Microbial Risk Analysis 31 (2026) 100370

Contents lists available at ScienceDirect

Microbial Risk Analysis

journal homepage: www.elsevier.com/locate/mran

A codex-informed hazard screening and qualitative safety assessment framework for essential oil-bacteriophage combinations in food systems

Rafail Fokas *, Apostolos Vantarakis *

Department of Public Health, Medical School, University of Patras, 26504 Patras, Greece

ARTICLE INFO

Keywords:
Screening framework
Hazard tiering
Uncertainty characterization
Evidence grading
Phage dossier

ABSTRACT

Background: This study investigates whether essential oil-bacteriophage combinations may be screened for food safety using a transparent qualitative approach, therefore filling a gap in which synergy is frequently reported for efficacy but seldom examined for safety.
Methods: Hazards of main essential oil elements were profiled in the OECD QSAR Toolbox, translated into qualitative concern tiers, and aggregated to the oil level using composition weighting and dominance principles. Bacteriophage safety was evaluated by dossier using eight criteria: genetic integrity, manufacturing and process quality, stability, host range, effectiveness in food-like settings, regulatory precedent, and uncertainty, with specific STOP criteria for genomic or manufacturing failures. A conservative maximum rule was used to combine the essential oil and phage tiers, which were then mapped to screening labels using an FAO/WHO-style matrix with likelihood categories for purely lytic, Good Manufacturing Practice-grade phages.
Results: When applied to oregano, thyme, and dittany against *Escherichia coli*, all combinations mapped to Low screening output, within the qualitative and conservative boundaries of the framework, despite oregano and dittany presenting Moderate–High essential oil tiers due to cautious QSAR alerts.
Conclusions: The methodology provides an early-stage safety screen that supports feasibility under rigorous inclusion criteria, while remaining preliminary and hypothesis-generating. Validation in food matrices and EO-phage compatibility (titre-stability) confirmation under intended-use circumstances will be required before practical implementation; formulation solutions may be considered if titre-stability data reveal a major loss of infectivity at the desired EO dose.

Introduction

Antimicrobial-resistant (AMR) foodborne bacteria are driving renewed interest in non-traditional, non-thermal interventions that control microbial hazards without compromising consumer safety (Chavan and Vashishth, 2025; Farrukh et al., 2025). Increasing regulatory scrutiny and consumer demand for alternatives have accelerated research into natural antimicrobials suitable for food-use applications (Sambu et al., 2022; Aldabayan, 2025).

Essential oils (EOs) are widely studied as natural antimicrobials, but their use in food systems is constrained by volatility, sensory impacts, matrix-dependent efficacy, and dose-limited safety considerations. As multi-constituent mixtures, EOs also introduce uncertainty because diminished susceptibility/tolerance can arise after repeated or sublethal exposure in a strain- and compound-dependent manner (Ben Miri, 2025; Falleh, 2025)

In addition to EOs, bacteriophages (phages) have gained popularity as targeted biocontrol agents, particularly against pathogenic bacteria. Phages have remarkable specificity, making them a good weapon for targeting specific pathogens without harming beneficial bacteria (WHO, 2025), and they have been designated Generally Recognized As Safe (GRAS) by regulatory bodies such as the United States Food and Drug Administration (FDA) (Pinto et al., 2020). Despite their acknowledged benefits, phage application faces practical challenges such as the emergence of resistant bacterial strains, variable efficacy based on environmental factors, stability issues within complex food matrices, and ongoing uncertainty about regulatory acceptance and consumer perception. In the European Union, food safety risk analysis is governed by Regulation (EC) No. 178/2002 (European Parliament, 2002), which defines risk assessment as a four-step procedure (hazard identification, hazard characterization, exposure assessment, and risk characterisation) and is supported by EFSA scientific views (EFSA, 2026). Within this

* Corresponding authors.
E-mail addresses: up1098253@upatras.gr (R. Fokas), avanta@upatras.gr (A. Vantarakis).

https://doi.org/10.1016/j.mran.2026.100370
Received 28 November 2025; Received in revised form 19 February 2026; Accepted 19 February 2026
Available online 21 February 2026

perspective, the current framework is clearly positioned as an early-stage screening tool designed to establish hazard characterisation and evidence requirements before any formal exposure assessment or risk characterisation. Given the complementary strengths and limitations of EOs and phages, recent studies have explored combined EO-phage application and reported synergistic antibacterial effects under some conditions (Fokas et al., 2025b). However, reported EO-phage "synergy" is heterogeneous and context-dependent, and may vary (or not exist) depending on EO chemotype/constituents, concentration, contact time, temperature, and target matrix; thus, synergistic performance should be treated as an evidentiary claim rather than a default expectation. Despite the rising efficacy research, a structured and transparent hazard-screening strategy for EO-phage combos is still lacking. Existing research typically reports microbial reductions without systematically incorporating (i) constituent-level toxicological alerts for complex EO mixtures and (ii) dossier-style prerequisites for food-use phage preparations (e.g., strictly lytic lifestyle, WGS-based exclusion of lysogeny/virulence/AMR genes, and manufacturing quality controls). This gap hinders the early prioritization of EO-phage candidates for in-food validation and regulatory discussion. To address this critical gap, a Codex informed, screening-level hazard assessment workflow was developed. The Codex paradigm offers an organized and methodical structure that is particularly suitable for emerging technologies and formulations where quantitative data remain limited. The objective is to develop a preliminary safety profile for a hypothetical EO-phage antibacterial combination through a structured synthesis of toxicological information, safety data, and previous evaluations conducted individually for essential oils and bacteriophages. This integrated appraisal highlights potential hazards, explores realistic exposure contexts, and identifies areas of uncertainty that warrant further investigation. Beyond filling current knowledge gaps, the work aims to support future research priorities, inform regulatory discussions, and contribute to the safe incorporation of EO-phage synergistic approaches within the global food safety landscape.

The scope is limited to food-use biocontrol EO-phage applications and does not address clinical/therapeutic phage use or medical safety endpoints. The methodology is not presented as a substitute for regulatory risk assessment, which typically requires exposure assessment and risk characterization and may include quantitative modelling when sufficient data are available. The framework operates as an early-stage qualitative screening tool rather than a definitive regulatory risk evaluation, and its outcomes should be interpreted within the limits imposed by the strict inclusion criteria applied: strictly lytic, genomically verified, Good Manufacturing Practice (GMP)-grade phages and well-characterized essential oils used at food-relevant levels. Although the methodology is pathogen-agnostic, *Escherichia coli* is utilized as a working example since there is a relatively strong database for both essential-oil activity and phage biocontrol in food-relevant scenarios, allowing for clear tier assignment. The given example is unique to STEC (with O157:H7 utilized as a conservative reference model), and the same approach may be used to *Salmonella* by changing organism-specific inputs.

This approach provides an initial, transparent structuring of plausible hazards and is intended to guide subsequent in-food validation and quantitative exposure assessment. The compatibility between EO and phages is not assumed to be uniformly antagonistic. Published evidence indicates that essential oils and their constituents can exert variable effects on bacteriophage infectivity depending on chemotype/constituent profile, concentration, contact time, temperature, and the food matrix (Ni et al., 2020; Rathod et al., 2021; Zinno et al., 2023). Accordingly, the workflow treats EO–phage compatibility (titre stability under intended-use conditions) as an evidence prerequisite rather than an *a priori* assumption. Where compatibility data are unavailable, the screening output is reported as Conditional, pending confirmation of titre stability under the target formulation and food-use conditions. Temperature–time profiles (e.g., immediate freezing vs refrigerated storage) are treated as boundary conditions; without efficacy and stability evidence under the intended temperature regime, no generalizable conclusion is drawn for frozen-chain products.

2. Materials and methods

This study applies a Codex-informed (FAO/WHO, 1999) screening-level hazard assessment for food-use applications, divided into three modules: essential oils, bacteriophages, and their integration, with food-use conditions serving as the reference scenario. Codex-informed denotes adherence to high-level Codex risk analysis principles (structured approach, transparency, explicit assumptions and uncertainty), rather than endorsement of specific scoring rules. The screening question is: under intended food-use settings, what is the screening-level consumer-health concern category for a specific food-grade EO-phage coupling, assuming it meets the stated inclusion requirements and is compatible with known evidence? In Codex terminology, EO and phage tiering operationalize hazard characterisation, whereas exposure assessment is not quantified but represented through scenario-based qualitative likelihood categories; risk characterisation is the resulting matrix-derived screening outcome (i.e., concern/risk band) used to prioritise combinations for subsequent in-food validation and, where possible, quantitative exposure assessment. Where data is insufficient for quantitative exposure modelling, the procedure documents qualitative likelihood categories for screening purposes and expressly flags evidentiary gaps that must be addressed through further exposure assessment and (where possible) QMRA. Temperature-time profiles (including instantaneous freezing and frozen storage) are viewed as scenario-defining circumstances; hence, application to frozen-chain items necessitates effectiveness and stability data established using the anticipated storage-thaw profile. The methodology (Fig. 1) follows hazard identification and characterization, uses predetermined qualitative likelihood categories instead of quantitative exposure modeling, and maps outcomes in a clear screening matrix. In this paper, concern tiers correspond to qualitative hazard-concern categories (not probabilities), whereas likelihood categories (EL/VL/L) refer to qualitative likelihood descriptors used exclusively in the screening matrix; uncertainty implies confidence in tier assignment (Low/Medium/High). Scientific grading denotes the assignment of predefined hazard-concern tiers to EO constituents (QSAR outputs) and to phage attributes (dossier/WGS/manufacturing evidence) before any risk-management discussion. Two binary STOP gates are then applied within the phage module: (i) Genomic safety (WGS consistent with a strictly lytic phage and absence of lysogeny, virulence/toxin or AMR determinants) and (ii) Manufacturing/process safety (GMP-grade production and purification meeting predefined impurity/bioburden specifications). Operationally, failure of either STOP gate results in “High or not acceptable” classification and the pairing is not progressed to scoring/integration.

2.1. Essential oils hazard characterization

We chose three essential oils (EOs) (Table 1) in advance: oregano (*Origanum vulgare* ssp. *hirtum*), thyme (*Thymus vulgaris*), and dittany (*Origanum dictamnus*) because they are consistently reported in the peer-reviewed literature (Fokas et al., 2025a, 2024) as the most potent food-relevant EOs, owing to their high phenolic monoterpene content (notably carvacrol and thymol) and consistently strong antimicrobial performance against *E. coli* and other foodborne pathogens. EO tiering in this workflow is batch-specific and based on the measured GC–MS profile of the intended food-grade oil. Table 1 provides illustrative Greek examples and is not claimed to be globally representative.

Bottom-up EO hazards were defined by assigning qualitative hazard concern tiers to main elements using the OECD QSAR Toolbox v4.8 (QSAR Toolbox, 2025), which were then aggregated to the oil level according to predetermined procedures. Each ingredient was profiled using a variety of toxicological profilers (chromosomal aberration, skin

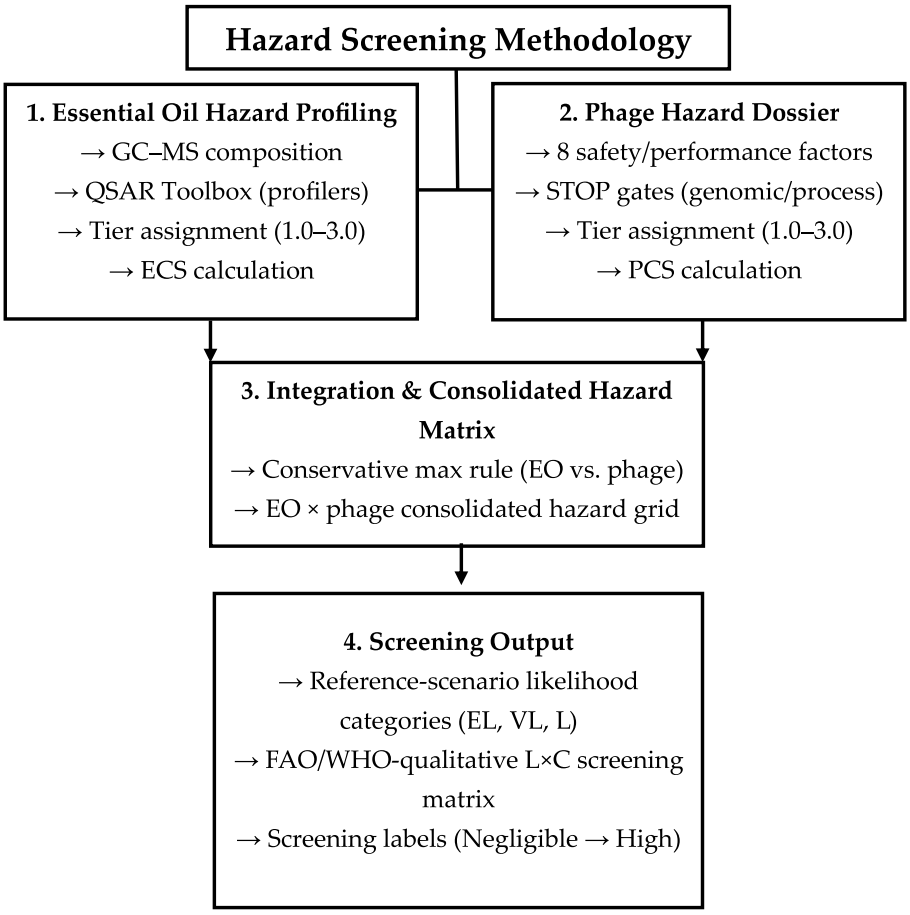


Fig. 1. Screening workflow for EO–phage combinations. EO constituents are tiered using OECD QSAR Toolbox outputs and composition-weighted to yield ECS; phages are tiered via dossier/WGS and manufacturing evidence to yield PCS, with Genomic and Manufacturing STOP gates (fail → "High or not acceptable"). Integration uses ICS and a Likelihood × Consequence screening matrix to produce categorical screening labels.
Abbreviations: ECS, Essential-oil Concern Score; PCS, Phage Concern Score; ICS, Intervention Composite Score; WGS, whole genome sequencing; GMP, Good Manufacturing Practice.

Table 1
Densities and GC–MS composition (major components, % area) of the three EOs, with total identified volatiles. Profiles are illustrative and may vary across cultivars/chemotypes and regions, tier assignment therefore requires batch-specific GC–MS specifications (Economou et al., 2011).

Essential Oil	Density	Major GC-MS Components (% area)	Total Identified Volatiles (%)	Key Reference
Oregano (*Origanum vulgare* ssp. *hirtum*)	0.95	Carvacrol (91.3 %)	97.3	(Fokas et al., 2025a)
Thyme (*Thymus vulgaris*)	0.92	Thymol (26.59) p-Cymene (33.53) 1.8-Cineole (6.25) Limonene (5.32)	90.6	
Dittany (*Origanum dictamnus*)	0.95	Carvacrol (54.81) p-Cymene (13.99) γ-Terpinene (8.33)	95.1	

sensitization, carcinogenicity-genotoxic and non-genotoxic, HESS repeated-dose toxicity, DART, acute oral toxicity, aquatic toxicity) and metabolism simulators (skin, rat liver S9, microbial). For each endpoint, the Toolbox output was recorded as ALERT/NO (and MET where applicable), and only results within the applicability domain (AD) were retained; discordant profiler outputs were handled conservatively by assigning the higher hazard concern tier. All examples included checks for applicability domains (AD) and inter-profiler concordance.

QSAR outputs (Table S1, S2, 2) were transformed into qualitative tiers using the following rule: ALERT in a relevant profiler indicates ≥ Moderate concern, whereas concordant ALERTs across different profilers/simulators elevate to Moderate-High. NO (no alert) indicates Low, while MET (metabolite plausibility) is supportive but does not boost the tier alone. The constituent-level uncertainty was conservatively set at Medium. This Medium uncertainty tier reflects the reliance on QSAR-based toxicological prediction rather than comprehensive *in vivo* datasets and therefore tempers the confidence with which negligible–low screening outputs can be interpreted. Tiers were converted to semi-quantitative values (Low = 1.0, Low-Moderate = 1.5, Moderate = 2.0, Moderate-High = 2.5, High = 3.0) and weighted averaged with GC–MS composition to produce the Essential-oil Concern Score (ECS)

$$\mathrm{ECS} = \sum_i \left(\frac{\%i}{100} x\, Si\right) + \left[1 - \sum_i \left(\frac{\%i}{100}\right)\right] x\ 1.0$$

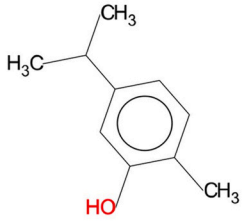
where the unidentified remainder is fixed at 1.0 (Low).

ECS values were assigned to EO Final Tiers (Table 3) based on predetermined thresholds (1.00–1.29 Low, 1.30–1.69 Low-Moderate; 1.70–2.09 Moderate, 2.10–2.49 Moderate-High; ≥ 2.50 High). To preserve worst-case interpretation, dominance rules were applied after ECS calculation: (i) any Moderate-High constituent ≥ 50 % classifies the EO as ≥ Moderate-High irrespective of ECS, (ii) any Moderate-High constituent 20–49 % classifies the EO as ≥ Moderate, (iii) if Low-Moderate/Moderate constituents collectively reach ≥ 60–70 %, the EO tier is raised

one level above the ECS-based tier. EO-level uncertainty = maximum constituent uncertainty, composite values are provided to one decimal with raw values in brackets, and color cues (if used) are illustrative and have no effect on tiering. Numeric codings are used solely as transparent screening indices (monotonic mappings of ordinal tiers) and are not interpreted as interval-scale measurements or quantitative screening estimates.

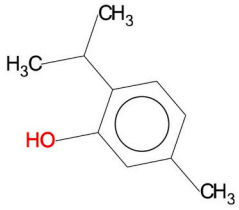
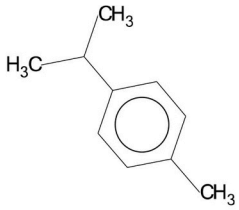
To contextualize the QSAR-derived concern tiers, established regulatory approaches for assessing the safety of flavouring substances were considered. In such frameworks, *in silico* profilers are used primarily to

Table 2
QSAR-based structural alerts and consolidated concern tiers for key EO constituents.

Compound	Key Structural Alerts (food-safety relevant)	Overall Concern	Uncertainty
Carvacrol 	Chromosomal aberration (OASIS alert, Skin metabolism simulator, Rat liver S9 simulators, Microbials simulators)	Moderate	Medium
	Bioaccumulation – metabolism alerts (Skin metabolism simulator, Rat liver S9 simulators, Microbial simulators)	Moderate	Medium
	Acute aquatic toxicity (Verhaar, Skin metabolism simulator, Rat liver S9 simulators, Microbial simulators)	Low	Medium
	Aquatic toxicity (ECOSAR, Skin metabolism simulator, Rat liver S9 simulator, Microbial simulators)	Low	Medium
	Acute oral toxicity (Skin metabolism simulator, Microbial simulators)	Moderate	Medium
	Developmental & reproductive toxicity - DART (Rat liver S9 simulator, Microbial simulators)	Low-Moderate	Medium
	Keratinocyte gene expression (Rat liver S9 simulator, Microbials simulators)	Low-Moderate	Medium
	Carcinogenicity (Rat liver S9 simulator, Microbial simulators)	Low-Moderate	Medium
	Skin sensitization (Rat liver S9 simulator, GHS, OASIS, Microbial simulators)	Low-Moderate	Medium

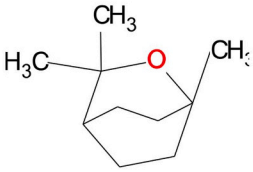
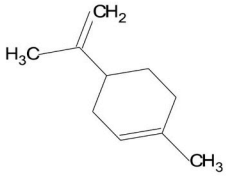
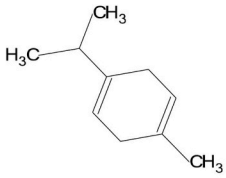
(*continued on next page*)

Table 2 (*continued*)

	Repeated dose toxicity (HESS, Skin metabolism, Rat liver S9 simulators, Microbial simulators)	Moderate-High	Medium
	Overall Carvacrol	Moderate-High concern	Medium uncertainty
Thymol 	Bioaccumulation – metabolism alerts (Skin metabolism simulator, Rat liver S9 simulators, Microbial simulators)	Low-Moderate	Medium
	Skin sensitization (GHS alert, Skin metabolism simulator)	Low-Moderate	Medium
	Carcinogenicity (Rat liver S9 simulator)	Low-Moderate	Medium
	Repeated dose toxicity (HESS, Skin metabolism, Rat liver S9 simulators)	Moderate-High	Medium
	Overall Thymol	Moderate-High concern	Medium uncertainty
p-Cymene 	Bioaccumulation – metabolism alerts (Skin metabolism simulator, Rat liver S9 simulators, Microbial simulators)	Low-Moderate	Medium
	Keratinocyte gene expression	Low-Moderate	Medium
	Carcinogenicity (Rat liver S9 simulator)	Low-Moderate	Medium
	Repeated dose toxicity (Skin metabolism simulator, Rat liver S9 simulators)	Low-Moderate	Medium
	Overall p-Cymene	Low-Moderate concern	Medium
	Bioaccumulation – metabolism alerts (Skin metabolism simulator, Rat liver S9 simulators, Microbial simulators)	Low-Moderate	Medium

(*continued on next page*)

Table 2 (*continued*)

1.8-Cineole 	Repeated dose toxicity (Rat liver S9 simulator)	Low-Moderate	Medium
	Overall Cineole	Low-Moderate concern	Medium uncertainty
Limonene 	Bioaccumulation – metabolism alerts (Skin metabolism simulator, Rat liver S9 simulators, Microbial simulators)	Low-Moderate	Medium
	Repeated dose toxicity (HESS, species-specific, conservative)	Moderate	Medium
	Overall Limonene	Moderate concern	Medium uncertainty
γ-Terpinene 	Bioaccumulation – metabolism alerts (Skin metabolism simulator, Rat liver S9 simulators, Microbial simulators)	Low-Moderate	Medium
	Repeated dose toxicity (Rat liver S9 simulator)	Low-Moderate	Medium
	Overall γ-Terpinene	Low-Moderate concern	Medium uncertainty

Overall Concern: hazard concern tier derived from QSAR alerts and regulatory precedent. Uncertainty: confidence in tier assignment; Medium indicates QSAR-based evidence without comprehensive *in vivo* datasets.

Table 3
EO-level aggregation: weighted scores, thresholds, dominance rules, and final tiers.

EO	Weighted score	Threshold tier	Dominant Rule	Final Tier	Uncertainty
Oregano	2.4 (2.37)	Moderate-High	≥ 50 % Moderate-High (Carvacrol 91.3 %)	Moderate-High	Medium
Thyme	1.7 (1.65)	Low-Moderate	20–49 % Moderate-High (Thymol 26.59 %)	Moderate	Medium
Dittany	1.9 (1.93)	Moderate	≥ 50 % Moderate-High (Carvacrol 54.81 %)	Moderate-High	Medium

ECS: from the final EO tier → map to 1.0–3.0 (Low=1.0, Low–Moderate=1.5, Moderate=2.0, Moderate-High=2.5, High=3.0).

flag structural motifs of potential toxicological relevance rather than to yield quantitative toxicity estimates. This provides a transparent, precautionary basis for interpreting Moderate or Moderate–High concern tiers arising from structural alerts. Major EO constituents such as carvacrol and thymol have previously undergone FEMA GRAS evaluations, confirming their safe use as flavouring ingredients at typical food-use levels (Cohen et al., 2021). These regulatory precedents provide external consistency and support the qualitative concern tiers derived from the QSAR profiler.

2.2. *Bacteriophages hazard characterization*

Bacteriophages were assessed using a dossier-based approach that mirrors current expectations for food-use phage preparations and phage therapy medicinal products. For transparency and biological plausibility, a strictly lytic *E. coli* O157:H7 phage belonging to the Myoviridae (T4-like) was used as the reference model for tiering, because T4-like phages include well-studied, genomically characterized representatives repeatedly proposed for food-safety applications (Liao et al., 2011). This organism is merely presented as a working example. The same dossier-based tiering logic may be modified for *Salmonella* (and other

foodborne targets) by replacing pathogen-specific phage dossiers with matrix-specific performance proof.

Genomic safety was treated as a binary prerequisite. Whole genome sequencing (WGS) is required (EFSA, 2024) to confirm a strictly lytic lifestyle and to exclude any genes associated with lysogeny (e.g., integrases, excisionases, repressor/CI-like regulators), virulence or toxin production, antimicrobial resistance, or generalized transduction. Phages failing this screen are excluded at the outset and classified as "High or not acceptable", regardless of any other attributes. In contrast, T4-like phages with clean WGS profiles and well-defined lytic replication were assigned a Low tier for genomic safety, consistent with published evaluations of safe food-use phages.

Manufacturing and process safety were also treated as a prerequisite and aligned with GMP expectations for biologicals (EMA, 2014). The phage must be produced on a well-characterized, non-pathogenic production strain, under controlled fermentation and purification conditions (Nour El-Din et al., 2025). Process controls should demonstrate effective reduction of host-derived impurities (e.g., lipopolysaccharide endotoxin, residual host DNA and proteins), microbiological purity of the final preparation, and batch-to-batch consistency within predefined specifications. Preparations meeting these criteria were assigned a Low tier for manufacturing and process safety, whereas failure to meet them triggers the second STOP gate and results in a High or not acceptable classification.

Once the two STOP-gate prerequisites were satisfied, the remaining six factors were scored: host range and specificity, efficacy in food-like conditions, resistance dynamics, stability, regulatory precedent, and uncertainty. Host range was generally tiered as Moderate because individual phages have narrow host spectra (Bull et al., 2022) and in practice, cocktails or rotation strategies are often required to cover the diversity of pathogenic *E. coli* strains in foods (Fazzino et al., 2020). Efficacy in food-like matrices was also tiered as Moderate, reflecting typical log reductions of approximately 1–3 log CFU (Fokas et al., 2025c) under realistic conditions and the need to use phages as part of multi-hurdle strategies rather than as stand-alone antimicrobials. Resistance dynamics were scored as Moderate because phage resistance can emerge but can be mitigated through cocktail design, rotation and surveillance (Fujiki et al., 2025).

Stability was tiered as Low-Moderate, as lytic *E. coli* phages are generally stable at refrigeration temperatures and near-neutral pH, but lose activity at high heat, extreme pH values or prolonged UV exposure, which restricts their use to chilled or minimally processed products (Bagińska et al., 2024). Regulatory precedent was scored as Low, reflecting the fact that several phage-based preparations targeting foodborne pathogens (e.g., *Listeria* and *Salmonella*) have been evaluated and accepted by regulatory authorities for use on foods, provided that genomic and manufacturing criteria are fulfilled (Fokas et al., 2025b). Finally, uncertainty was scored as Moderate to acknowledge remaining gaps related to matrix-specific performance, strain-to-strain variability and the limited number of long-term, real-world applications.

Each factor was assigned a qualitative tier (Low, Low-Moderate, Moderate, Moderate-High, High), which was mapped to a numeric score (1.0–3.0). The Phage Concern Score (PCS) was calculated as the arithmetic mean of the eight factor scores, provided both STOP gates were passed; otherwise, the phage was not considered further. The arithmetic mean is only used to calculate aggregate coded tier scores. For log-scaled outcomes (e.g., log reductions/titres), summaries should be generated on the log scale (geometric mean), not as arithmetic means of raw data. Under the assumptions above for a strictly lytic, GMP-grade, genomically verified *E. coli* phage, the PCS corresponded to a Low-Moderate overall concern level (Table 4), which was then integrated with the EO tiers in the consolidated matrix. Numeric codings are used solely as transparent screening indices (monotonic mappings of ordinal tiers) and are not interpreted as interval-scale measurements (see Supplementary Table S4 for robustness to alternative codings).

2.3. Combined EO–phage consolidated matrix

The consolidated framework incorporated essential oil and bacteriophage variables into a single hazard-screening matrix constructed using predefined procedures. EO hazards were represented as an ECS using the tier-to-score mapping (Low = 1.0, Low–Moderate = 1.5, Moderate = 2.0, Moderate–High = 2.5, High = 3.0). These scores were then translated into EO Final Tiers according to fixed thresholds and dominance rules, as described above.

Bacteriophage hazards were evaluated across eight factors: genomic safety, host range, efficacy in food-like settings, resistance dynamics, stability, manufacturing and process safety, regulatory precedent, and uncertainty. Each factor employed the same qualitative tiers and contributed to a Phage Concern Score (PCS), calculated as the arithmetic mean.

Cell-specific tiers for each EO × phage-factor pairing followed a conservative worst-case logic: the cell tier was set to the higher of the EO Final Tier or the phage-factor tier. This approach aligns with Codex qualitative principles, in which the most influential hazard dominates the cell-level interpretation. Two STOP gates were defined *a priori*: failure to satisfy genomic safety or manufacturing/process-safety prerequisites resulted in automatic classification of the cell as High or not acceptable, irrespective of other scores. When both STOP gates were passed, row-level summaries were generated using an Intervention Composite Score (ICS) defined as max (ECS, PCS). To evaluate sensitivity to the assumed likelihood categories, a simple scenario analysis is provided (Supplementary Table S3) where likelihood categories are shifted upward by one level (e.g., EL→VL, VL→L) to represent plausible deviations from the reference dossier or use-conditions; resulting screening labels are reported alongside the reference scenario.

In a secondary, visible layer, screening outputs were mapped as Likelihood × Consequence using qualitative likelihood categories (predefined reference levels) for the reference scenario (strictly lytic, WGS-verified, dossier-supported, GMP-grade phage preparation). These likelihoods are not empirical frequencies they represent scenario-based

Table 4
Bacteriophage factor tiers and Phage Concern Score (PCS).

Parameter	Qualitative tier	Numeric score	One-line rationale
Genomic safety (lysogeny / virulence / AMR / transduction)	Low	1.0	Strictly lytic, clean WGS required. STOP: any red-flag gene ⇒ exclude.
Host range & specificity	Moderate	2.0	Single phages are narrow. Cocktails needed to cover pathogenic diversity.
Efficacy (food-like conditions)	Moderate	2.0	Consistent but partial reductions (~1–3 logs), use in multi-hurdle strategies.
Resistance dynamics (emergence & control)	Moderate	2.0	Resistance can arise, cocktails/rotation & surveillance keep it manageable.
Stability (formulation, pH, temperature, UV)	Low-Moderate	1.5	Stable at chill/food pH; sensitive to high heat/UV → apply on raw/chilled.
Manufacturing & process safety (endotoxin, sterility, residuals)	Low	1.0	GMP purification controls LPS, sterile, well-characterized lots.
Regulatory precedent	Low	1.0	Established acceptance for well-characterized food-use phage preparations
Uncertainty (evidence, strength, matrix/strain variability)	Moderate	2.0	Performance varies by matrix/strain; validate and monitor.
Overall phage concern score	Low-Moderate	1.6 (1.5625)	

Table 5
Combined hazard-screening matrix for EO–phage pairs under strictly lytic, GMP-grade assumptions.

EO (final tier)	Phage factor	Hazard (cell tier)	Likelihood (anchor)	Screening output (L×C)
Oregano (2.5 (Moderate–High))	Genomic safety	2.5 (High)	EL	Negligible
	Manufacturing / process	2.5 (High)	VL	Very Low
	Stability (formulation, pH, T, UV)	2.5 (High)	VL	Very Low
	Regulatory precedent	2.5 (High)	VL	Very Low
	Host range & specificity	2.5 (High)	L	Low
	Efficacy (food-like)	2.5 (High)	L	Low
	Resistance dynamics	2.5 (High)	L	Low
	Uncertainty	2.5 (High)	L	Low
Thyme (2.0 (Moderate))	Genomic safety	2.0 (Moderate)	EL	Negligible
	Manufacturing / process	2.0 (Moderate)	VL	Negligible
	Stability (formulation, pH, T, UV)	2.0 (Moderate)	VL	Negligible
	Regulatory precedent	2.0 (Moderate)	VL	Negligible
	Host range & specificity	2.0 (Moderate)	L	Very Low
	Efficacy (food-like)	2.0 (Moderate)	L	Very Low
	Resistance dynamics	2.0 (Moderate)	L	Very Low
	Uncertainty	2.0 (Moderate)	L	Very Low
Dittany (2.5 (Moderate–High))	Genomic safety	2.5 (High)	EL	Negligible
	Manufacturing / process	2.5 (High)	VL	Very Low
	Stability (formulation, pH, T, UV)	2.5 (High)	VL	Very Low
	Regulatory precedent	2.5 (High)	VL	Very Low
	Host range & specificity	2.5 (High)	L	Low
	Efficacy (food-like)	2.5 (High)	L	Low
	Resistance dynamics	2.5 (High)	L	Low
	Uncertainty	2.5 (High)	L	Low

Legend — Tier → score: Low=1.0, Low-Moderate=1.5, Moderate=2.0, Moderate-High=2.5, High=3.0. Cell hazard tier = max (oil final tier, phage-factor tier). For L × C binning, 2.5 is treated as a High consequence proxy and 2.0 as a Moderate consequence proxy. Qualitative likelihood categories (reference scenario): EL=Extremely unlikely; VL=Very low; L=Low. These are screening-level reference categories (not empirical probabilities) and should be recalibrated for other dossiers or intended-use conditions. Screening labels (qualitative matrix): Negligible, Very Low, Low, Moderate, High. Interpretation note: likelihood categories represent reference-scenario plausibility (not measured frequency) and consequence bins are hazard proxies; thus, Table 5 provides screening labels for prioritisation rather than empirical frequency × severity risk estimates. Sensitivity note: a one-level stress-test (EL→VL; VL→L; L unchanged) is reported in Supplementary Table S3.

plausibility levels intended to support transparent screening and must be recalibrated if the phage dossier, food matrix, storage profile, or application mode differs. Accordingly, upward or downward reclassification of likelihood categories under plausible alternative scenarios will shift matrix outputs, and all categorical outputs reported here apply only to the stated reference scenario. EO–phage compatibility and ΔPFU performance were treated as evidentiary prerequisites: where titre stability or efficacy is not demonstrated under intended-use conditions, the output is reported as an evidence gap requiring confirmation rather than a definitive screening label. Consequence was binned as a hazard-proxy category on the EO Final Tier (e.g., 2.5 mapped to High consequence, 2.0 to Moderate consequence), providing a transparent proxy for maximum toxicological hazard impact within the combined system. A FAO/WHO-style qualitative matrix was then applied to derive categorical screening outputs (negligible, very low, low, moderate, high). A one-level likelihood stress-test (EL→VL; VL→L; L unchanged) is reported in Supplementary Table S3 to illustrate sensitivity of categorical outputs to the assumed reference likelihood categories.

3. Results

QSAR screening of six major EO constituents revealed Moderate-High concern for carvacrol and thymol (due to repeated-dose toxicity alerts and carvacrol's chromosomal aberration), Moderate concern for limonene (due to repeated-dose alert), and Low-Moderate concern for p-cymene, 1.8-cineole, and γ-terpinene. Aquatic-toxicity warnings were typically low and had little impact on food safety rankings. All components had medium uncertainty since the evidence was QSAR-based. The constituent-level levels were then utilized to calculate EO-level weighted scores.

Oregano had a GC–MS weighted score of 2.4 (2.37), thyme 1.7 (1.65), and dittany 1.9 (1.93) (Table 3). Using the predefined dominance rules, oregano and dittany were classified Moderate-High because a Moderate-High constituent exceeded 50 % (carvacrol 91.3 % and 54.81 %, respectively), whereas thyme, despite a Low-Moderate weighted average, was elevated to Moderate because a Moderate-High constituent accounted for 20–49 % (thymol 26.59 %). Uncertainty is medium across all EOs.

The matrix shows a consistently negligible–low screening output band across all EO-phage pairings under rigorous lytic, GMP-grade phage assumptions. No veto gates were activated. Although oregano and dittany are classified as Moderate-High consequence (EO tier 2.5), their risks remain low due to Extremely/Very Low likelihood categories for genetic integrity, manufacturing, regulatory compliance, and stability. Thyme (EO tier 2.0, Moderate consequence) has the lowest overall risk, with genomics, manufacturing, stability, and regulatory mapping at Negligible and the remaining aspects at Very Low.

4. Discussion

Under the stated reference scenario and screening rules, the framework mapped all evaluated EO–phage pairings to a low screening concern band (negligible–low). This interpretation applies strictly within the conservative inclusion criteria and qualitative boundaries of the framework and should not be read as a regulatory safety conclusion.

This result is significant given the conservative methods used. The evaluation erred on the side of caution by using a "conservative maximum rule" to integrate risks, in which the greater hazard level between each EO and phage predominate. Nonetheless, no combination surpassed the low screening-output band. In practice, this means that neither the chemical contents of the EOs nor the biological properties of the phage caused any substantial safety issues under the settings tested. These findings reflect feasibility rather than definitive safety and should be interpreted as preliminary until supported by in-food validation and quantitative exposure assessment. The significant observation is that EO–phage combinations may produce antibacterial synergy while not elevating consumer-health concerns within this conservative framework, supporting their further exploration as food biopreservation candidates. This outcome arises partly from the conservative design of the framework, including alert-driven QSAR tiering for EO constituents and strict STOP-gate exclusion criteria for phages. The Codex-informed qualitative matrix produced a negligible–low screening output under the stated reference scenario; this reflects the framework's screening inputs and assumptions rather than a quantitative exposure-based risk estimate. For example, oregano and thyme oils are high in carvacrol and thymol, chemicals known to be strong antimicrobials (Khwaza and Aderibigbe, 2025) but with minimal acute toxicity and relatively modest genotoxic potential (Cohen et al., 2021) (carvacrol is an allowed food additive (Benincá et al., 2024)). Our QSAR hazard profile revealed no red-flag toxicants among the major EO constituents, consistent with published evaluations that apply *in silico* warnings (per ICH M7 criteria) to identify genotoxic or higher-concern compounds. The adoption of a purely lytic, GMP-produced phage minimized inherent hazards (Bretaudeau et al., 2020). These phages do not integrate into bacterial genomes, preventing transmission of toxin or resistance genes (Elois et al., 2023). Taken together, our data indicate that the intrinsic safety margins of culinary EOs and well-characterized phages may be maintained even when combined, supporting prioritisation for further validation under representative food-use conditions. However, the negligible–low screening output does not imply absence of hazard, it reflects that combined hazards did not exceed those of each component alone under the assumptions applied. The Codex-aligned screening matrix yields negligible–low screening output under the stated reference scenario; this should not be interpreted as a quantitative likelihood of adverse health impacts.

Our findings support and expand on the expanding body of research revealing synergistic antimicrobial effects when EOs are combined with bacteriophages. Previous studies have shown that such combinations reduce pathogens more effectively than each treatment alone. Abdallah et al. (2021) found that combining thyme EO (1 %) with a lytic anti--*Staphylococcus aureus* phage reduced *Staphylococcus aureus* on chicken fillets by around 87 % compared to either treatment alone. Elafify et al. (2025) found that combining a phage cocktail with sub-inhibitory amounts of cinnamon or thymol resulted in >5-log CFU/mL reductions of *E. coli*. This also prevented the formation of phage-resistant mutant. Studies show that EOs can enhance phage infection by destabilizing bacterial cell membranes or defensive systems, whereas phages offer highly targeted bacterial death - a complementing method of action (Fokas et al., 2025b). The screening outputs did not indicate elevated concern beyond that of each component alone under the stated assumptions, addressing a common gap in synergy studies that report efficacy without a transparent hazard-screening structure. This addresses a missing element in prior synergy studies, which focused on efficacy but did not incorporate structured hazard or screening or transparent safety evaluation. Most published synergy studies implicitly assumed safety based on the long history of EOs in foods and the known host-specificity of lytic phages, but they did not assess safety using a Codex-informed, principles-based screening approach (Abdallah et al., 2021; Elafify et al., 2025; Ghosh et al., 2016; Kim et al., 2024). For example, writers frequently state that the EOs examined are natural flavor compounds (Angane et al., 2022) or that the phages utilized are naturally occurring (Narayanan et al., 2024) and GRAS (Rivera-Lopez et al., 2025), but a thorough hazard characterisation and screening-level safety evaluation were beyond their scope.

This study's technique is, to our knowledge, the first to incorporate computational toxicology and phage safety "dossiers" within a Codex-informed qualitative screening evaluation for a multi-hurdle antimicrobial intervention. The uniqueness is in the combination of two previously distinct realms of food safety assessment: chemical hazard profile of natural product ingredients and biological hazard evaluation of bacteriophages. Rather than relying merely on general GRAS status or past usage, we found and evaluated toxicological alarms (e.g., structural

flags for genotoxicity or chronic toxicity) for dozens of specific compounds in each oil. By implementing weighting and dominance rules at the EO level, our method ensured that major constituents (such as carvacrol in oregano/dittany and thymol in thyme) with higher concentrations had a proportionally greater influence on the hazard outcome, while also instituting a dominance criterion that required any single component with an unacceptable high hazard to override others. This is a significant methodological advance since EOs are complex mixtures, which our approach captures in a risk-centric manner. Second, we created a dossier-based phage safety assessment based on eight essential variables, drawing inspiration from regulatory expectations for new microbial agents. These factors included the phage's lifecycle traits (strictly lytic), genomic analysis (absence of virulence, toxin, or antibiotic resistance genes), host range specificity, manufacturing purity (endotoxin levels, absence of microbial contaminants), formulation stability, dose considerations, and historical or regulatory status. Implementing "STOP gates" in this assessment, such as automatically excluding phages with temperate/lysogenic or integrase/toxin genes, ensured that only phages fulfilling high safety norms were regarded acceptable. To integrate the two streams (EO and phage hazards), a conservative maximum-hazard approach was used: rather than assuming dilution or additive effects, the greatest hazard level between the EO and the phage determined the combined hazard class. This precautionary principle assures that the pair's hazard classification is not underestimated, a single dangerous component on either side would indicate the need for risk management. We converted the combined hazard and qualitative probability into an FAO/WHO screening matrix (FAO, 2020). In doing so, we anchored the likelihood assessment in real-world expectations: given that the phages are strictly lytic, host-specific, and manufactured under GMP, the probability of an adverse effect was deemed very low ("rare/unlikely"), whereas the severity of any hypothetical hazard from either component was at worst moderate (e.g., mild irritancy). Likelihood × consequence screening matrices can be structurally influenced by the chosen likelihood categories, which may cap outputs if set *a priori* at low levels; therefore, the reported categorical outputs are conditional on the stated reference scenario and should be recalibrated for other dossiers or intended-use conditions. Dominance and max operators are used as precautionary hazard-screening rules and do not model mixture toxicology (dose/response addition) or interaction under joint exposure. Accordingly, the framework does not infer additivity, synergy, or antagonism for consumer hazard; interaction-relevant questions require explicit assumptions and quantitative exposure assessment.

Given the qualitative and conservative nature of the framework, the findings should be viewed as an initial screening step rather than a definitive safety determination. Future study should include *in vivo* and *in situ* validation, such as animal studies and food challenge trials, to evaluate the safety, microbial reduction, sensory effects, and toxicokinetic of co-ingested EOs and phages at realistic doses. Where titre-stability testing under intended-use conditions indicates a material loss of phage infectivity at the target EO dose, formulation and delivery must be optimized to protect phage fitness at low EO levels, such as through co-encapsulation (Gondil and Chhibber, 2021), edible films (Rindhe et al., 2024), or active packaging (Wagh et al., 2025), with all materials verified as food-grade and in line with the negligible–low screening profile under the stated assumptions. The methodology should be stress-tested against a broader range of targets, including *E. coli* spectrum, *Listeria* and *Staphylococcus*, as well as EOs with varying chemistries, while long-term or repeated-exposure studies track microbiome endpoints and any subtle hazards associated with chronic consumption. Early and ongoing regulatory interaction is required to define dossier expectations, legal categorization, and acceptance criteria, allowing for faster submissions that include QSAR results, phage genomes, and effectiveness data. Finally, consumer acceptance necessitates specialized study on perceptions, labeling, and communication, with a focus on natural origin, clean-label positioning, and the benign fate of exclusively lytic phages, to ensure that technological safety benefits transfer into practical commercial adoption.

The food safety community may reliably leverage EO-phage synergy by pursuing the recommendations, which include *in vivo* validations, enhanced formulations, expanded investigations, regulatory alignment, and consumer outreach. This may enable the development of natural and effective biopreservatives that tackle both microbial spoilage and antimicrobial resistance in the food supply. With a solid safety basis in place, it is evident that these combination actions for enhanced food security and public health may be thoroughly explored and implemented. These conclusions remain contingent on confirming studies that evaluate real-world use conditions.

5. Conclusions

This study presents a clear, screening-level approach for organizing safety-related considerations for food-grade EO-phage combos. Under the indicated inclusion criteria (batch-characterized food-grade essential oils and strictly lytic, genomically validated, GMP-grade phages), the illustrative EO-phage pairings were classified as low concern within the reference scenario's predefined likelihood anchors. The approach generates hypotheses but does not substitute *in vivo* toxicology, quantitative exposure assessment, or food matrix validation. To move forward into actual use, compatibility (titre stability) and performance under intended-use conditions, including applicable storage temperature-time profiles (e.g., instant freezing), must be confirmed. Finally, regulatory consultation is required to formalize dossier expectations and decision criteria for food-grade EO-phage preparations.

Funding

This research was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 5th Call for HFRI PhD Fellowships (Fellowship Number: 19437).

Institutional review board statement

“Not applicable”

Informed consent statement

“Not applicable.”
Data Availability Statement

Abbreviations

The following abbreviations are used in this manuscript:

AD	Applicability Domain
AMR	Antimicrobial Resistance
DART	Developmental and Reproductive Toxicity
ECS	Essential-oil Concern Score
EO	Essential Oil
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GC–MS	Gas Chromatography–Mass Spectrometry
GMP	Good Manufacturing Practice
GRAS	Generally Recognized as Safe
HESS	Hazard Evaluation Support System
ICS	Intervention Composite Score
LPS	Lipopolysaccharide
OECD	Organization for Economic Co-operation and Development
PCS	Phage Concern Score
QRA	Qualitative Risk Assessment
QSAR	Quantitative Structure–Activity Relationship
WGS	Whole Genome Sequencing
WHO	World Health Organization

CRediT authorship contribution statement

Rafail Fokas: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Apostolos Vantarakis:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

Acknowledgements

The publication of the article in OA mode was financially supported by HEAL-Link

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mran.2026.100370.

Data availability

No data was used for the research described in the article.

References

Abdallah, K., Tharwat, A., Gharieb, R., 2021. High efficacy of a characterized lytic bacteriophage in combination with thyme essential oil against multidrug-resistant *Staphylococcus aureus* in chicken products. Iran. J. Vet. Res. 22, 24–32. https://doi.org/10.22099/IJVR.2020.38083.5543.

Aldabayan, Y.S., 2025. Effect of artificial food additives on lung health—an overview. Medicina B Aires 61, 684. https://doi.org/10.3390/MEDICINA61040684.

Angane, M., Swift, S., Huang, K., Butts, C.A., Quek, S.Y., 2022. Essential oils and their major components: an updated review on antimicrobial activities, mechanism of action and their potential application in the food industry. Foods. 11, 464. https://doi.org/10.3390/FOODS11030464.

Bagińska, N., Grygiel, I., Orwat, F., Harhala, M.A., Jędrusiak, A., Gębarowska, E., Letkiewicz, S., Górski, A., Jończyk-Matysiak, E., 2024. Stability study in selected conditions and biofilm-reducing activity of phages active against drug-resistant *Acinetobacter baumannii*. Sci. Rep. 14, 4285. https://doi.org/10.1038/s41598-024-54469-z, 2024.

Ben Miri, Y., 2025. Essential oils: chemical composition and diverse biological activities: a comprehensive review. Nat. Prod. Commun. 20. https://doi.org/10.1177/1934578X241311790.

Benincá, T., Schmidt, L., Thomé Cardoso, L., Rossini Augusti, P., da Silva Malheiros, P., 2024. Carvacrol as a food additive: toxicological aspects and the role of nanotechnology in enhancing its antimicrobial and antioxidant properties. Food Res. Int. 197, 115256. https://doi.org/10.1016/J.FOODRES.2024.115256.

Bretaudeau, L., Tremblais, K., Aubrit, F., Meichenin, M., Arnaud, I., 2020. Good Manufacturing Practice (GMP) compliance for phage therapy medicinal products. Front. Microbiol. 11, 465744. https://doi.org/10.3389/fmicb.2020.01161.

Bull, J.J., Wichman, H.A., Krone, S.M., 2022. Modeling the directed evolution of broad host range phages. Antibiotics 11, 1709. https://doi.org/10.3390/ANTIBIOTICS11121709.

Chavan, P., Vashishth, R., 2025. Antimicrobial resistance in foodborne pathogens: consequences for public health and future approaches. Discov. Appl. Sci. 7, 1–19. https://doi.org/10.1007/S42452-025-07015-Z.

Cohen, S.M., Eisenbrand, G., Fukushima, S., Gooderham, N.J., Guengerich, F.P., Hecht, S. S., Rietjens, I.M.C.M., Rosol, T.J., Davidsen, J.M., Harman, C.L., Lu, V., Taylor, S.V., 2021a. FEMA GRAS assessment of natural flavor complexes: *origanum* oil, thyme oil and related phenol derivative-containing flavoring ingredients. Food Chem. Toxicol. 155. https://doi.org/10.1016/j.fct.2021.112378.

Economou, G., Panagopoulos, G., Tarantilis, P., Kalivas, D., Kotoulas, V., Travlos, I.S., Polysiou, M., Karamanos, A., 2011. Variability in essential oil content and composition of *Origanum hirtum L.*, *Origanum onites L.*, *Coridothymus capitatus (L.)* and *Satureja thymbra L.* populations from the greek island Ikaria. Ind. Crops. Prod. 33, 236–241. https://doi.org/10.1016/J.INDCROP.2010.10.021.

EFSA, 2026. Risk Assessment. URL. https://www.efsa.europa.eu/en/glossary/risk-assessment. accessed 02 February2026.

EFSA, 2024. EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain. EFSa J. 22. https://doi.org/10.2903/J.EFSA.2024.8912.

Elafify, M., Mahmoud, A.A., Wang, X., Zhang, S., Ding, T., Ahn, J., 2025. Synergistic antimicrobial efficacy of phage cocktails and essential oils against *Escherichia coli*. Microb. Pathog. 200. https://doi.org/10.1016/j.micpath.2025.107330.

Elois, M.A., Silva, R., Pilati, G.V.T., Rodríguez-Lázaro, D., Fongaro, G., 2023. Bacteriophages as biotechnological tools. Viruses. 15, 349. https://doi.org/10.3390/V15020349.

EMA, 2014. Good Manufacturing Practice. | European Medicines Agency (EMA). URL. https://www.ema.europa.eu/en/human-regulatory-overview/research-development/compliance-research-development/good-manufacturing-practice#registration-of-manufacturers-of-active-substances-10395. accessed 2 November 2025.

European Parliament and Council of the European Union, 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council. https://eur-lex.europa.eu/eli/reg/2002/178/oj/eng. accessed 02 February 2026.

Falleh, H., 2025. Demystifying the power of essential oils: a review of their antibacterial properties and potential as natural food preservatives. EXCLI. J. 24, 828. https://doi.org/10.17179/EXCLI2025-8439.

FAO, 2020. Guide to Ranking Food Safety Risks at the National Level. https://doi.org/10.4060/cb0887en.

FAO/WHO, 1999. Principles and Guidelines for the Conduct of Microbiological Risk Assessment. URL: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%3A%2F%2Fworkspace.fao.org%2Fsites%2Fcodex%2FStandards%2FCXG%2B30-1999%2FCXG_030e_2014.pdf. accessed 19 February 2026.

Farrukh, M., Munawar, A., Nawaz, Z., Hussain, N., Hafeez, A.B, Szweda, P., 2025. Antibiotic resistance and preventive strategies in foodborne pathogenic bacteria: a comprehensive review. Food Sci. Biotechnol. 34, 2101–2129. https://doi.org/10.1007/S10068-024-01767-X.

Fazzino, L., Anisman, J., Chacón, J.M., Harcombe, W.R., 2020. Phage cocktail strategies for the suppression of a pathogen in a cross-feeding coculture. Microb. Biotechnol. 13, 1997. https://doi.org/10.1111/1751-7915.13650.

Fokas, R., Anastopoulou, Z., Vantarakis, A., 2025a. Antimicrobial activity of greek native essential oils against *Escherichia coli* O157:H7 and antibiotic resistance-strains harboring pnorm plasmid, mecA, mcr-1 and blaOXA genes. Antibiotics 14, 741. https://doi.org/10.3390/ANTIBIOTICS14080741, 2025.

Fokas, R., Giormezis, N., Vantarakis, A., 2025b. Synergistic approaches to foodborne pathogen control: a narrative review of essential oils and bacteriophages. Foods. 14, 1508. https://doi.org/10.3390/FOODS14091508.

Fokas, R., Kotsiri, Z., Vantarakis, A., 2025c. Can bacteriophages be effectively utilized for disinfection in animal-derived food products? A systematic review. Pathogens. 14, 291. https://doi.org/10.3390/PATHOGENS14030291/S1.

Fokas, R., 2024. Comparative in vitro evaluation of the antimicrobial properties of essential oils from lamiaceae, cistaceae, and asteraceae families against *Enterococcus faecalis*. https://doi.org/10.26502/jfsnr.2642-110000165.

Fujiki, J., Yokoyama, D., Yamamoto, H., Kimura, N., Shimizu, M., Kobayashi, H., Nakamura, K., Iwano, H., 2025. Biocontrol of phage resistance in pseudomonas infections: insights into directed breaking of spontaneous evolutionary selection in phage therapy. Viruses. 17, 1080. https://doi.org/10.3390/V17081080, 2025.

Ghosh, A., Ricke, S.C., Almeida, G., Gibson, K.E., 2016. Combined application of essential oil compounds and bacteriophage to inhibit growth of *Staphylococcus aureus* in vitro. Curr. Microbiol. 72, 426–435. https://doi.org/10.1007/S00284-015-0968-6.

Gondil, V.S., Chhibber, S., 2021. Bacteriophage and endolysin encapsulation systems: a promising strategy to improve therapeutic outcomes. Front. Pharmacol. 12. https://doi.org/10.3389/fphar.2021.675440.

Khwaza, V., Aderibigbe, B.A., 2025. Antibacterial activity of selected essential oil components and their derivatives: a review. Antibiotics 14, 68. https://doi.org/10.3390/ANTIBIOTICS14010068.

Kim, J., Kim, S., Wang, J., Ahn, J., 2024. Synergistic antimicrobial activity of essential oils in combination with phage endolysin against *Salmonella typhimurium* in cooked ground beef. Food Control 157, 110187. https://doi.org/10.1016/J.FOODCONT.2023.110187.

Liao, W.-C., Ng, W.V., Lin, I.-H., Syu, W.-J., Liu, T.-T., Chang, C.-H., 2011. T4-Like genome organization of the *Escherichia coli* O157:H7 lytic phage AR1. J. Virol. 85, 6567–6578. https://doi.org/10.1128/JVI.02378-10.

Narayanan, K.B., Bhaskar, R., Han, S.S., 2024. Bacteriophages: natural antimicrobial bioadditives for food preservation in active packaging. Int. J. Biol. Macromol. 276. https://doi.org/10.1016/j.ijbiomac.2024.133945.

Ni, P., Wang, L., Deng, B., Jiu, S., Ma, C., Zhang, C., Almeida, A., Wang, D., Xu, W., Wang, S., 2020. Combined application of bacteriophages and carvacrol in the control of *Pseudomonas syringae* pv. *actinidiae* planktonic and biofilm forms. Microorganisms. 8, 8. https://doi.org/10.3390/MICROORGANISMS8060837, 2020.

Nour El-Din, H., Kettal, M., Lam, S., Granados Maciel, J., Peters, D.L., Chen, W., 2025. Cell-free expression system: a promising platform for bacteriophage production and engineering. Microb. Cell Fact. 24, 42. https://doi.org/10.1186/S12934-025-02661-9, 2025-.

Pinto, G., Almeida, C., Azeredo, J., 2020. Bacteriophages to control Shiga toxin-producing *E. coli* – safety and regulatory challenges. Crit. Rev. Biotechnol. 40, 1081–1097. https://doi.org/10.1080/07388551.2020.1805719.

QSAR Toolbox, 2025. URL. https://qsartoolbox.org/. accessed 10 January2025.

Rathod, N.B., Kulawik, P., Ozogul, F., Regenstein, J.M., Ozogul, Y., 2021. Biological activity of plant-based carvacrol and thymol and their impact on human health and food quality. Trends. Food Sci. Technol. 116, 733–748. https://doi.org/10.1016/J.TIFS.2021.08.023.

Rindhe, S., Khan, A., Priyadarshi, R., Chatli, M., Wagh, R., Kumbhar, V., Wankar, A., Rhim, J.W., 2024. Application of bacteriophages in biopolymer-based functional food packaging films. Compr. Rev. Food Sci. Food Saf. 23, e13333. https://doi.org/10.1111/1541-4337.13333.

Rivera-Lopez, E.O., Tirko, N.N., Dudley, E.G., 2025. Regulatory landscape and the potential of bacteriophage applications in the United States' Food Industry. J. Food Prot. 88, 100510. https://doi.org/10.1016/J.JFP.2025.100510.

Sambu, S., Hemaram, U., Murugan, R., Alsofi, A.A., 2022. Toxicological and teratogenic effect of various food additives: an updated review. Biomed. Res. Int. 2022, 6829409. https://doi.org/10.1155/2022/6829409.

Wagh, R.V., Priyadarshi, R., Khan, A., Riahi, Z., Packialakshmi, J.S., Kumar, P., Rindhe, S.N., Rhim, J.W., 2025. The role of active packaging in the defense against foodborne pathogens with particular attention to bacteriophages. Microorganisms. 13, 401. https://doi.org/10.3390/MICROORGANISMS13020401/S1.

WHO, 2025. Bacteriophages and their use in Combating Antimicrobial Resistance. URL: https://www.who.int/europe/news-room/fact-sheets/item/bacteriophages-and-their-use-in-combating-antimicrobial-resistance. accessed 10 January 2025.

Zinno, P., Guantario, B., Lombardi, G., Ranaldi, G., Finamore, A., Allegra, S., Mammano, M.M., Fascella, G., Raffo, A., Roselli, M., 2023. Chemical composition and biological activities of essential oils from origanum vulgare genotypes belonging to the carvacrol and thymol chemotypes. Plants 12, 1344. https://doi.org/10.3390/PLANTS12061344/S1.