerges through lipid membrane alterations, an evolutionary pathway that frequently incurs a fitness cost. Of interest, these alterations could increase susceptibility to phage-mediated lysis. This reciprocal collateral sensitivity would prevent the emergence of pan-resistant phenotypes, ensuring that the adaptation to one agent would strategically facilitate the lethality of the other, a well-documented phenomenon in antibiotic-antibiotic (Pal et al., 2015) and phage-antibiotic combinations (Mangalea and Duerkop, 2020). In parallel, the combination of phages and EOs can lead to "defensive paralysis". By impairing efflux pumps and quorum-sensing (QS) circuits, EOs could compromise the bacteria's ability to coordinate collective anti-phage or antimicrobial defenses (Dhara and Tripathi, 2020; Jiang et al., 2023; Topa et al., 2020), arguably achieving a greater bactericidal

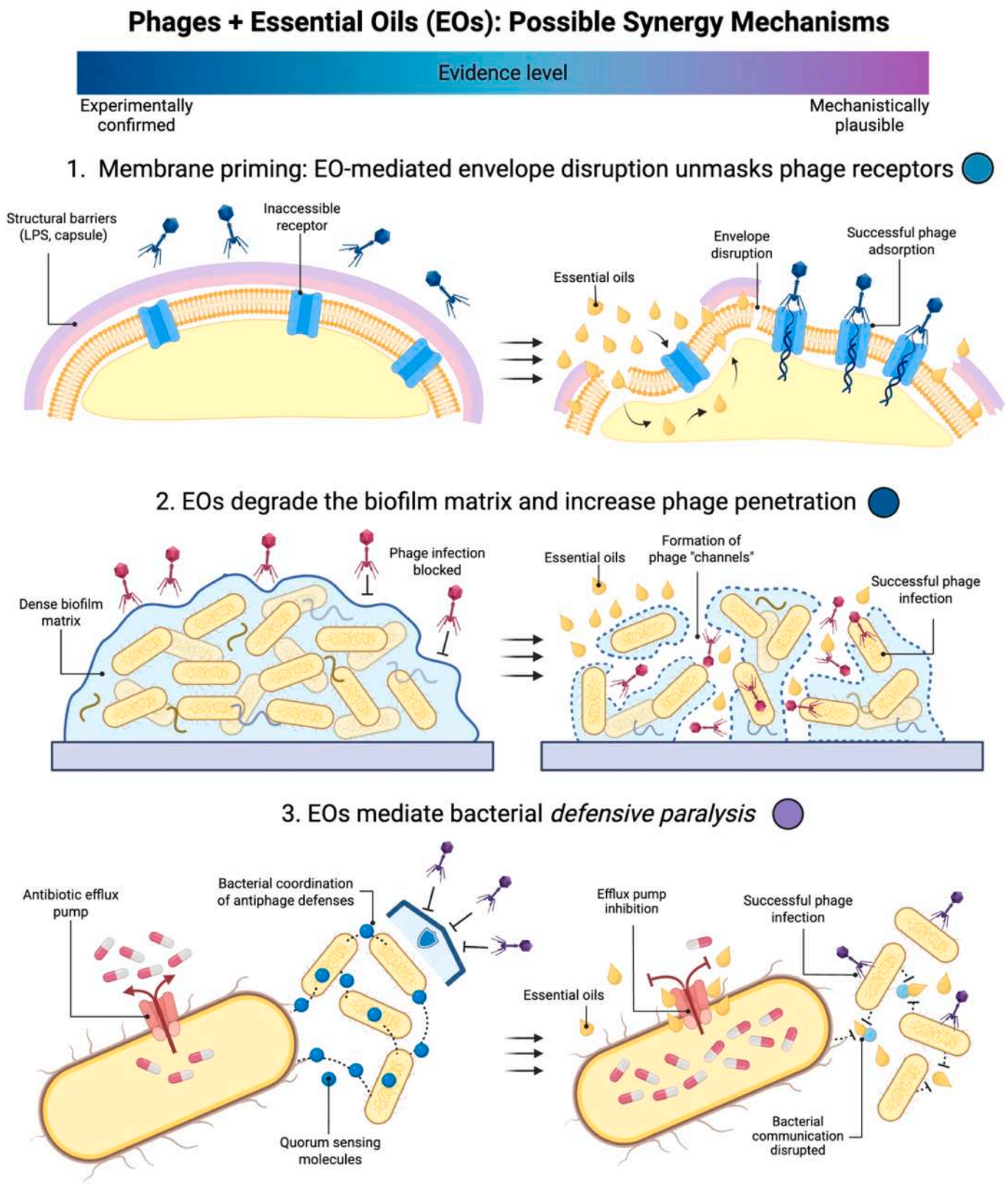


Fig. 1. Synergy mechanisms between bacteriophages and essential oils (EOs). EOs can disrupt the bacterial envelope and unmask previously hidden phage receptors (1), degrade the biofilm matrix to increase phage penetration (2), and induce a *defensive paralysis* state in bacteria, broadly increasing susceptibility to antimicrobials (3). A subjective assessment on the evidence level for each mechanism is color coded with the circles next to each subtitle. Created with BioRender.

effect at the population level.

While we acknowledge that this section presents a compelling yet hypothetical theoretical framework instead of established findings, there is preliminary evidence to warrant further investigation. (Elafify et al., 2025) reported that treatment of *E. coli* with a two-phage cocktail resulted in a post-treatment phage-resistant mutant frequency of 88%. However, with the addition of cinnamon EO or thymol, the emergence of phage-resistance dropped to 8% and 6%, respectively. Furthermore, the relative fitness of these post phage-EO treatment populations was significantly reduced compared to those that received single treatments. These experiments could be expanded to include the quantification of other fitness costs, collateral sensitivity profiling with antibiotics, or longitudinal serial passage assays that track the evolutionary trajectories of bacteria when faced with the double phage-EO challenge.

4.3. How to avoid phage-EO antagonism

Understanding the mechanisms through which phages and EOs may achieve antimicrobial synergy allows us to similarly predict the conditions that could lead to unwanted antagonism between them. First, if used at high doses, EOs may outright kill the bacterial host before phage replication occurs (Fokas et al., 2025). Finetuning the dose of EO, to achieve an adjuvant, phage-enhancing, non-bactericidal role will be paramount. Second, phage viability could be compromised in the presence of EOs (Chao et al., 2000). Tailed phages, which lack a lipid envelope and would therefore be impervious to the membrane-disrupting actions of EOs, are primarily considered for therapeutic applications. However, the full effects of EOs on capsid integrity or tail fiber stability have not been elucidated. Minimum preclinical stability testing should consist of phage titer measurement after short- and long-term exposure but could be complemented by morphological assessment by transmission electron microscopy. Third, by disrupting bacterial membranes, EOs could be destroying instead of unmasking phage receptors. Phage receptors are highly specific, and even small changes in receptor architecture, including those mediated by EO activity on the bacterial envelope, may render phages incapable of adsorption. We believe that phage receptor identification is a prerequisite for the accurate prediction of synergy in phage-based combination treatments (Gordillo Altamirano and Barr, 2021). Fourth, the unpredictable pharmacokinetics of EOs may result in a temporal and spatial misalignment between their effect and that of phages, especially in complex environments such as biofilms (Chaachouay, 2025; Iskandar et al., 2025), an issue that needs to be thoroughly questioned during preclinical research.

4.4. Current evidence gaps and methodological limitations

The use of phage–EO combinations as an antibacterial strategy is a nascent field, as demonstrated by the limited results of our structured search (Table 2). From this small sample of studies, formal meta-analysis is not feasible given the heterogeneity of experimental designs, phage characteristics, and EO chemotypes. There is a lack of standardization in the protocols for laboratories to assess phage susceptibility and phage interactions with bioactive compounds (Kline et al., 2025). Without uniform methodologies, FICI results are neither reproducible nor comparable between institutions (Gomara and Ramon-Garcia, 2019). Furthermore, FICI threshold definitions to confirm synergy varied across studies ($\leq$0.5, $<$0.5, $\leq$0.75), undermining cross-study comparison. Inter-laboratory reproducibility is further compromised by absent reference phage strains, inconsistent EO chemotype reporting, and non-harmonized assay conditions. We identified that the studies were primarily focusing on descriptive *in vitro* assays that provided little-to-no mechanistic insight. It would be ideal to expand the analysis of synergy kinetics by moving beyond static assays to strategies such as time-kill curves, which account for variables such as the emergence of phage-resistance under dual stress. Publication bias toward positive outcomes may be implicated in the apparent universality of synergy, and that distortion should be accounted for. Crucially, we did not encounter studies reporting *in vivo* or clinical data, precluding our ability to gauge the influence of pharmacokinetic behavior or immune interactions that will surely skew results from the controlled laboratory conditions. To address these gaps, we propose a minimum reporting framework for future phage–EO combination studies in Table 3.

4.5. Lessons from phage-antibiotic combinations

Phage–antibiotic synergy (PAS) provides the most directly applicable methodological framework for phage–EO development. Both strategies exploit non-overlapping mechanisms to overcome resistance but differ substantially in their pharmacological profiles. Antibiotics act through specific molecular targets while EO constituents exert pleiotropic biophysical effects on membrane integrity, efflux pump function, and quorum sensing, making cross-resistance mechanistically less likely. PAS is driven by a unique combination of antibiotic mechanism of action, stoichiometry, and bacterial resistance strategies, all heavily influenced by the host microenvironment, and the specific phage-host pairs involved (Kunz Coyne et al., 2024). This principle is equally applicable to phage–EO combinations where the EO chemotype, concentration, and dominant mechanism(s) of action are additionally important. While some studies in PAS have suggested that the order and/or timing of administration (sequential versus simultaneous) influence the outcomes, others have rejected this premise (Fungo et al., 2023; Kumaran et al., 2018). For phage–EO combinations, a staggered approach, with EO administration first to prime the membrane, may be more appropriate but this hypothesis would require careful testing. In a nutshell, studies on PAS constitute an excellent guiding light for the development of phage-EO combinations, although there is substantial

Table 3
Minimum reporting framework for phage–EO synergy studies.

Parameter	Rationale	Reference
Phage and bacterial strain identity	Enables replication and cross-study comparison	(ANON, 2019; Turner et al., 2021)
Phage stability in EO	EOs, especially at high concentrations, may inactivate phages	(Chao et al., 2000)
Phage propagation	Titre variability is a primary source of inter-laboratory irreproducibility	(Glonti and Pirnay, 2022)
Phage receptor	Predicts phage-resistance trade-offs and effects of EO membrane priming	(Gordillo Altamirano and Barr, 2021)
EO identity	EO chemotype varies substantially by origin and extraction method	(Yingngam, 2022)
EO solubilization method	Solvents may independently affect membrane integrity and phage viability	(Cao et al., 2023)
MIC determination	Standardised MIC methodology is essential for valid FICI calculation	(Wiegand et al., 2008)
FICI methodology or method to infer synergy	FICI threshold, and method inconsistency are primary barriers to cross-study comparison	(Fatsis-Kavalopoulos et al., 2024; Ma and Motsinger-Reif, 2019; Odds, 2003)
Biofilm assessment and method	Biofilm architecture fundamentally alters pharmacodynamics of both phage and EO	(Cleaver and Garnett, 2023)
Statistical analysis	Synergy claims should be supported by robust replication and statistical testing	(Demidenko and Miller, 2019)
Outcome reporting	Publication bias toward positive synergy inflates perceived field-wide efficacy	(Joober et al., 2012)

idiosyncrasy to both approaches.

5. Translational roadmap: From discovery to clinical integration

The full clinical integration of novel antimicrobial strategies, such as phages and EOs, requires a robust, stepwise translational pipeline that addresses biological, pharmacological, and regulatory barriers at each stage. Documented preclinical synergy does not guarantee therapeutic success. This is due to a fundamental predictive gap, as demonstrated by (Henry et al., 2013). In their study focusing on phage therapy, they posited that *in vitro* success is a poor predictor of actual efficacy in compartmentalized infections. In human patients, the host microenvironment can greatly alter phage-bacteria dynamics, and this would include the interactions with antibiotics, EOs, and other adjuvants. Fig. 2 systematically maps the critical objectives and challenges encountered across the *in vitro*, *in vivo*, and clinical phases of development for these advanced combination therapies, as well as the foundational logistical, and geopolitical issues to be resolved.

5.1. Priority clinical niches and infection models for phage-EO studies

Section 2.5. emphasized some of the critical areas that stand to benefit from research and development of clinical phage therapy. Considering the simplicity of the delivery route, EO tolerability at the site of administration, and regulatory precedent, we believe that chronic wound infections would be an ideal niche for first-in-human phage-EO combination studies. Topical delivery averts the systemic pharmacokinetic barriers described for EOs, decreases the risk of rapid immune clearance of phages, and better manages the narrow therapeutic window imposed by EOs. Preliminary assessments of tea tree, lavender, eucalyptus, tangerine, and peppermint EOs have suggested tolerability at topical concentrations (Araujo et al., 2026; Infante et al., 2022) that

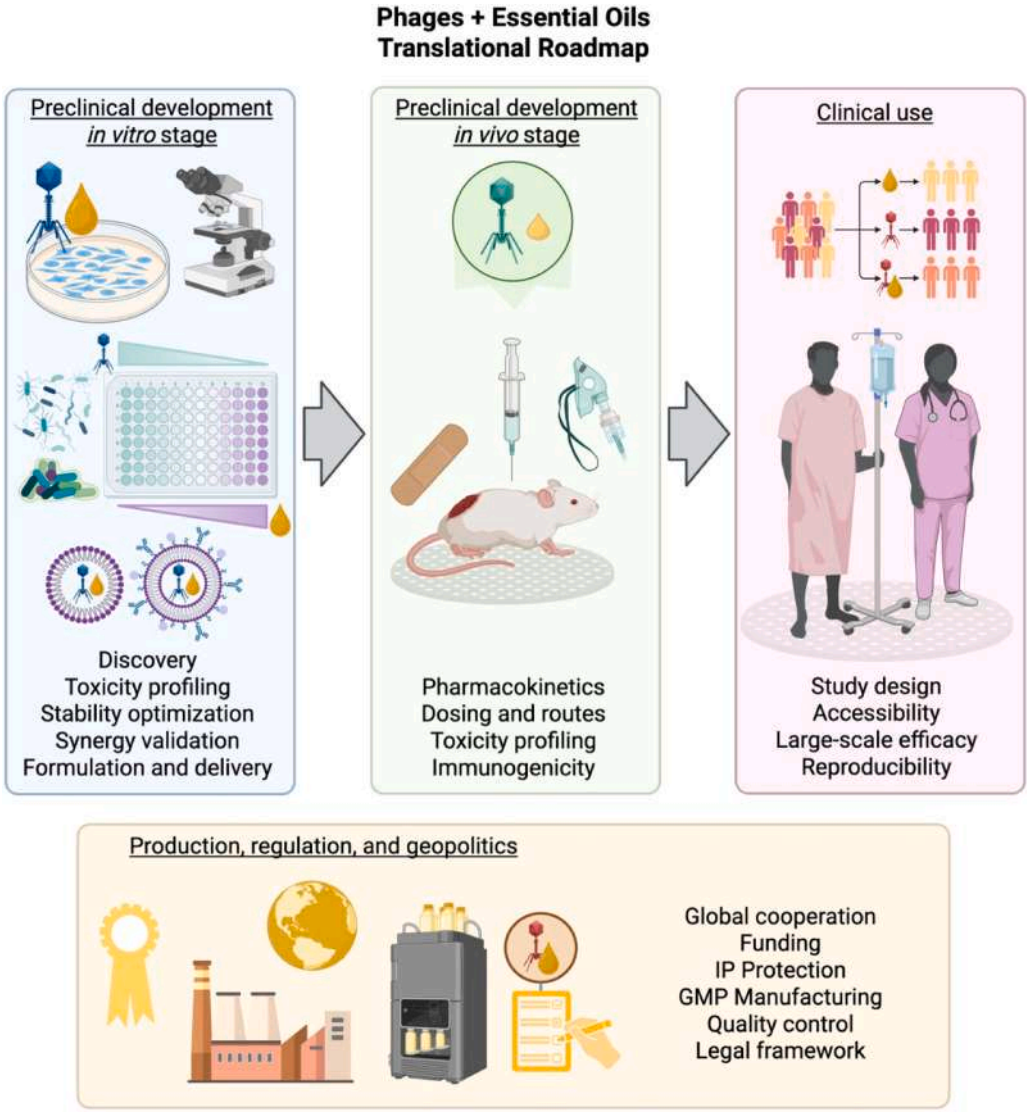


Fig. 2. Translational roadmap for phage-EO combination therapies. Despite the exciting potential of phage-essential oil combinations as powerful antimicrobials, challenges need to be overcome at every stage of the translational pipeline. We propose a tiered approach for research priorities. Tier 1 (blue): Fundamental, immediate priority, 1–3 years. These are experimental pre-requisites for any translational progress and include the systematic characterization of phage viability across EO constituents, concentrations, and contact times, and adoption of the minimum reporting framework proposed in Table 3. Tier 2 (green): Translation, medium priority, 3–7 years. These studies bridge *in vitro* findings to *in vivo* validation. They include animal model studies in priority clinical niches, PK/PD modelling, formulation and administration route development, and toxicological profiling of EOs at adjuvant-relevant concentrations. Tier 3 (red): Clinical, long-term priority, 7 + years. Contingent on successful completion of Tiers 1 and 2, involves the development of adaptive clinical trial designs that accommodate the biological variability of both phage and EO components. Foundational requirements for the entire initiative (yellow) include global cooperation, funding, and logistical, legal, and regulatory issues.

need to be confirmed through extensive cytotoxicity assays. EOs could be incorporated into hydrogels, nanofiber dressings, lipid carriers, or other vehicles (Barnes et al., 2021; Kielholz et al., 2023) for optimized delivery. Phage therapy for wound infections has an extensive clinical precedent up to randomized trials (Jault et al., 2019; Rhoads et al., 2009), providing a regulatory framework that a combination product could build upon. Finally, this model offers the practical advantage of demonstrating a mechanistic rationale of the Biophysical Adjuvant Model: biofilm disruption by EO constituents prior to phage application can be directly visualized by confocal microscopy and quantified by CFU reduction, providing clear mechanistic endpoints alongside clinical outcomes.

Another amenable niche for trialing phage-EO combination therapy concerns urinary tract infections, including those associated with indwelling catheters. EOs such as linalool and eucalyptol have been assessed for short-term cytotoxicity on urothelial cell lines, demonstrating not only a favourable safety profile, but also intrinsic anti-inflammatory properties (Horvath et al., 2022). On the other hand, respiratory infections, such as ventilator-associated pneumonia, or those seen in patients with cystic fibrosis and bronchiectasis would present an almost insurmountable challenge for the use of phage-EO combinations. While nebulized use of phage therapy has strong evidence to support its safety (Chan et al., 2025), EO inhalation has been implicated in rare but severe cases of exogenous lipoid pneumonia (Hassan et al., 2025; Yeung et al., 2021). Finally, gastrointestinal infections present the most complicated pharmacokinetic barrier for EOs, demanding dedicated microencapsulation strategies to overcome pH and enzymatic inactivation, and premature absorption of the compounds (Yang et al., 2025). However, they would highlight a potential boost in safety. Certain compounds, such as cinnamon oil, have been shown to inhibit prophage induction and toxin production, such as Shiga toxin in *E. coli* O157:H7 (Sheng et al., 2016). This property is desirable, as it diminishes the likelihood of prophage-mediated transduction, particularly at sites populated with a rich microbiota, like the gut.

5.2. PK/PD considerations and formulation strategies

The application of EOs, alone or as adjuvants to phages, must be evaluated using the Selectivity Index (SI), a preclinical analogue of the Therapeutic Index (TI), where the effective or synergistic concentrations of a compound are compared to their cytotoxic or clinical safety thresholds, respectively (Muller and Milton, 2012). These parameters have been preliminarily shown to vary widely for different EOs (Belete et al., 2025). Narrow therapeutic windows pose a significant challenge to clinical translation, following the principle that synergy at high concentrations is clinically irrelevant if it exceeds the cytotoxic threshold for human cell lines.

The pharmacokinetic integration of a replicating biological agent with a volatile small molecule has no established modelling framework. Conceptual models developed for phage–antibiotic combinations (Assafiri et al., 2025), provide a starting point but require adaptation for three EO-specific parameters: rapid volatilization and clearance, concentration-dependent membrane effects on phage adsorption kinetics, and the non-linear relationship between EO concentration and bacterial susceptibility. Future modelling should prioritize characterizing the temporal window during which EO-mediated membrane priming enhances phage adsorption. This pharmacodynamic “synchrony window” will also determine optimal dosing intervals.

Some of these concerns can be dealt with using modern delivery platforms for phage–EO co-administration. Although, to the best of our knowledge, no published study has yet demonstrated successful co-formulation of intact phage with EO constituents in a single delivery vehicle, we highlight three promising approaches. First, nano-emulsification could reduce EO droplet size to 20–200 nm, improving aqueous dispersibility while reducing volatility, though preparation conditions must be validated for phage viability (Samananda Singh, 2024). Second, liposomal encapsulation enables differential loading of hydrophilic phage in the aqueous core and lipophilic EO constituents in the bilayer, providing temporally controlled dual release (Liu et al., 2024). Third, embedding and immobilization in different biopolymer matrices can increase localized concentration, and facilitate high treatment efficacy (Liu et al., 2024).

5.3. Regulatory and manufacturing issues

The clinical translation of phage–EO therapeutics will require navigation of regulatory frameworks that were developed independently for biological and botanical medicinal products. As a result, such combinations are likely to be treated as multi-component or combination therapies, with regulatory oversight determined by the constituent providing the primary mode of action (Tian et al., 2022). In the applications proposed by this work, bacteriophages would be expected to supply the direct antibacterial effect, while EOs would function as adjuvants. This classification implies that developers may need to satisfy quality, safety, and efficacy requirements applicable to both biological agents (Niazi, 2025) and complex botanical mixtures (Hoffman, 2015), increasing the scope of chemistry, manufacturing, and controls documentation compared with single-modality products. To the best of our knowledge, there is no established precedent for a comparable dual regulatory burden although the Adaptive Licensing or Advanced Therapy Medicinal Products (ATMP) framework could be a suitable starting point (Iglesias-Lopez et al., 2019).

Manufacturing considerations further complicate development. Phage therapeutics already present challenges related to upstream propagation, removal of bacterial host debris and endotoxin (Subedi et al., 2025), and maintenance of infective titre during formulation and storage (Huang et al., 2025), associated with Good Manufacturing Practices (GMP). The incorporation of EOs introduces additional constraints linked to their volatility, susceptibility to oxidation, and interactions with packaging materials and excipients. Moreover, EOs are chemically heterogeneous natural products whose composition varies according to plant chemotype, geographical origin, cultivation practices, extraction method, and even seasonality (Barra, 2009; Benomari et al., 2023; Lima et al., 2021; Yingngam, 2022). Such variability can influence both antimicrobial activity and host toxicity, necessitating robust chemical fingerprinting and tightly defined specification ranges to ensure batch-to-batch consistency. Experience from early phage therapy trials has demonstrated that inadequate control of formulation stability and potency can directly undermine clinical outcomes (Jault et al., 2019). These lessons are directly applicable to phage–EO systems, in which physicochemical compatibility between components must be demonstrated prior to large-scale manufacturing and clinical evaluation.

5.4. The role of geopolitics

Besides these biological, regulatory and logistical hurdles, the clinical translation of phage therapy, and its combination with EOs, must surmount geopolitical challenges. Despite the success of interventions under Expanded Access (compassionate use) protocols, the global clinical adoption of phage therapy faces a critical fragmentation. In the Global North, robust frameworks such as those of the FDA in the United States and the EMA in the European Union have advanced to categorize phages as medicinal biological products subject to GMP (Good Manufacturing Practices) standards. On the other hand, the Global South, especially in South America, reveals a worrying asymmetry. The regional landscape, led by Brazil and Argentina, shows remarkable technical specialization in genomics and agro-industrial applications (Najjar et al., 2025). However, the lack of regulatory harmonization between agencies such as ANVISA and INVIMA (national health regulatory agencies in Brazil and Colombia, respectively) slows the transition from the laboratory to the patient's bedside. To mitigate the AMR crisis,

it is imperative to establish unified research and biosafety infrastructures and ethical frameworks. We must ensure that therapeutic innovation is not limited by GDP or political geography (Fowoyo, 2024; Gordillo Altamirano and Barr, 2019; Maimaiti et al., 2022; Pirnay et al., 2022).

6. Future directions: A structured research agenda

There are strong biological arguments to encourage research into the therapeutic use of phage-EO combinations. Our review has also identified critical evidence gaps and methodological limitations to this endeavor. Collectively, they define a structured research agenda (Table 4) that summarizes seven priority areas requiring systematic investigation before phage-EO combinations can progress from experimental concept to clinical application, organized by estimated timeline and anchored in the frameworks proposed in this review.

7. Conclusions

The evidence reviewed here supports a fundamental reframing of how EOs should be positioned within the antimicrobial combination therapy landscape. Rather than evaluating EO as standalone broad-spectrum agents constrained by toxicity and regulatory barriers, the Biophysical Adjuvant Model proposed here reconceptualizes them as precision modulators of phage–bacteria interaction dynamics. We should think of EOs as agents whose therapeutic value lies not in independent bactericidal activity but in their capacity to prime bacterial membranes, modulate resistance landscapes, and disrupt biofilm architecture in ways that amplify phage predation efficiency.

This reframing has immediate practical implications. It shifts the relevant EO concentration range from bactericidal to sub-MIC adjuvant concentrations, potentially resolving the cytotoxicity constraints that have stalled EO clinical development. It redefines the primary outcome measure for phage–EO synergy studies from FICI-based synergy scores to phage adsorption rate enhancement and resistance frequency reduction. It also establishes a mechanistically grounded framework for selecting phage–EO pairings based on membrane-activity profiles rather than empirical checkerboard screening.

The field currently stands at an inflection point. The original research studies we highlighted in Table 2 have established proof-of-concept; the minimum reporting framework proposed in Table 3 provides the methodological infrastructure for the next generation of studies; and the prioritized research agenda outlined in Table 4 identifies the experiments that must be completed before clinical translation can be responsibly pursued. The transition from an interesting idea to a strategic asset in the global response to antimicrobial resistance depends on the field's willingness to adopt rigorous, standardized, mechanistically grounded experimental approaches. We believe the Biophysical Adjuvant Model provides the conceptual framework necessary to make that transition.

Table 4
Future research priorities for phage-EO combination therapy.

Priority	Specific Action	Timeline
Standardized synergy assays	Adopt minimum reporting framework (Table 3); replace checkerboard with time-kill assays for phage-containing combinations; harmonize FICI threshold	Immediate
Phage stability characterization	Systematic phage viability assessment across EO constituent concentrations and contact times for clinically relevant phage–pathogen pairings	1–3 years
Resistance frequency assays	Quantify resistance emergence frequency under phage alone, EO alone, and combination exposure to validate collateral sensitivity hypothesis	1–3 years
Animal models	Phage–EO combination studies in chronic wound and catheter-associated UTI biofilm models using standardized endpoints: biofilm eradication rate, inflammatory biomarkers, resistance emergence frequency	3–5 years
PK/PD modelling	Characterize the pharmacodynamic synchrony window; develop integrated phage–EO PK/PD models building on phage–antibiotic frameworks	3–5 years
Formulation development	Validate nanoemulsion, liposomal, and embedding co-formulation platforms for simultaneous phage viability and EO activity retention	5–7 years
Clinical translation	Phase I safety study — topical phage–EO combination for chronic wound infections as a prioritized first-in-human model	7 + years

CRediT authorship contribution statement

DX Romero-Calle: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **F Gordillo Altamirano:** Data curation, Formal analysis, Investigation, Supervision, Validation, Visualization, Writing – original draft. **CA Carrasco Villanueva:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors acknowledge the use of generative AI (Oreate AI) for the first draft of Fig. 1. Using this first draft as guidance, the figure was then crafted and edited manually by the authors, using BioRender. No AI or large language models (LLMs) were used in the writing or editing of the text of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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