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Review

Beyond Taxonomy in Dairy Microbiology: Microbiome–Resistome–Mobilome–Phageome Interactions Shaping Quality and Safety

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Abstract

Dairy microbial ecosystems cannot be adequately described by total microbial counts, pathogen detection, or taxonomic profiling alone. Milk and dairy products contain interconnected microbial and genetic layers—the microbiome, resistome, mobilome, and phageome—shaped by animal-associated reservoirs, farm and processing environments, starter cultures, technological operations, and storage. This structured critical narrative review integrates these four components within a common microbial–genetic framework and examines how dairy processing reconfigures their interactions. Particular attention is given to cooling, heat treatment, fermentation, ripening, packaging, and storage and to the distinction between molecular detection and biological relevance. Across the reviewed evidence, the significance of microorganisms, antimicrobial resistance genes, mobile genetic elements, and phage-associated sequences depends on viability, functional activity, host assignment, genomic context, transfer potential, persistence, and exposure. Shotgun and long-read metagenomics, metatranscriptomics, metaproteomics, metaviromics, and viability-resolved methods provide increasingly detailed information, but interpretation remains constrained by methodological heterogeneity and limited experimental validation. Future progress requires standardized workflows, longitudinal sampling, stronger ARG–MGE–host resolution, validated phage–host interactions, and studies linking microbial identity and genetic mobility with technological outcomes and dairy safety.

Keywords: dairy microbiome; resistome; mobilome; phageome; antimicrobial resistance; bacteriophages; metagenomics; dairy processing; microbial ecology; food safety

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1. Introduction

The composition, quality, and microbiological safety of milk and dairy products are shaped by a succession of interconnected stages, beginning with primary production and extending through milk collection, transportation, reception, processing, and subsequent storage. At the farm level, animal management, feeding systems, and production practices

influence the physicochemical and nutritional characteristics of raw milk and may affect the properties of derived dairy products, including yogurt [1]. Technological processing and product formulation introduce further variability. For instance, the incorporation of pumpkin pomace powder into whey cheese has been shown to affect nutritional, phytochemical, structural, and sensory characteristics [2], while red beet peel powder supplementation of a whey-fruit beverage modified physicochemical, rheological, antioxidant, sensory, and microbiological parameters during refrigerated storage [3]. Alongside these compositional and technological factors, microbial communities represent a major determinant of dairy product quality, functionality, and safety.

A further dairy-formulation study evaluated betalain-containing Phytolacca americana berry preparations as natural colorants in cheese, highlighting an additional example of product-specific formulation effects on physicochemical and sensory attributes [4].

At primary production, the microbiota associated with raw milk originates from multiple reservoirs, including the mammary gland, teat and skin surfaces, the farm environment, and milking equipment [5,6]. Following collection, milk is subjected to cooling, transportation, and reception at the dairy-processing facility. These successive operations connect farm-associated microbial populations with collection equipment, transport systems, unloading facilities, and storage tanks, thereby creating additional opportunities for microbial persistence, redistribution, or environmental introduction. The microbiological characteristics of milk entering processing therefore depend not only on its initial microbial composition but also on the conditions encountered throughout the pre-processing chain. The relative contribution of individual reservoirs may vary according to production practices, equipment hygiene, temperature control, and the duration of handling and storage.

At the processing facility, milk encounters further microbial selection pressures associated with thermal treatment, fermentation, starter-culture addition, equipment contact, and product-specific technological operations. In fermented dairy products, starter and non-starter microorganisms contribute to acidification, proteolysis, aroma formation, texture development, and microbial stability, whereas other microorganisms may be associated with spoilage or foodborne disease [6,7]. Microorganisms persisting on processing surfaces and within biofilms may contribute to microbial persistence and redistribution within production environments [8]. Subsequent ripening, packaging, and refrigerated storage further influence microbial survival, community composition, and functional activity. Consequently, the microbial communities recovered from finished dairy products reflect successive microbial inputs and selective processes rather than the original raw-milk microbiota alone (Figure 1).

Culture-independent analytical approaches have substantially improved the characterization of microbial communities across these interconnected stages. Amplicon sequencing enables community profiling, although taxonomic composition alone provides limited information about microbial functions and genetic interactions. Shotgun metagenomics offers additional resolution by identifying microbial populations alongside genes associated with metabolic functions, technological traits, antimicrobial resistance, virulence, bacteriocin production, and phage-related processes [9]. Its application to artisanal Idiazabal cheese production demonstrated that feed, teat skin, dairy surfaces, ingredients, raw milk, whey, and cheese constitute interconnected microbial reservoirs. Source-tracking and functional analyses further indicated that farm- and processing-associated sources contribute not only to cheese microbiota but also to antimicrobial resistance and virulence-associated genetic profiles [10]. These findings support the interpretation of dairy products as the outcome of a dynamic microbial continuum extending from primary production to finished-product storage.

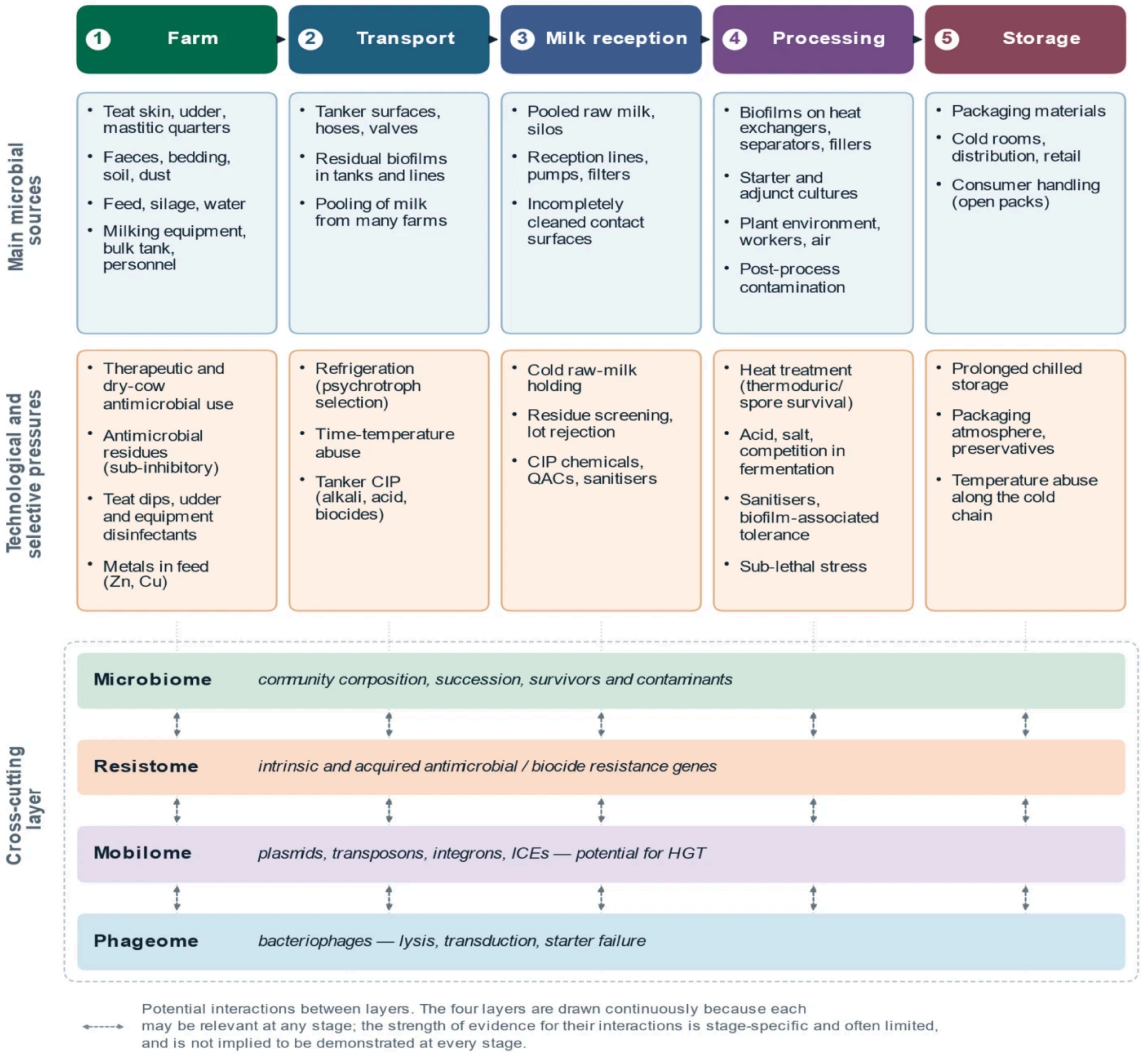


Figure 1. Microbial sources and selective pressures along the dairy production chain. Schematic representation of the successive stages from primary production and milk transportation to reception, processing, and storage. The figure highlights potential microbial reservoirs, routes of microbial introduction, and technological selection pressures shaping dairy microbial communities. The microbiome, resistome, mobilome, and phageome are represented as interconnected dimensions that may influence dairy product quality and microbiological safety. Their representation does not imply that horizontal gene transfer or other microbial interactions occur at every.

The relationship between microbial community structure and antimicrobial resistance is particularly relevant across this production continuum. Using metagenomic and culture-dependent approaches, Yi et al. investigated microbial populations and antimicrobial resistance across farm, pre-pasteurization, post-pasteurization, and processing environments in on-farm dairy plants [11]. Indicator organisms persisted across processing stages, antimicrobial-resistant psychrotrophic bacteria were detected at several sampling points, and plasmid-mediated resistance genes were identified in cultured isolates and metagenomic datasets [11]. At a broader food-production level, an investigation involving 1780 samples from 113 processing facilities identified antimicrobial resistance genes in raw materials, finished foods, and processing environments, with numerous resistance determinants associated with mobile genetic elements, particularly plasmids [12]. Dairy-specific metagenomic studies of cheeses, starter cultures, and probiotic preparations have similarly demonstrated differences in resistome and mobilome composition according to product characteristics and processing conditions. Raw-milk cheeses manufactured without added

starter cultures exhibited greater diversity of antimicrobial resistance genes and mobile genetic elements, whereas milk thermization and starter addition were associated with lower diversity and abundance of these determinants [13]. Accordingly, technological operations may modify both microbial community structure and the genetic repertoire detected within dairy ecosystems.

Nevertheless, the presence of antimicrobial resistance genes in milk, processing environments, or finished products does not necessarily indicate that their bacterial hosts are viable, that the genes are expressed, or that horizontal gene transfer can occur. A systematic review addressing antimicrobial resistance genes in milk highlighted substantial heterogeneity among studies concerning sampling strategies, analytical techniques, quantification, and genomic interpretation [14]. Similar limitations arise when antimicrobial resistance is examined across the dairy production chain, where meaningful interpretation requires information on microbial host identity, viability, genetic location, persistence, and mobility [15]. Plasmids, transposons, integrons, insertion sequences, and other mobile genetic elements are therefore essential components of the dairy mobilome because they provide information on the genomic context and potential dissemination of resistance determinants. However, the identification of resistance genes on potentially mobile elements must be distinguished from the experimental demonstration of horizontal gene transfer.

Bacteriophages constitute another important component of dairy microbial ecosystems, particularly during processing and fermentation. Their technological relevance is well established because phage infection of starter bacteria can impair acidification and fermentation performance. Their persistence depends on phage-specific characteristics, host susceptibility, and the surrounding dairy matrix. A recent analysis of more than 1000 thermal-inactivation observations involving dairy-associated phages demonstrated considerable variability in heat resistance, indicating that conventional dairy heat treatments do not produce uniform phage inactivation [16]. Beyond fermentation disturbances, bacteriophages may influence bacterial population structure through host-specific infection and lysis, while temperate phages and prophages contribute to microbial genomic diversity. Phage-mediated transduction represents a potential mechanism of genetic exchange, although the detection of phage-associated genetic material does not independently establish successful transfer. Conversely, selected lytic phages are increasingly investigated as targeted agents for controlling undesirable microorganisms. Thus, phages may influence both technological performance and microbial–genetic interactions throughout dairy processing and storage.

Previous research has considerably advanced the understanding of dairy microbial ecology, antimicrobial resistance, genetic mobility, and bacteriophage dynamics. Importantly, several studies have already integrated multiple analytical dimensions. For example, shotgun metagenomic analysis of Idiazabal cheese production connected microbial sources with community composition, antimicrobial resistance determinants, and technologically relevant functions [10], while comparative investigations of cheeses, starter cultures, and probiotic preparations revealed associations among microbial composition, resistance genes, mobile genetic elements, and processing conditions [13]. These contributions demonstrate that combining microbial and genetic information is not, in itself, a novel approach. Nevertheless, interpreting the relationships among these components across consecutive dairy-production stages remains challenging, particularly when distinguishing changes in microbial community composition from changes in resistance-gene carriage, genetic mobility, and bacteriophage-mediated population dynamics.

The contribution of the present review is therefore not the identification of previously unknown microbial interactions, but the development of a process-oriented interpretive framework connecting four complementary analytical dimensions: microbial host composition, antimicrobial resistance determinants, genetic mobility, and phage–host interactions.

The framework considers how these dimensions are shaped by the successive stages of dairy production, from farm-associated reservoirs and milk transportation to processing environments, fermentation, ripening, and storage. It emphasizes that conclusions derived from individual components may change when additional biological evidence is considered. For example, reductions in bacterial diversity or resistance-gene abundance do not necessarily establish proportional reductions in genetic dissemination potential, because the identity of surviving hosts, genomic location of resistance determinants, and persistence of mobile elements may differ. Similarly, the technological implications of detecting bacteriophages depend on whether their hosts are desirable starter microorganisms, spoilage-associated bacteria, or microorganisms of food-safety concern.

By integrating these dimensions with technological conditions and experimentally supported biological endpoints, this review aims to distinguish changes in microbial composition from changes in functional and genetic potential, identify evidence gaps that cannot be resolved through single-layer analyses, and support more targeted interpretation of dairy product quality and microbiological safety. The proposed framework is intended as a critical synthesis and decision-support perspective rather than a new mechanistic model or a validated quantitative risk-assessment tool.

2. Literature Search Strategy

This review was conducted as a structured critical narrative review following established recommendations for transparent literature synthesis [17]. Literature searches were performed in Web of Science Core Collection, Scopus, PubMed, and ScienceDirect and were last updated on 18 September 2026. The search focused primarily on peer-reviewed publications published between 2020 and 2026, while earlier studies were retained when they provided essential methodological or conceptual background.

Search terms were organized into five thematic groups covering (i) dairy microbiome and microbial ecology, (ii) resistome and antimicrobial resistance, (iii) mobilome and horizontal gene transfer, (iv) bacteriophages and phageome, and (v) omics-based characterization of dairy microbial communities. Each thematic group was combined with dairy-related terms, including milk, dairy, cheese, yogurt, fermented milk, and whey. Additional terms describing processing conditions, including fermentation, pasteurization, ripening, storage, and processing environments, were applied when relevant.

Original research articles and recent reviews addressing microbial community composition, antimicrobial resistance determinants, mobile genetic elements, phage–host interactions, or processing-related microbial changes in milk and dairy products were considered. Titles and abstracts were assessed for thematic relevance, followed by full-text evaluation where appropriate. Non-peer-reviewed publications and studies without a direct relationship to dairy microbial ecology, genetic mobility, product quality, or microbiological safety were excluded. Reference lists of key publications were also examined to identify additional relevant studies. The selected literature was critically synthesized according to relationships among microbial composition, functional genetic potential, gene mobility, phage–host interactions, and dairy processing.

To improve methodological transparency, the 127 references cited in the final manuscript were retrospectively classified according to their primary thematic relevance. Of these, 44 references addressed dairy microbiome and microbial ecology, 17 focused on resistome and antimicrobial resistance, 11 on mobilome and horizontal gene transfer, 27 on bacteriophages and phageome, and 17 on omics-based characterization and analytical approaches. An additional 11 references provided cross-cutting methodological, contextual, or risk-assessment background. Each reference was assigned to one primary category to avoid double counting, although some publications addressed multiple thematic areas. The the-

matic distribution and reference-level classification of all 127 publications are provided in Supplementary Table S1.

As this study was designed as a structured critical narrative review rather than a systematic review, a PRISMA flow diagram was not prepared. The original search and screening records were not retained; therefore, the numbers of initially retrieved, screened, and excluded records cannot be reliably reconstructed. The thematic counts reported in Supplementary Table S1 refer exclusively to the 127 publications cited in the final manuscript and should not be interpreted as retrospective systematic screening results. This limitation is acknowledged to ensure transparency regarding the literature selection process.

3. The Dairy Microbial Ecosystem: From Taxonomic Composition to Functional Networks

Dairy microbial ecosystems comprise microorganisms originating from milk, animals, farm and processing environments, and cultures intentionally introduced during manufacture. Their composition changes during processing, fermentation, ripening, and storage, while their biological relevance also depends on the functional genes carried by microbial hosts, the genetic elements associated with their potential transfer, and bacteriophages interacting with bacterial populations. These components can therefore be considered as interconnected layers of the same ecosystem rather than independent microbial features. The relationships among the microbiome, resistome, mobilome, and phageome, together with their main implications for dairy quality and microbiological safety, are summarized in Figure 2.

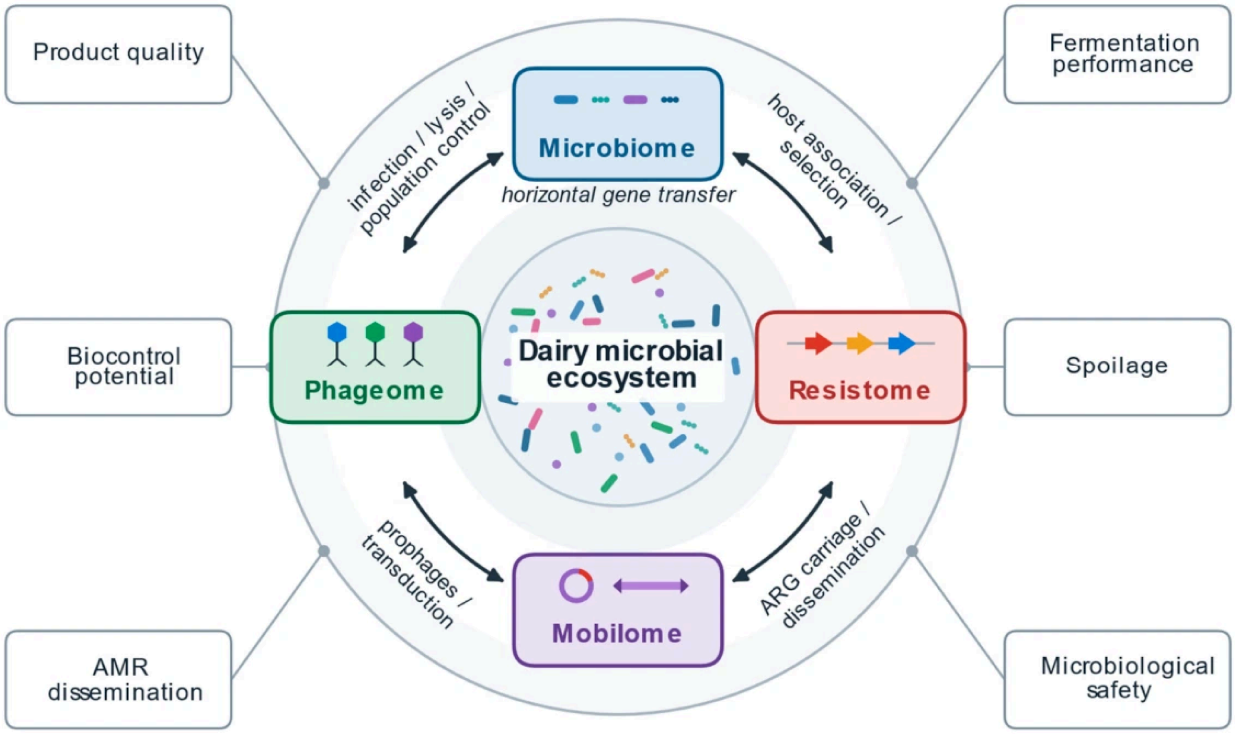


Figure 2. The dairy microbiome as an interconnected genetic–ecological network. The illustrated relationships represent established biological mechanisms rather than newly proposed interactions. The interpretive contribution of the framework lies in examining these mechanisms jointly under dairy-processing conditions and determining how evidence from each component modifies conclusions regarding microbial persistence, resistance-dissemination potential, fermentation performance, and microbiological safety.

The dairy microbial ecosystem comprises interacting microbial communities and their associated resistance determinants, mobile genetic elements, and bacteriophages. The microbiome provides the microbial hosts, whereas the resistome comprises antimicrobial resistance determinants and the mobilome includes mobile genetic elements involved in their carriage and potential dissemination. The phageome influences bacterial populations through infection and lysis and may participate in genetic exchange through lysogeny and transduction. Horizontal gene transfer connects microbial and genetic components through conjugation, transformation, and transduction. Interactions within this network may influence fermentation performance, product quality, spoilage, microbiological safety, AMR dissemination, and biocontrol potential.

The interpretive value of the four-component framework can be illustrated by considering antimicrobial resistance during cheese manufacture. In a comparative metagenomic investigation, raw-milk cheeses produced without added starter cultures exhibited greater diversity and abundance of antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs) than cheeses manufactured using milk thermization or starter cultures [13]. From a microbiome-only perspective, the observed changes could be interpreted primarily as a reduction in community diversity following technological selection. Resistome analysis adds information on the resistance determinants retained within the resulting community, while mobilome analysis reveals whether some of these determinants are associated with potentially transferable genetic elements. Consequently, a reduction in microbial diversity alone cannot establish that the remaining community has proportionally lower resistance-dissemination potential. Such an interpretation requires information on ARG–MGE linkage, bacterial host identity, and, where possible, experimentally demonstrated transfer.

The phageome introduces a further, distinct interpretive dimension. Phages may influence the survival and relative abundance of bacterial hosts through infection and lysis, interfere with starter-culture performance, and contribute to genetic exchange under appropriate biological conditions. Therefore, changes in phage populations could modify the technological or ecological consequences associated with the bacterial community remaining after processing. However, the contribution of phages to resistance dissemination cannot be inferred from their detection alone and requires evidence of phage–host association and functional transfer.

This example illustrates how integrated interpretation can change the research question from whether processing reduces microbial diversity or ARG abundance to which microbial hosts and resistance determinants persist, whether their genomic context supports potential dissemination, and whether phage–host interactions affect fermentation performance or microbial succession. The comparison is an interpretive synthesis of published evidence, not a demonstration that all four components were experimentally linked within a single dairy-processing study. Its practical contribution is to distinguish conclusions directly supported by available measurements from additional hypotheses requiring targeted validation.

3.1. The Dairy Microbiome

The microbiota of milk and dairy products originates from several sources and is progressively modified during manufacture [5,6]. Raw milk contributes considerable bacterial and fungal diversity, but the communities recovered from mature dairy products result from subsequent ecological selection. In three Savoyard raw-milk cheese systems, raw milk and brines represented important sources of microbial diversity, while curds and rinds developed distinct communities during processing and ripening. The ripening stage exerted a marked selective effect on bacterial populations, whereas fungal taxa were more widely distributed among dairy samples [18]. These findings are consistent with studies of cheese ecosystems in which starter microorganisms, non-starter lactic acid bacteria, yeasts, moulds,

and surface-associated populations develop under the combined effects of acidification, salting, water activity, oxygen availability, temperature, nutrient availability, and ripening time [7].

The processing environment provides additional microbial inputs. Source-tracking during artisanal Idiazabal cheese manufacture identified microbial connections among feed, teat skin, dairy surfaces, ingredients, raw milk, whey, and cheese [10]. The contribution of these reservoirs' changes during manufacture because technological conditions favor some populations while restricting others. Consequently, the microbiota of the final product represents the result of successive microbial inputs and selection events rather than a direct continuation of the community initially present in raw milk.

The microbial profile obtained also depends on the analytical method. In a small-scale white cheese processing facility, shotgun metagenomics detected 177 lactic acid bacterial species, compared with 12 species recovered by culturomics [19]. Although culture-based methods remain necessary for phenotypic characterization and experimental validation, sequencing approaches provide broader taxonomic coverage and allow simultaneous investigation of functional genetic traits [9]. Differences can also occur among sequencing platforms. In long-ripened Gouda cheeses, full-length 16S rRNA sequencing, short-read metagenomics, and long-read metagenomics identified broadly similar dominant species but produced different estimates of their relative abundance [20]. Interpretation of the dairy microbiome should therefore consider both the biological system and the methodological resolution used to characterize it.

3.2. The Dairy Resistome

The dairy resistome comprises antimicrobial resistance determinants associated with microorganisms present in milk, dairy products, and their production environments. These determinants may occur in commensal, environmental, technological, or pathogenic bacteria and may represent either intrinsic or acquired resistance [12–15]. Metagenomic approaches have substantially increased their detection because they do not require prior cultivation; however, the occurrence of an antimicrobial resistance gene does not alone establish its phenotypic expression, transferability, or relevance to consumer exposure.

Processing can modify the resistome through its effects on microbial survival and community composition. Metagenomic analysis of starter cultures, probiotic preparations, and cheeses showed differences in resistance-gene profiles according to product type and technological conditions. Raw-milk cheeses manufactured without added starters displayed greater ARG and mobile genetic element diversity than products subjected to milk thermization or starter addition [13]. In cheeses produced in Vermont, resistance determinants were detected in both rind and core samples during ripening, whereas pasteurized cheeses showed a more restricted resistome; their ARG profiles were influenced by the initial microbial community and ripening period [21].

These observations support a close relationship between microbiome composition and the resistance determinants detected within it. Nevertheless, resistome data require contextual interpretation. Host identity, cellular viability, genomic location, expression, and association with potentially mobile elements provide different levels of information and should not be inferred from ARG abundance alone [14,15]. The resistome therefore describes resistance-associated genetic potential, while its biological relevance depends on the organisms and genomic structures in which these determinants occur.

3.3. The Dairy Mobilome

The mobilome comprises genetic elements capable of movement within or between genomes, including plasmids, transposons, integrons, insertion sequences, and integrative

elements. In dairy microbial communities, these structures are particularly relevant when they carry antimicrobial resistance determinants because they may increase opportunities for horizontal dissemination. The relationship between resistome and mobilome is therefore central to distinguishing gene occurrence from potential gene mobility.

Shotgun metagenomic analysis of raw cow and buffalo milk from the Brazilian Amazon identified resistance-associated contigs together with plasmids, integrons, multi-AMR gene arrangements, and viral sequences [22]. Such co-occurrence provides evidence of genetic association but does not demonstrate that transfer occurs under dairy production conditions. The level of evidence consequently differs among detection of an ARG, localization of that ARG on a mobile element, identification of its bacterial host, and experimental confirmation of transfer.

The importance of this distinction was demonstrated by Xu et al. [23], who combined metagenomic sequencing with bacterial isolation, antimicrobial susceptibility testing, PCR, biofilm assays, and conjugation experiments in 539 samples collected from 42 intensive dairy farms. In addition to characterizing the bacteriome and resistome, the authors experimentally confirmed horizontal transfer of *bla*SHV from *Klebsiella pneumoniae* to *Escherichia coli* [23]. This provides direct evidence that at least some resistance determinants present in dairy production systems can move between bacterial hosts.

Horizontal gene transfer can occur through conjugation, transformation, and transduction. The relative contribution of these mechanisms depends on the bacterial populations, genetic elements, and environmental conditions involved. Population density, cellular stress, biofilm formation, viability, and contact between potential donors and recipients may therefore influence transfer opportunities. In this context, the mobilome represents the principal genetic interface between community structure and the potential dissemination of adaptive traits within dairy microbial ecosystems.

3.4. The Dairy Phageome

Bacteriophages constitute a substantial component of dairy microbial communities. Their technological importance has traditionally been associated with infection of starter bacteria, which may impair acidification and fermentation performance [16]. Metagenomic studies now indicate that dairy-associated phages also interact with broader bacterial populations involved in fermentation and ripening.

Analysis of bacterial and viral metagenomes from artisanal Canastra cheese recovered more than 1200 viral operational taxonomic units and identified numerous phage–bacterium associations at strain-level resolution [24]. Phage diversity differed among producers, and genomic data indicated persistent interactions between phages and bacterial members of endogenous starter and cheese communities. These findings support an ecological role for phages in modulating bacterial populations rather than limiting their significance to starter-culture failure.

Changes in phage communities have also been observed during cheese maturation. In a French smear-ripened cheese, the relative abundance of *Lactococcus*-associated phages decreased during ripening while phages associated with ripening bacteria became more prominent. Viral and bacterial succession were closely related, and several dominant phages were repeatedly detected in products from the same production environment across multiple years [25]. The phageome can therefore display both short-term succession during maturation and longer-term persistence within a production system.

Temperate phages and prophages also establish a link between the phageome and genetic mobility. Transduction provides one possible mechanism for bacterial DNA transfer, but the presence of resistance genes in viral fractions should not be interpreted as evidence that transfer has occurred. Blanco-Picazo et al. [26] detected 11 ARGs in viral fractions

obtained from dairy products and a nut-based beverage and showed that some ARG-containing phage particles retained infectivity under laboratory conditions. These findings demonstrate the occurrence of resistance determinants in infective viral particles but do not establish their transfer frequency or significance under dairy-processing or gastrointestinal conditions [26].

The phageome therefore has several distinct roles within dairy microbial ecosystems: it can affect bacterial population dynamics through infection and lysis, contribute to genetic exchange under appropriate conditions, interfere with starter-culture performance, and provide a basis for targeted microbial control. Considered together with the microbiome, resistome, and mobilome, these interactions form the microbial–genetic network represented in Figure 2.

The four components described above are functionally connected. Changes in the microbiome modify the available microbial hosts and consequently the genetic repertoire detected within the community. The resistome represents part of this repertoire, whereas the mobilome provides information on the potential movement of genetic determinants between organisms. Phages further modify bacterial populations and can contribute to genetic exchange through lysogeny and transduction. This framework provides the basis for examining how animal-associated, environmental, and technological reservoirs introduce these components into the dairy chain and how subsequent processing reshapes their composition and connectivity.

4. Microbial and Genetic Reservoirs Along the Dairy Chain

Microorganisms and their associated genetic determinants enter dairy production through multiple, partly interconnected reservoirs. These include animal-associated niches, feed and the farm environment, milking and processing equipment, production surfaces, water and brines, and starter and adjunct cultures intentionally introduced during manufacture. Their relative contribution varies according to farm management, technological conditions, product type, and sampling strategy [5,10,11]. Source attribution is therefore more informative when taxonomic similarity is supported by longitudinal sampling, strain-level resolution, or source-tracking analyses. Reliable strain-level source attribution requires genomic resolution appropriate to the bacterial species and the epidemiological context. Core-genome multilocus sequence typing (cgMLST) should report the allelic distance between isolates, the proportion of successfully assigned loci, and the typing scheme used, whereas SNP-based comparisons should specify the genomic reference, sequence quality, genomic regions analyzed, and pairwise SNP distances. No universal cgMLST or SNP threshold can establish a common source across different bacterial species; interpretation requires species-specific criteria supported by phylogenetic relationships, temporal sampling, and production-chain traceability. For metagenome-assembled genomes (MAGs), reconstruction quality should be documented according to established MIMAG standards, including genome completeness and contamination. High-quality draft MAGs require >90% completeness and <5% contamination, together with the specified rRNA and tRNA criteria, whereas medium-quality drafts require ≥50% completeness and <10% contamination. These quality categories do not independently establish strain identity or transmission. Consequently, source attribution should rely on concordant genomic, spatial, temporal, and epidemiological evidence rather than taxonomic similarity or genetic distance alone. The same distinction applies to genetic determinants: the occurrence of an antimicrobial resistance gene or mobile genetic element in an environmental reservoir does not demonstrate its subsequent transfer to milk or to a dairy-associated microorganism.

4.1. Animal-Associated Reservoirs

Animal-associated microbial populations represent the first potential biological source encountered during milk production. Microorganisms may be recovered from teat skin, the teat canal, mammary-associated samples, and freshly expressed milk, although their relative contribution and biological origin remain difficult to separate from environmental and sampling effects [5]. Recent longitudinal analysis of milk and teat-skin microbiota from three dairy farms showed that both sampling location and stage of lactation explained more variation in bacterial community structure than intramammary cephalosporin treatment at dry-off. Milk and teat skin also contained distinct microbial communities, indicating that proximity between these niches does not imply identical composition [27].

Interpretation of an intrinsic mammary microbiome requires particular caution because bovine milk is a low-microbial-biomass matrix. Dean et al. [28] used extensive negative controls and source-attribution approaches to evaluate milk, internal and external mammary epithelia, air, sampling materials, and laboratory controls. A large proportion of sequence reads recovered from milk and mammary-associated samples could be attributed to contamination originating from extraction reagents, sampling procedures, or teat-associated sources. These observations do not exclude microorganisms from the mammary system but show that low-biomass microbiome studies require stringent controls before taxa are assigned to an endogenous reservoir [28].

Teat surfaces appear more consistently as contributors to the microbiota of conventionally collected raw milk. In a 2025 source-tracking study based on 84 farm and milk samples collected in two seasons, teat-surface microbiota and environmental sources associated with automatic milking equipment and milk tanks showed the strongest relationship with raw-milk communities, followed by fecal sources [29]. These results support the view that animal-associated and milking-environment reservoirs should not be treated as independent compartments. Rather, microorganisms reaching raw milk may reflect successive contact between animal surfaces and the equipment through which milk is collected and stored.

The animal reservoir may also contain resistance determinants and mobile genetic elements. Raw-milk metagenomes have revealed complex resistome–mobilome profiles [22,23], but their attribution to the mammary gland, teat surface, fecal contamination, or the wider farm environment remains dependent on study design. Consequently, detection in raw milk should not automatically be interpreted as evidence of an intramammary origin.

4.2. Farm-Associated Environmental Reservoirs

Feed, bedding, feces, manure, water, soil, and air provide additional microbial inputs before and during milking. Their contributions are not equivalent. In a controlled feeding study, Eliasson et al. [30] compared forage, silage, partial mixed rations, used bedding, and bulk-tank milk. Milk microbial communities showed greater similarity to used bedding than to feed, whereas transfer of dominant microorganisms from silage and feed to milk was limited. The study also highlighted the susceptibility of low-biomass milk samples to environmental contamination during microbiome analysis [30].

Farm-associated reservoirs are relevant not only to community composition but also to food safety. Perdomo and Calle [31] detected important differences among microbial communities according to sample type within a dairy farm and reported *Listeria monocytogenes*, *Cronobacter* spp., and *Escherichia coli* O157:H7 across environmental sampling zones, with seasonal differences in prevalence. Bedding and fecal material also contained comparatively high loads of *Staphylococcus aureus* in the system studied [31]. These findings illustrate that environmental niches surrounding the animal can contain both background microbiota and

microorganisms of safety significance, although their presence in the farm environment does not demonstrate contamination of milk.

Manure constitutes a particularly important genetic reservoir because antimicrobial-resistant bacteria, ARGs, mobile genetic elements, and extracellular DNA may coexist within the same material. Sakib et al. [32] separately quantified intracellular and extracellular fractions of six ARGs and three integron-associated markers in fresh dairy manure and a manure reception pit. The ratio of extracellular to intracellular DNA increased after manure entered the pit, and recurring associations between ARGs and integron markers were observed [32]. The findings extend the concept of a farm resistome beyond viable bacterial cells by demonstrating that extracellular DNA can persist as part of the environmental genetic reservoir. They do not, however, demonstrate subsequent uptake of these determinants by dairy-associated bacteria.

Available data indicate that the farm environment provides a heterogeneous collection of microbial and genetic reservoirs. The contribution of individual sources depends on housing, hygiene, season, animal contact, and production practices and should therefore be determined within the specific production system rather than assumed from the presence of identical taxa.

4.3. Milking and Dairy-Processing Environments

Milking systems provide a direct interface between animal-associated microorganisms and collected milk. Teat liners, milk hoses, filters, pipelines, bulk tanks, residual cleaning water, and other equipment may retain microorganisms after sanitation and contribute repeatedly to subsequent milk batches. In 16 Alpine dairy farms, Bücher et al. [33] investigated sources of dairy propionic acid bacteria associated with quality defects in long-ripened cheeses. These organisms were detected at multiple points in the barn and milking system, and residual cleaning water was identified as an important contamination source in some farms. The study also showed that inappropriate cleaning conditions and farm-specific practices affected contamination patterns [33].

Longitudinal tracking further demonstrates that persistent environmental populations may bridge primary production and processing. Wilson et al. [34] followed milk, water, and 384 environmental swabs over six months in a washed-rind cheese operation. Residual viable populations were detected on parts of the milking system after sanitation, while farm and processing-facility surfaces harbored distinct but partly shared communities. Their results indicate that milk transport and processing do not simply transmit the entire farm microbiota; some populations may be lost, while others can persist or become enriched in specific production niches [34].

The scale of the processing-environment reservoir has become clearer through large metagenomic surveys. De Filippis et al. [35] analysed 1250 samples from 73 cheese-making facilities in four European countries, including raw materials, cheeses, food-contact and non-food-contact surfaces, operators' hands and clothing, brine, and whey. More than 6100 high-quality metagenome-assembled genomes were reconstructed. Environmental surfaces showed microbial compositions and functional profiles distinct from raw materials and final products, and facility-specific strains provided evidence of persistent local microbial signatures [35]. Genes potentially involved in technological functions and bacteriocin production were found in environmental microbiomes, while several antimicrobial-resistance gene families were more abundant on processing surfaces than in cheese-related materials or final products. Thus, a dairy processing environment may act simultaneously as a reservoir of technologically useful organisms and genetic functions and as a reservoir requiring surveillance from a food-safety perspective.

Similar conclusions emerge from smaller dairy-specific investigations. Source tracking in artisanal Idiazabal cheese production identified brine and food-contact surfaces among important reservoirs of microorganisms and functional genes contributing to cheese characteristics, while grass, teat skin, and other inputs contributed to resistance-associated profiles [10]. In a white-cheese production facility, Kahraman Ilıkkan et al. [36] combined MALDI-TOF MS with shotgun metagenomics across milk, curd, finished cheese, and processing equipment. Environmental and equipment samples contained diverse microorganisms and substantial ARG profiles, emphasizing that processing surfaces can retain microbial and genetic determinants even when the food itself undergoes technological hurdles [36].

This broader interpretation is supported by the multi-food metagenomic investigation of Quijada et al. [12], in which raw materials, products, and environmental surfaces from 113 food-processing facilities showed distinct but interconnected resistomes and approximately 40% of detected AMR genes in assembly-based analyses were associated with mobile genetic elements, predominantly plasmids. Although the study was not restricted to dairy production, it provides strong evidence that production environments can shape the genetic context in which resistance determinants persist.

The processing environment should therefore not be considered exclusively as a contamination source. Depending on product and technology, resident communities may contribute to ripening, aroma formation, ecological competition, spoilage, persistence of undesirable microorganisms, and antimicrobial-resistance reservoirs. The relevant question is consequently not whether a facility contains microorganisms, but which strains and genetic functions persist, where they persist, and whether they can reach the product.

4.4. Starter and Adjunct Cultures as Controlled Microbial Reservoirs

Starter and adjunct cultures differ from the preceding reservoirs because they are intentionally introduced to direct fermentation and product development. Their contribution is generally more predictable, but they also carry genomes, accessory genes, prophages, mobile elements, and phage-defense systems that become part of the dairy microbial network.

Long-term use and propagation can select starter populations highly adapted to milk. Somerville et al. [37] characterized more than 100 community samples and more than 100 isolates from historical and modern Swiss cheese starter cultures. These cultures displayed low species- and strain-level genetic diversity, stable phenotypic traits, and evidence of genome decay and pseudogenization, including changes associated with transposable elements. The findings support the interpretation of traditional starter cultures as domesticated microbial communities adapted to a relatively narrow dairy niche [37].

Safety and technological performance nevertheless require strain-level characterization. Gephart et al. [38] combined whole-genome sequencing and phenotypic testing of LAB isolated from artisanal cheeses to identify candidate starter strains. The analysis considered genes associated with carbohydrate metabolism, proteolysis, exopolysaccharide production, antimicrobial activity, antibiotic resistance, prophages, and biogenic-amine potential. This illustrates how candidate starter organisms can be evaluated not only for fermentation properties but also for genetic features relevant to safety and stability [38].

Adjunct cultures can also deliberately reintroduce microbial diversity that may be reduced by standardized processing. Dreier et al. [39] prepared natural milk adjunct cultures from raw or thermized milk and used them together with a commercial starter in model semi-hard cheeses. Differences in adjunct composition were associated with changes in cheese microbial diversity, proteolysis, volatile compounds, and flavour characteristics [39]. Such cultures therefore represent controlled microbial reservoirs that can modify ecological succession and product functionality.

The presence of starter microorganisms does not imply that commercial cultures are major AMR reservoirs. Metagenomic comparison of commercial probiotics, starter cultures, and cheeses found substantially lower ARG and mobile-element diversity in commercial cultures than in raw-milk cheeses in the systems studied [13]. At the same time, starter strains remain major targets for bacteriophages, and their phage susceptibility and defense systems form an important part of the phageome–microbiome relationship [16]. This dual role makes starters distinct from uncontrolled environmental reservoirs: they are selected for reliable technological performance but remain biologically active participants in the wider microbial–genetic network.

A distinction should be made between in-house propagated production starter cultures and commercial Direct Vat Set (DVS) cultures. Production starters may be exposed to environmental microorganisms and bacteriophages during preparation, propagation, or handling, whereas DVS cultures are manufactured as defined commercial preparations intended for direct inoculation. Potential contamination associated with DVS use should therefore be distinguished from the intended starter composition and assessed in relation to handling, inoculation procedures, and the processing environment. In both cases, the genetic characteristics and phage susceptibility of the selected starter strains remain relevant to the microbiome–resistome–mobilome–phageome framework.

The reservoirs summarized in Table 1 should not be considered as independent or equally important sources. Animal-associated niches, farm environments, milking equipment, processing surfaces, and intentionally introduced cultures are interconnected through repeated microbial exchange. Likewise, the detection of identical taxa, antimicrobial resistance genes, mobile genetic elements, or phage sequences in different compartments does not by itself demonstrate transmission between them. Reliable source attribution therefore requires increasing analytical resolution, from taxonomic similarity to strain-level tracking, genomic linkage, and, where possible, experimental confirmation of transfer.

These reservoirs define the microbial and genetic material entering the dairy production chain. Their subsequent persistence, elimination, or redistribution is determined by technological conditions such as cooling, heat treatment, fermentation, salting, ripening, and storage. The following section therefore examines how dairy processing acts on the microbiome, resistome, mobilome, and phageome and reshapes the relationships among these components.

Table 1. Major microbial and genetic reservoirs contributing to the dairy microbiome–resistome–mobilome–phageome network.

Reservoir	Representative Sources	Principal Contribution	Resistome/Mobilome Relevance	Phage Relevance	Interpretation/Limitation	References
Animal-associated	Milk-associated niches, teat canal, teat and udder skin	Direct or indirect contribution of host-associated microorganisms to raw milk	Raw milk may contain ARGs and MGEs, but their precise anatomical origin is often unresolved	Host-associated bacteria may carry prophages or support phage populations	Low microbial biomass and contamination can complicate attribution of an intrinsic milk microbiome	[5,22,23,27–29]
Feed and bedding	Forage, silage, mixed ration, used bedding	Environmental microbial input to animal surfaces and milking environment	Potential indirect contribution of resistant microorganisms and genetic determinants	Phages may accompany bacterial populations, but dairy-specific source attribution remains limited	Shared taxa do not demonstrate direct transfer to milk	[10,30]
Feces and manure	Feces, fresh manure, manure pits	Reservoir of environmental and enteric microorganisms	ARGs, integrons and extracellular resistance-associated DNA may persist	Viral communities may be present, but transfer to milk requires separate evidence	Environmental occurrence does not establish transfer into dairy products	[22,31,32]
Milking environment	Teat liners, hoses, filters, pipelines, tanks, residual cleaning water	Recurrent microbial introduction during milk collection	Equipment-associated populations may contribute resistant organisms or genetic determinants	Can support phage persistence where susceptible hosts are present	Contribution is strongly affected by hygiene and farm-specific practices	[11,29,33,34]
Processing environment	Vats, cutting equipment, cheesecloth, drains, food-contact and non-food-contact surfaces	Persistent resident microbiota, cross-contamination, technological or spoilage functions	Processing surfaces may contain ARG carriers and MGEs	Environmental phages can persist and interact with starter or ripening organisms	Presence on a surface does not demonstrate transfer to the product	[8,10,12,35,36]
Brines and processing liquids	Brine, whey, process water	Selection and redistribution of salt-tolerant and dairy-adapted communities	Can contain genetic determinants associated with resident microbial populations	May serve as reservoirs or routes for phage persistence	Contribution varies strongly with reuse and processing practices	[10,34,35]
Starter cultures	Defined and undefined starter consortia	Controlled acidification and early fermentation	Commercial cultures may carry accessory genes, but some studies report comparatively low ARG/MGE burdens	Major hosts for fermentation-disrupting phages; may contain prophages and defense systems	Strain-level characterization is required	[13,16,37,38]
Adjunct cultures	NSLAB, natural milk/whey starters, selected ripening cultures	Controlled diversification of ripening, aroma and functionality	Genetic composition varies according to strains used	Can alter the available phage–host population	Benefits and risks depend on strain selection and characterization	[37–39]

5. Dairy Processing as a Driver of Microbial and Genetic Network Reconfiguration

The microbial and genetic components introduced into the dairy chain do not remain unchanged during processing. Cooling, heat treatment, fermentation, ripening, packaging, and storage impose successive physicochemical and biological pressures that alter microbial survival, population structure, and interactions among microorganisms. These changes can also modify the resistance determinants associated with surviving populations, the relative representation of mobile genetic elements, and the availability of bacterial hosts for bacteriophages. Processing should therefore be regarded as a sequence of selective events rather than exclusively as a means of microbial reduction. The principal stage-dependent changes affecting the microbiome, resistome, mobilome, and phageome are represented conceptually in Figure 3, distinguishing heat-treated dairy products from those manufactured without milk heat treatment.

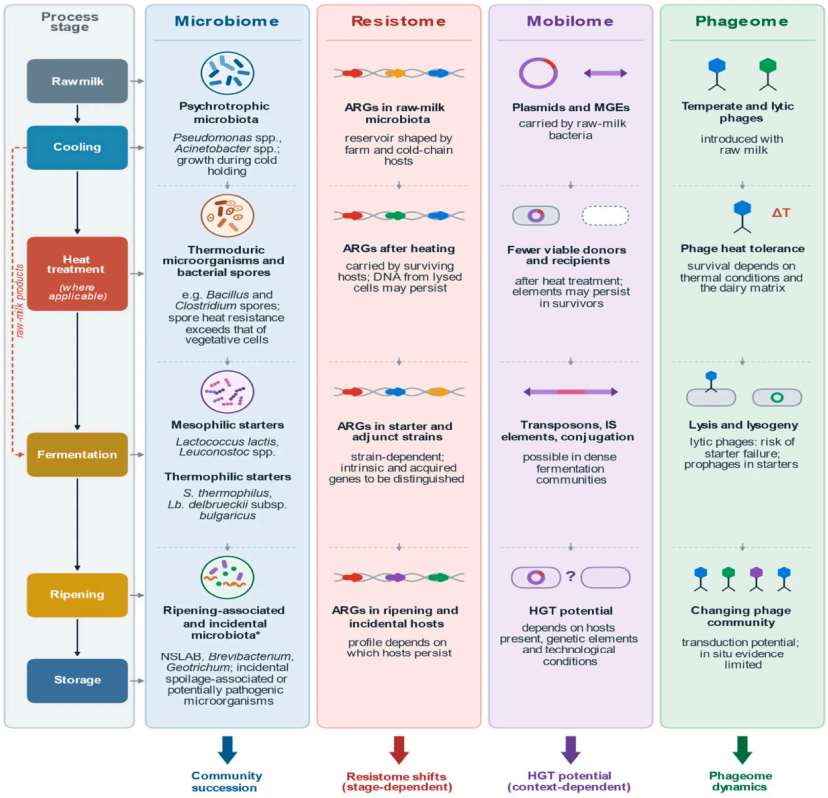


Figure 3. Dairy processing as a selective force shaping the microbiome–resistome–mobilome–phageome network. Conceptual representation of stage-dependent microbial and genetic changes during milk cooling, heat treatment (where applicable), fermentation, ripening, and storage. The diagram distinguishes psychrotrophic, mesophilic, thermophilic, thermoduric, and incidental microbiota and illustrates potential changes in antimicrobial resistance determinants, mobile genetic elements, and bacteriophage communities. The dashed pathway represents raw-milk products manufactured without heat treatment, while individual processing stages depend on the product and its technological history. The illustrated microbial–genetic interactions are context-dependent and do not imply experimentally demonstrated horizontal gene transfer at every stage. Links between columns indicate potential relevance, not interactions demonstrated at every stage. ARGs, antimicrobial resistance genes; MGEs, mobile genetic elements; HGT, horizontal gene transfer; IS, insertion sequence; NSLAB, non-starter lactic acid bacteria. Temperature-based categories (psychrotrophic, mesophilic, thermophilic) and origin-based incidental microbiota are not mutually exclusive.

Successive technological stages, from raw-milk cooling and heat treatment to fermentation, ripening, and storage, impose distinct selective pressures on dairy microbial communities and their associated genetic and viral components. Refrigerated storage can favor psychrotrophic populations, whereas heat treatment reduces susceptible vegetative cells and selects for surviving thermotolerant populations. Fermentation promotes starter-driven community restructuring, followed by further microbial succession during ripening and storage. These changes in community composition can be accompanied by shifts in the resistome, including the relative abundance and distribution of antimicrobial resistance genes (ARGs), and by changes in the mobilome, including plasmids, transposons, insertion sequences (IS), and opportunities for horizontal gene transfer (HGT). The phageome is also reshaped during processing through changes in host availability, lytic interactions, lysogeny, and phage-community composition. The scheme represents conceptual trends and potential interactions rather than quantitative changes at each processing stage.

5.1. Refrigerated Storage as a Pre-Processing Selective Pressure

Cooling of raw milk restricts the growth of many mesophilic microorganisms but does not prevent microbial activity. Prolonged refrigerated storage favors psychrotrophic populations capable of growth at low temperatures, particularly *Pseudomonas* spp., which are frequently associated with the deterioration of refrigerated raw milk and dairy products [40]. Quantitative evidence indicates considerable variability among studies: *Pseudomonas* spp. have been reported to account for approximately 65–70% of isolated psychrotrophic bacteria in some investigations, whereas a study of refrigerated raw milk reported a proportion of 17.9% of the psychrotrophic population [41,42]. These differences reflect the influence of sampling conditions and analytical approaches and indicate that the contribution of *Pseudomonas* cannot be represented by a single universal prevalence value. Beyond bacterial proliferation, psychrotrophic microorganisms may produce extracellular proteases and lipases with considerable heat stability, allowing their effects on milk proteins and lipids to persist even after subsequent thermal treatment [40].

The temperature at which raw milk is held before manufacture can also influence downstream microbial community development. Giagnoni et al. [43] evaluated raw-milk storage at 4, 7, and 9 °C under industrial conditions and subsequently examined the bacterial community of semi-fresh cheese manufactured using pasteurized milk and *Streptococcus thermophilus* starter cultures. Storage temperature was more strongly associated with cheese microbial composition than maturation time, and differences remained detectable despite subsequent pasteurization and starter inoculation. These findings suggest that pre-processing conditions may leave a detectable microbial legacy, even when milk undergoes thermal treatment before cheesemaking.

The consequences of psychrotrophic growth can extend beyond community composition. Chang et al. [44] recovered 106 *Pseudomonas* isolates from a pasteurized-milk production chain and found proteolytic, lipolytic, and biofilm-forming phenotypes in a substantial proportion of the isolates. Enzymatic activity was affected by incubation and thermal conditions, but simulated UHT treatment did not completely abolish the activity of some extracellular spoilage enzymes [44]. Thus, cooling acts simultaneously as a preservation step and as an ecological filter that may favor organisms whose metabolic products remain technologically relevant after the cells themselves have been reduced or eliminated.

5.2. Thermal Processing and Milk Pretreatment

Heat treatment produces one of the strongest reductions in viable microbial populations during dairy manufacture. Its effect, however, is selective rather than absolute. Susceptibility varies among vegetative cells, thermoduric organisms, bacterial spores, and bacteriophages,

while extracellular enzymes and microbial DNA may respond differently from viable cells. Consequently, the microbial or genetic signals detected after treatment do not necessarily represent the same biological state as those present before heating.

Pasteurization alters both abundance and community structure but can leave detectable populations of heat-tolerant microorganisms. Analysis of commercial pasteurized milk identified substantial bacterial diversity and enabled the recovery of thermoresistant lactic acid bacteria, demonstrating that heat treatment does not produce a microbiologically homogeneous matrix [45]. The effect on resistance-associated genetic content is similarly dependent on the initial community and treatment conditions. Metagenomic comparisons of dairy products have associated milk thermization and pasteurization with reduced ARG and MGE diversity relative to some raw-milk products, although resistance determinants can remain detectable after treatment [13,21]. Detection alone should not be interpreted as survival of an ARG-carrying viable cell, because extracellular DNA or DNA released from inactivated microorganisms may also contribute to sequencing signals.

Pretreatments applied before cheesemaking can further alter subsequent microbial development. Rodríguez et al. [46] compared industrial Herrgårds cheese production using microfiltration followed by pasteurization, pasteurization alone, and protein fortification followed by pasteurization. Pretreatment affected community development through manufacture and ripening, while microfiltration strongly reduced signals from several undesirable microorganisms, including *Clostridium tyrobutyricum* [46]. The communities continued to change after pretreatment, indicating that initial microbial reduction represents the beginning of subsequent selection rather than the final ecological state of the product.

Bacteriophages show additional variability. Thermal resistance differs considerably among dairy phages and according to the surrounding matrix, and commonly applied heat treatments cannot be assumed to inactivate all phage populations uniformly [16]. The effect of heating therefore extends across different layers of the network: it reduces susceptible microbial hosts, modifies the bacterial community supporting the resistome and mobilome, and changes but may not eliminate the phage reservoir.

The effects of refrigerated raw-milk storage should also be interpreted according to the production system. In farmstead cheese production, milk may be processed on-site shortly after milking, reducing intermediate handling and transportation steps, although on-farm storage conditions remain relevant. Conversely, raw milk processed at a separate dairy facility may undergo collection, refrigerated transportation, reception, and intermediate storage before manufacture. These operations may influence microbial community composition through temperature-dependent growth and potential environmental contamination, depending on equipment hygiene, handling practices, and storage duration. Importantly, raw-milk products should be distinguished from products manufactured using thermized or pasteurized milk, as thermal treatment introduces additional microbial selection pressures. Consequently, the microbiological effects of pre-processing storage cannot be generalized across different dairy production systems without considering their specific technological histories.

5.3. Fermentation and Starter-Driven Selection

Fermentation represents a transition from largely uncontrolled microbial inputs to strong selection driven by intentionally introduced cultures and by the physicochemical changes they generate. Starter growth lowers pH, consumes available substrates, produces inhibitory metabolites, and increases competition for ecological niches. These changes favor microorganisms adapted to the fermentation environment while restricting many members of the original raw-milk community.

Natural starter systems illustrate the extent to which fermentation conditions determine microbial assembly. In natural milk cultures used for Montasio PDO cheese, *Streptococcus thermophilus* was dominant across dairies, while subdominant populations varied among cultures; incubation temperature was identified as the principal factor associated with differences in biodiversity [47]. Functional correlations between specific microbial populations and organic acids, residual sugars, and volatile compounds also demonstrated that changes in community structure were associated with technological functions rather than representing taxonomic variation alone [47].

Starter addition can simultaneously influence other components of the microbial–genetic network. In metagenomic comparisons, cheeses produced from thermized milk with added starters contained less diverse ARG and MGE profiles than some raw-milk cheeses manufactured without starter cultures [13]. This association does not demonstrate that starter cultures directly eliminate resistance determinants; rather, starter-driven community selection may reduce or replace microbial hosts contributing to the original resistome and mobilome.

Starter dominance also modifies the host population available to bacteriophages. Yu et al. [48] followed phage populations through industrial Gouda-type cheese manufacture and reported substantial stage-dependent changes in phage diversity and abundance, including phages associated with starter bacteria [48]. The findings indicate that fermentation can simultaneously select bacterial hosts and the viruses capable of infecting them. This reciprocal selection is particularly relevant in undefined or multispecies cultures, where phage susceptibility, bacterial competition, and strain replacement may occur at the same time.

Fermentation should therefore be considered both a technological operation and an ecological restructuring event. The resulting community provides the biological basis for subsequent ripening and determines, to a considerable extent, which bacterial hosts, resistance determinants, mobile elements, and bacteriophages remain available for later stages.

5.4. Ripening as Microbial and Functional Succession

Ripening imposes prolonged selection under conditions of decreasing water activity, changing pH, salt exposure, nutrient depletion, oxygen gradients, and accumulation of microbial metabolites. These conditions generate both temporal and spatial succession, particularly in cheeses where rind, under-rind, and core provide distinct ecological niches.

In Asiago PDO cheese, microbial composition differed between dairies and changed during manufacture and ripening. Starter selection affected subsequent non-starter lactic acid bacteria, while metagenomic analysis connected specific taxa with amino-acid metabolic and catabolic pathways relevant to flavour development [49]. Similar multi-omics analysis of Apulian Caciocavallo showed stage- and location-dependent differences among rind, under-rind, and core communities, accompanied by changes in metabolite profiles [50]. These observations indicate that ripening represents functional succession as well as taxonomic succession.

The processing chain can continue to influence mature cheese long after initial fermentation. In Gouda cheese produced from pasteurized milk, microbial and metabolite profiles changed during ripening for up to 100 weeks, and cheese brine was identified as a source of particular non-starter lactic acid bacteria associated with subsequent metabolic activity [51]. Thus, microbial assembly during maturation reflects not only starter populations but also earlier processing reservoirs and selection occurring after curd formation.

Resistome and phageome profiles can change concurrently with bacterial succession. ARG profiles in cheese have been associated with ripening period and initial microbiota [21],

while viral metagenomics of smear-ripened cheese demonstrated a transition from phages associated with early bacterial populations toward phages linked to ripening organisms [25]. These parallel changes are important because the biological significance of a resistance determinant or phage depends partly on the presence and abundance of its microbial host. Ripening can therefore alter not only the individual layers represented in Figure 2 but also the connections among them.

5.5. Storage, Packaging, and Post-Processing Recontamination

Microbial selection continues after manufacture. Refrigerated storage favors organisms capable of persistence or growth under low-temperature conditions, while package atmosphere, oxygen availability, carbon dioxide concentration, and residual substrate availability further affect community development. Post-processing contamination can introduce additional microorganisms after earlier reduction steps, particularly when filling, cutting, portioning, or packaging exposes the product to equipment and environmental surfaces.

Metagenomic analysis of pasteurized milk stored for 14 days at 4 °C showed a marked succession of the residual microbiota. *Acinetobacter* and *Corynebacterium* were prominent during earlier storage, while *Pseudomonas* progressively increased and became dominant during later refrigeration. Predicted functional profiles also changed with the community, including carbohydrate- and amino-acid-associated functions [52]. The result illustrates that a low-abundance population surviving or entering after pasteurization can become technologically dominant under favorable storage conditions.

Packaging modifies this selection. Renoldi et al. [53] compared vacuum and modified-atmosphere packaging during 120 days of refrigerated storage of portioned PDO semi-hard cheese. Packaging affected spoilage-microorganism proliferation and volatile production, while vacuum packaging restricted yeast proliferation more effectively under the experimental conditions [53]. In Stelvio PDO cheese, modified-atmosphere and vacuum packaging also produced different volatilome trajectories, and volatile compounds were strongly associated with yeasts and non-starter lactic acid bacteria [54]. These results support the interpretation of packaging as an additional ecological filter rather than an inert final step.

Long shelf life does not eliminate microbiological influence even in products subjected to severe heat treatment. Analysis of normal and deteriorated UHT milk identified *Pseudomonas*, *Streptococcus*, and *Acinetobacter* among core taxa associated with product condition. In deteriorated samples, *Pseudomonas* abundance correlated with heat-resistant protease content, whereas *Acinetobacter* was associated with heat-resistant lipase activity [55]. Such findings must be interpreted cautiously because detection of bacterial DNA in UHT products does not establish viability of the detected taxa. They nevertheless emphasize that product deterioration can reflect microbial activity occurring before heat treatment, residual enzymatic activity, post-process contamination, or combinations of these mechanisms.

Processing environments therefore remain relevant after the principal lethality steps. Studies of on-farm processing plants and cheese facilities have demonstrated microbial persistence on equipment and environmental surfaces and have identified processing-associated ARG reservoirs [11,34,36]. Recontamination can consequently reintroduce organisms into a product whose microbial community was previously reduced. The final microbiological state of a dairy product is therefore determined by the cumulative sequence of selection, removal, persistence, and reintroduction occurring throughout the production chain.

Cooling, pretreatment, heat processing, fermentation, ripening, packaging, and storage do not act independently on the dairy microbial system. Each operation changes the community inherited by the following stage and can thereby alter the microbial hosts carrying resistance determinants, the genetic elements available for transfer, and the bacteriophages

able to propagate. As represented in Figure 2, microbial reduction at one stage does not imply elimination of the wider microbial–genetic network. This stage-dependent reconfiguration provides the basis for a more detailed consideration of the resistome–mobilome axis, particularly the distinction between gene detection, genomic mobility, and experimentally demonstrated transfer.

6. The Resistome–Mobilome Axis: From Detection to Transfer Potential

Antimicrobial resistance in dairy ecosystems cannot be interpreted solely from the number or diversity of antimicrobial resistance genes (ARGs) detected in a sample. The biological significance of a resistance determinant depends on the microorganism carrying it, its chromosomal or extrachromosomal location, association with mobile genetic elements (MGEs), expression, and ability to move between bacterial hosts. Recent dairy studies increasingly combine phenotypic susceptibility testing, metagenomics, whole-genome sequencing, long-read sequencing, and transfer assays to resolve these relationships [14,15,23]. These approaches provide different levels of evidence and should not be interpreted as equivalent. Detection establishes genetic presence, whereas dissemination requires additional genomic and biological conditions.

6.1. Major Resistance Determinants and Their Dairy-Associated Hosts

Resistance determinants reported in dairy-associated microorganisms encompass several antimicrobial classes, including β-lactams, tetracyclines, macrolides, aminoglycosides, sulfonamides, trimethoprim, phenicols, glycopeptides, and fluoroquinolones [14,15]. Their occurrence differs among microorganisms, dairy matrices, production environments, and geographical regions. The same phenotypic resistance may also originate from intrinsic characteristics, acquired resistance genes, chromosomal mutations, or combinations of these mechanisms. Identification of the microbial host is therefore necessary for interpreting resistome data.

Enterococci illustrate this complexity because they are frequently recovered from animals, milk, cheeses, and dairy-processing environments but differ considerably in their resistance profiles and genomic backgrounds. Long-read whole-genome sequencing of enterococci from dairy cattle farms allowed Ocejo et al. [56] to distinguish *Enterococcus lactis* from the closely related *E. faecium*. Resistance determinants were distributed differently among *E. faecalis*, *E. faecium*, and *E. lactis*, demonstrating that species- and strain-level resolution can substantially alter the interpretation of dairy-associated AMR reservoirs [56].

The same principle applies to Enterobacteriaceae. Complete genome analysis of multidrug-resistant *Escherichia coli* isolated from dairy cattle feces identified determinants associated with β-lactam, aminoglycoside, tetracycline, sulfonamide, trimethoprim, and fluoroquinolone resistance within IncY plasmids. These plasmids also contained insertion sequences, transposons, integron-associated regions, and genes related to conjugative transfer [57]. Such genomic organization is compatible with mobility, but it should not be considered direct evidence that transfer occurs under dairy-production conditions.

Changes in bacterial populations during manufacture may also modify the resistance determinants detected in the final product. Santamarina-García et al. [58] characterized more than 200 lactic acid bacterial isolates along an artisanal raw ewe-milk PDO cheese production chain and reported changes in both LAB populations and the distribution of ARGs and MGEs between feces, raw milk, whey, fresh cheese, and ripened cheese. The findings indicate that technological selection can modify the resistant bacterial population entering and persisting through production [58].

Dairy enterococci may simultaneously carry ARGs and markers associated with mobile elements. Vinchurkar et al. [59] detected resistance determinants associated with amino-

glycosides, tetracyclines, macrolides, and chloramphenicol together with transposon- and integron-related markers in *Enterococcus* isolates from dairy niches. The co-occurrence of ARGs and MGEs increases the relevance of genomic context, although it does not demonstrate that the corresponding determinants are transferable.

Experimental studies provide stronger evidence. Morandi et al. [60] compared enterococci isolated from bovine raw milk and feces and found distinct population structures between the two sources. Selected *E. faecalis* strains carrying *tetM* were able to transfer this determinant to *Lactobacillus delbrueckii* subsp. *lactis* under experimental conditions, whereas transfer was not observed for all tested strains [60]. Transfer potential is therefore strain-dependent and cannot be inferred from the presence of an ARG alone.

6.2. Chromosomal Versus Mobile Resistance: Why Genetic Context Matters

Resistance determinants located on chromosomes and those carried by plasmids, transposons, integrons, integrative elements, or other MGEs do not have the same potential for dissemination. Chromosomal determinants are primarily inherited vertically, whereas resistance genes associated with transferable elements may move between genetically distinct bacterial hosts under appropriate conditions. Establishing genomic location is therefore an important step between ARG detection and assessment of mobility.

Long-read sequencing is particularly valuable for this purpose because repetitive sequences, plasmids, and composite resistance regions may be difficult to reconstruct accurately using short-read data alone. In raw-milk *E. coli* isolates from Gujarat, Mangroliya et al. [61] used Nanopore whole-genome sequencing and identified plasmid-associated resistance genes including *bla*TEM-1, *sul2*, *dfrA14*, *aph*(6)-Id, *aph*(3″)-Ib, and *tet(B). IncF-group plasmids, insertion sequences, and the transposons Tn2 and Tn10 were also identified [61]. Such information provides considerably greater biological resolution than detection of the same ARGs by targeted PCR alone.

Host assignment represents another important stage of interpretation. Whole-genome metagenomic analysis of milk from cows with subclinical mastitis enabled reconstruction of near-complete genomes belonging to several bacterial species, including *Pseudomonas veronii*, *Staphylococcus xylosus*, *E. coli*, *Klebsiella pneumoniae*, and *Staphylococcus hominis*, together with resistance and virulence determinants associated with these genomic backgrounds [62]. Genome-resolved approaches can therefore connect ARGs with putative bacterial hosts without prior cultivation, although assembly quality and linkage of MGEs to hosts remain methodological constraints.

Whole-genome sequencing can also resolve lineages that are obscured at the species level. Zhou et al. [63] characterized *Staphylococcus aureus* isolates from bulk-tank milk and identified multiple MLST–*spa* lineages, resistance determinants, plasmid replicons, and differences in antimicrobial susceptibility. Livestock-associated MRSA was identified within the sampled population, although the authors noted that findings from a geographically restricted dataset should not be generalized beyond the studied production system [63].

Culture-based surveillance combined with susceptibility testing and targeted PCR remains useful, particularly where genomic resources are limited. Khan et al. [64] identified resistance determinants including *mecA*, *vanA*, *bla*TEM, *bla*CTX-M-15, *qnrS*, and *aac*(6′)-Ib-cr in *S. aureus* and *E. coli* isolates recovered from raw milk. Such approaches provide information on local resistance patterns but generally offer less detail on ARG location, host-genome context, and transfer potential than complete genome or plasmid-resolved sequencing [64].

The evidence obtained therefore depends strongly on analytical resolution. Detection of an ARG indicates its presence; assignment to a bacterial host provides additional biological context; localization on a plasmid or another MGE suggests mobility; and identification of

transfer-associated machinery strengthens this inference. None of these observations alone demonstrates that transfer has actually occurred.

6.3. Horizontal Gene Transfer in Dairy Systems

Horizontal gene transfer occurs through conjugation, transformation, and transduction. The biological requirements and available evidence differ among these mechanisms, and their relative contribution to ARG dissemination in dairy environments remains unevenly characterized.

Conjugation currently provides some of the clearest experimental evidence of resistance-gene mobility in dairy-associated bacteria. Xu et al. [23] combined metagenomic sequencing with bacterial isolation, antimicrobial susceptibility testing, PCR, biofilm assays, and conjugation experiments across 539 samples obtained from 42 dairy farms. Transfer of *bla*SHV from *Klebsiella pneumoniae* to *E. coli* was experimentally demonstrated, confirming that at least some resistance determinants identified within dairy-production systems can move between bacterial hosts [23]. The transfer of *tetM* from *E. faecalis* to *L. delbrueckii* subsp. *lactis* reported by Morandi et al. [60] provides a second example involving bacteria directly relevant to dairy ecosystems.

Environmental conditions can further modify conjugative transfer. Ma et al. [65] investigated ESBL-producing *E. coli* recovered from raw milk and dairy-farm environments and established both *E. coli*–*E. coli* and *E. coli*–*K. pneumoniae* conjugation models. Under the tested conditions, low-to-moderate Cu^{2+} exposure increased intrageneric transfer frequency by approximately two-fold, while the same response was not observed in the intergeneric model. Changes in oxidative stress, cellular aggregation, membrane properties, and surface charge were associated with this effect [65]. These findings indicate that transfer potential is influenced not only by the ARG or plasmid involved but also by environmental conditions acting on donor and recipient cells.

Evidence for natural transformation in dairy systems is less direct. Extracellular DNA containing ARGs and integron-associated markers has been detected in dairy-farm environments [32], demonstrating that extracellular resistance-associated genetic material can persist outside viable bacterial cells. However, extracellular DNA represents only a potential substrate for transformation. Detection does not establish uptake by a competent recipient, genomic incorporation, or subsequent expression. The contribution of natural transformation to resistance dissemination in dairy matrices therefore remains less clearly demonstrated than conjugation.

Transduction provides a mechanistic connection between the mobilome and phageome. ARGs have been detected in viral fractions obtained from dairy products, and some ARG-containing phage particles retained infectivity under laboratory conditions [26]. Nevertheless, an infective viral particle containing a resistance determinant does not establish successful transduction of that determinant to another bacterial host. Direct evidence of ARG transfer by phages under dairy-processing or product conditions remains limited.

6.4. From ARG Detection to Demonstrated Transfer: A Hierarchy of Evidence

Differences in molecular and experimental approaches mean that apparently similar reports of antimicrobial resistance may represent substantially different levels of biological evidence. Metagenomics can detect antimicrobial resistance genes (ARGs) without necessarily resolving their bacterial hosts or genomic locations. Genome-resolved analysis can associate ARGs with bacterial chromosomes or mobile genetic elements (MGEs), while long-read sequencing can improve the reconstruction of plasmids and complex resistance regions. However, genomic association does not establish functional mobility, and experi-

mental transfer assays are required to determine whether a resistance determinant can be acquired by a recipient organism.

The methodological challenges associated with reconstructing plasmids and identifying their bacterial hosts extend beyond dairy ecosystems. A global analysis of metaplasmidomes across multiple environments demonstrated considerable plasmid diversity, incomplete representation in reference databases, and the occurrence of ARG-carrying plasmids across different microbial hosts and ecological settings [66]. These findings highlight the limitations of short-read sequencing and reference-dependent approaches for resolving ARG–plasmid–host relationships. Similar interpretive challenges arise in dairy-associated enterococci, where intrinsic and acquired resistance determinants coexist. A recent review identified substantial variation in antimicrobial resistance profiles according to bacterial species, dairy matrices, geographical origin, and analytical methodology, emphasizing the importance of distinguishing intrinsic resistance from acquired determinants with potential for horizontal dissemination [67].

Recent mobility-aware and genomics-informed approaches have emphasized that ARG occurrence alone is insufficient for assessing antimicrobial resistance dissemination. Cheng et al. [68] proposed integrating genomic information, genetic mobility, transmission pathways, clinical relevance, and evidence uncertainty into AMR risk assessment. Similarly, Klümper et al. [69] emphasized the incorporation of ARG mobility into environmental surveillance and risk assessment. Building upon these established approaches, the present review organizes dairy-associated evidence into six interpretive levels that distinguish molecular identification, genomic context, inferred transfer potential, and experimentally demonstrated horizontal gene transfer. Unlike broader AMR risk-assessment frameworks, this hierarchy specifically emphasizes the transition from genomic evidence to transfer demonstrated under conditions representative of dairy production and processing.

For interpretive purposes, evidence supporting ARG mobility is organized into six levels of increasing biological support:

Level 1—ARG detection: A resistance determinant is identified using PCR, metagenomics, or another molecular method, without necessarily establishing its bacterial host, genomic location, expression, or transferability.

Level 2—Host assignment: The ARG is reliably associated with a bacterial species, strain, or reconstructed genome, providing information on its microbial reservoir but not necessarily its genomic mobility.

Level 3—Mobile genetic context: The ARG is localized on, or supported by convincing sequence-level linkage to, a plasmid, transposon, integrative element, or another potentially mobile genetic structure. Detection of ARGs and MGE markers in the same sample, without demonstrated linkage, is insufficient for this level.

Level 4—Genetic transfer potential: The ARG-containing genetic structure possesses identifiable mobility-associated features, such as conjugation or mobilization machinery, compatible with a plausible mechanism of horizontal transfer. Such genomic evidence supports transfer potential but does not demonstrate that transfer occurs.

Level 5—Experimental transfer: Acquisition of the resistance determinant by a recipient organism is experimentally demonstrated under controlled in vitro or ex vivo conditions and supported by appropriate phenotypic and/or molecular confirmation.

Level 6—Transfer under representative dairy conditions: Horizontal transfer is experimentally demonstrated within a dairy matrix or under conditions that reproduce relevant features of dairy production, processing, fermentation, ripening, or storage, with confirmation of recipient acquisition and appropriate experimental controls.

These levels represent an evidence hierarchy rather than a validated quantitative risk-scoring system. They are not intended to assign numerical risk values, predict transfer

probabilities, or establish thresholds for microbiological safety. Furthermore, the levels should not be interpreted as a universal sequence of experimental procedures, since different analytical approaches may provide complementary evidence. Their purpose is to distinguish the strength of available evidence for genetic mobility and realized transfer, while recognizing that the public-health relevance of an ARG also depends on its clinical importance, microbial host, expression, persistence, and potential exposure pathways. Accordingly, a higher evidence level does not necessarily indicate a greater antimicrobial resistance risk.

The specific contribution of the proposed hierarchy lies in its application to dairy-associated microbial ecosystems, where processing conditions may substantially modify bacterial viability, community composition, and opportunities for horizontal gene transfer. In particular, Levels 3 and 4 distinguish genetic association from inferred transfer potential, Level 5 requires experimental demonstration of transfer, and Level 6 extends this evidence to technologically and ecologically relevant dairy conditions. This distinction is important because transfer demonstrated in laboratory culture cannot automatically be extrapolated to milk, whey, curd, or cheese, where physicochemical conditions and microbial interactions may influence donor–recipient contact, survival, and transfer efficiency.

Although Level 6 represents the highest context-specific level of evidence within the proposed hierarchy, its achievement remains insufficiently documented in the dairy studies considered in this review. Nevertheless, such investigations are experimentally feasible in principle. A suitable design would involve characterized donor and recipient strains, an identified ARG-containing genetic element, and controlled experiments conducted in representative dairy matrices. Relevant experimental conditions should reflect the technological process being investigated, including temperature, pH, water activity, salt concentration, fermentation or storage duration, and the composition of the surrounding microbial community. Depending on the research objective, experiments could examine transfer during milk storage, fermentation, curd formation, cheese ripening, or within dairy-associated biofilms.

Reliable demonstration of transfer would require recovery and identification of recipient-derived transconjugants or other confirmed transfer products, followed by molecular or genomic verification of ARG acquisition. Appropriate donor-only, recipient-only, and other mechanism-specific controls would be necessary to exclude contamination, donor carryover, and misinterpretation of recipient growth. Where feasible, longitudinal sampling and determination of transfer frequencies relative to recoverable donor and recipient populations would provide additional quantitative information. The use of representative dairy matrices and defined processing conditions would strengthen ecological relevance, although laboratory simulations would still require careful consideration before extrapolation to industrial production systems.

Consequently, Level 6 should be regarded as a clearly defined experimental objective rather than a level routinely achieved in current dairy AMR research. Its inclusion highlights the evidence gap between demonstrating transfer under simplified laboratory conditions and establishing whether comparable events occur under conditions relevant to dairy manufacture. Addressing this gap would improve the interpretation of ARG persistence and dissemination across dairy production chains and provide more appropriate evidence for subsequent exposure assessment and quantitative microbial risk assessment.

Representative dairy-associated studies and the corresponding strength of evidence are summarized in Table 2.

The studies summarized in Table 2 illustrate the substantial differences between detecting resistance determinants, resolving their genetic contexts, and experimentally demonstrating horizontal transfer. Molecular and genomic approaches provide extensive information

on ARG occurrence and association with microbial hosts or potentially mobile elements, whereas studies directly confirming transfer are less common. Importantly, the available examples do not establish that transfer demonstrated under controlled conditions necessarily occurs in representative dairy matrices. Interpretation must therefore remain specific to the evidence generated by each study, particularly when distinguishing ARG–MGE co-occurrence from confirmed genetic linkage and functional mobility.

Table 2. Representative dairy-associated ARG–MGE–host relationships and strength of evidence supporting antimicrobial resistance mobility.

Iry Source or Matrix	Host/Bacterial Group	Resistance Determinant(s)	Genetic Context/Evidence	Experimental Transfer	Evidence Level *	References
Intensive dairy farms/raw milk	*Klebsiella pneumoniae*	*blaSHV*	ARG identified in a characterized dairy resistome and evaluated by conjugation	Yes; transfer to *E. coli*	5	[23]
Bovine raw milk	*Enterococcus faecalis*	*tetM*	ARG assigned to the bacterial host; strain-dependent transfer phenotype	Yes; transfer to *L. delbrueckii* subsp. *lactis*	5	[60]
Dairy cattle farms	*E. faecalis*, *E. faecium*, *E. lactis*	Multiple resistance determinants	Long-read WGS with plasmid-level characterization	Not tested directly	3–4	[56]
Dairy cattle feces	*Escherichia coli*	*tet(A)*, *aph* genes, *blaTEM-1*, *blaCTX-M-15*, *sul2*, *qnrS1*, *dfrA14*	IncY plasmids, insertion sequences, transposons, integron-associated regions, and conjugation-associated genes	Not directly demonstrated	4	[57]
Raw ewe-milk cheese production chain	LAB	Multiple resistance determinants	ARGs and MGE markers detected by HT-qPCR across successive production stages; direct ARG–host and ARG–MGE linkage not established.	Not tested	1–3	[58]
Dairy niches	*Enterococcus* spp.	Aminoglycoside-, tetracycline-, macrolide-, and chloramphenicol-associated genes	ARGs detected together with transposon- and integron-associated markers; direct genetic linkage requires confirmation	Not tested	To be verified	[59]
Raw milk	*E. coli*	*blaTEM-1*, *sul2*, *dfrA14*, *aph(6)-Id*, *aph(3″)-Ib*, *tet(B)*	Plasmids, Tn2, Tn10, and multiple insertion sequences resolved by WGS	Not tested	3–4	[61]
Raw milk/dairy-farm environment	ESBL-producing *E. coli*	ESBL-associated ARGs	Plasmid-associated resistance investigated using conjugation models under Cu^{2+} exposure	Yes; intrageneric and intergeneric conjugation tested	5	[65]
Dairy products, viral fraction	Phage-associated fraction	Multiple ARGs	ARGs detected in viral fractions; selected particles retained infectivity; ARG localization on transferable phage genomes not established	ARG transfer not demonstrated	To be verified	[26]

* Evidence levels: 1, ARG detection; 2, reliable host assignment; 3, confirmed or strongly supported ARG–MGE linkage; 4, genomic evidence of transfer potential; 5, experimentally demonstrated transfer under controlled conditions; 6, transfer demonstrated under representative dairy conditions. Each study was assigned a single level based on the highest level explicitly supported for the specific ARG–host–genetic element relationship summarized. Where evidence was heterogeneous or insufficient to establish a higher level, a conservative classification was applied. These levels describe the strength of biological evidence and do not constitute quantitative AMR risk scores.

Overall, the resistome–mobilome relationship should be interpreted through complementary lines of molecular, genomic, and experimental evidence rather than ARG abundance alone. Identifying viable microbial hosts, resolving ARG-containing genetic structures, determining whether transfer machinery is present, and experimentally assessing transfer under relevant conditions are essential for establishing the biological significance of resistance determinants in dairy ecosystems. This evidence-based perspective also provides a foundation for integrating the phageome, since bacteriophages may influence bacterial

community structure and, under appropriate circumstances, participate in genetic exchange through transduction. However, phage-associated ARG detection must remain distinct from experimentally demonstrated phage-mediated transfer.

7. The Dairy Phageome: Technological Threat, Ecological Regulator, Genetic Actor, and Biocontrol Tool

Bacteriophages represent a distinct component of dairy microbial ecosystems because their effects depend directly on the presence, abundance, and physiological state of susceptible bacterial hosts. In industrial fermentations, infection of starter cultures can delay acidification or cause fermentation failure, whereas in complex cheeses phages may contribute to microbial succession through selective lysis. Temperate phages additionally interact with bacterial genomes through lysogeny, while strictly lytic phages directed against undesirable microorganisms can be deliberately applied as biocontrol agents. The dairy phageome should therefore not be considered exclusively as a source of technological failure, but as a dynamic component connecting microbial ecology, bacterial evolution, genetic exchange, and food-safety interventions [16,24,25,48].

7.1. Phages as Technological Hazards and Persistent Dairy Reservoirs

The industrial impact of dairy phages is most apparent when virulent phages infect lactic acid bacteria used as starter cultures. High starter-cell densities, repeated use of genetically similar strains, and continuous availability of susceptible hosts provide favorable conditions for phage amplification. Whey, brines, production surfaces, aerosols, raw materials, and processing residues can subsequently contribute to phage dissemination between production cycles [16].

Long-term surveillance demonstrates that phage populations can remain associated with individual processing facilities for extended periods. Jolicoeur et al. [70] examined whey samples collected during Cheddar production in a Canadian cheese factory from 2001 to 2020. Phages were isolated from 932 samples, and molecular characterization identified at least 241 distinct lactococcal phages. Although many were detected only once, numerous phages reappeared over time, including individual types recovered over multiple years. The study also showed the practical value of factory-specific phage monitoring for adjusting starter-culture rotation [70].

A similar pattern has recently been documented for *Streptococcus thermophilus*. Marangon et al. [71] characterized 25 phages isolated over 15 years from a yogurt-production facility and identified members of both *Moineauvirus* and *Brussowvirus*. Persistence was associated with differences in host range, genomic characteristics, and resistance to environmental stresses. The study also detected anti-CRISPR-associated features and substantial genomic variation among persistent phages, illustrating that industrial phage populations continue to evolve during long-term exposure to starter strains and control measures [71].

Whey constitutes an especially relevant reservoir because large phage populations generated during fermentation may enter the whey fraction following curd separation. Subsequent concentration, membrane processing, and drying do not necessarily eliminate all infectious particles. A recent review focused specifically on whey-powder manufacture concluded that phages can survive multiple processing operations and that contaminated whey-derived ingredients may reintroduce phages into later fermentations when reused in dairy manufacture [72]. These observations connect circular reuse of dairy side streams with the need for specific phage-management procedures.

The resistance of individual phages to technological treatments can differ markedly. Herasimovich et al. [73] characterized the virulent *Lactococcus* Ceduovirus BIM BV-114 isolated from industrial cheese brine. Conventional pasteurization conditions were in-

sufficient for complete inactivation, while complete loss of infectivity in skimmed milk required considerably more severe heating; the matrix also protected the phage against UV-C compared with buffer conditions [73]. The results support the broader conclusion that phage resistance cannot be predicted from bacterial lethality parameters and that the suspending dairy matrix affects inactivation [16].

Nonthermal control methods are consequently receiving increased attention. Jacob et al. [74] evaluated UV-C inactivation of 16 dairy phages deposited on dry surfaces and observed phage-dependent kinetics, including tailing at higher doses for several isolates. Thus, even when a treatment produces substantial reductions, residual infective particles may remain and later encounter susceptible starter bacteria [74]. Together with the stage-dependent phage changes reported during Gouda-type cheese production [48], these findings support phage management based on multiple barriers rather than reliance on a single thermal or sanitation step.

7.2. Phage–Host Recognition, Bacterial Defense, and Co-Evolution

Phage infection begins with recognition of a compatible bacterial host. Consequently, phage susceptibility is generally a strain-level characteristic rather than a property that can be assigned uniformly to a bacterial species. Chandran et al. [75] isolated *Limosilactobacillus fermentum* strains from traditional dahi and tested them against a lytic phage recovered from dairy effluent. Ten of eleven strains were resistant, whereas one was susceptible, illustrating the substantial intra-species variability relevant to starter selection [75]. Host recognition depends on specific interactions between phage receptor-binding structures and bacterial surface molecules. Kampff et al. [76] demonstrated that the cell-surface rhamnose-glucose polysaccharide of *S. thermophilus* functions as the receptor for bacteriophage P738. Mutations affecting a glycosyltransferase within the *rgp* locus modified the terminal structure of this polysaccharide and reduced phage binding and infection [76]. Such findings provide a molecular explanation for differences in host range and show how relatively small changes in bacterial surface structures can modify phage susceptibility.

Once infection has been initiated, dairy bacteria can deploy multiple defense mechanisms. In *Lactococcus*, Grafakou et al. [77] functionally characterized three plasmid-encoded systems, Rhea, Aristaios, and Kamadhenu, and confirmed their activity against *Skunavirus* phages in milk-based conditions. Transfer of defense-associated plasmids can therefore modify the phage-resistance phenotype of industrial strains, providing a direct connection between the mobilome and phageome [77].

The defensive repertoire of *S. thermophilus* is even broader than the well-characterized CRISPR-Cas and restriction-modification systems. Kelleher et al. [78] analyzed 27 strains and identified multiple additional defense systems, including Kiwa, Gabija, Dodola, sirtuin-associated systems, and abortive-infection mechanisms. Up to approximately 2.5% of the analyzed genomic content was associated with predicted phage defense, indicating substantial evolutionary investment in resistance to viral predation [78].

CRISPR spacer content also records previous interactions with foreign genetic material. Analysis of 11 *S. thermophilus* strains isolated from traditional yogurts identified 21 CRISPR loci, and spacer sequences showed matches to both phages and plasmids [79]. CRISPR-based immunity therefore illustrates another point of contact between the phageome and mobilome: the same defense machinery can record and respond to different classes of invading DNA [79].

The interaction remains evolutionary rather than static because phages develop mechanisms that circumvent host defenses. Pastuszka et al. [80] demonstrated that the phage-encoded AcrIIA3 protein inhibits the CRISPR3-Cas system of *S. thermophilus*, adding to other anti-CRISPR mechanisms already described in dairy streptococcal phages [80]. More

recent genome-scale analysis extended this relationship considerably. Leprince et al. [81] examined 263 *S. thermophilus* genomes, identified 28 defense systems, and experimentally tested 18 systems against dairy phages. Seventeen showed activity against at least one streptococcal phage, while combinations of accessory defenses with CRISPR increased resistance against particular phage groups. In parallel, bioinformatic analysis identified six families of known anti-CRISPR proteins targeting type II-A CRISPR-Cas systems in 40% (77/191) of the analyzed Streptococcus thermophilus phage genomes, with AcrIIA6 being the most prevalent (36%, 69/191) [81].

These observations indicate an ongoing cycle of host defense and viral counter-defense. CRISPR spacer acquisition, restriction-modification, abortive-infection systems, receptor modification, and newly described defense islands can reduce infection, while phages may escape through receptor adaptation, mutation of CRISPR targets, altered restriction-site composition, or anti-CRISPR proteins [77,80–82]. The resulting co-evolution is technologically relevant because starter cultures that are resistant to currently circulating phages may not remain resistant indefinitely. The use of strain rotation, continuous phage monitoring, and complementary defense systems is therefore more appropriate than reliance on a single resistance mechanism [70,81,82].

7.3. Phages as Ecological Regulators and Mediators of Genetic Exchange

Bacteriophages influence dairy microbial ecosystems through selective infection, bacterial lysis, lysogeny, and, under appropriate conditions, genetic exchange. Their ecological significance therefore extends beyond their abundance or taxonomic diversity and depends on interactions with bacterial hosts, resistance determinants, and mobile genetic elements. These relationships provide a mechanistic connection between the dairy phageome, microbiome, resistome, and mobilome.

Metaviromic investigations have revealed complex and dynamic phage–host associations in dairy products. In artisanal Canastra cheese, more than 1200 viral operational taxonomic units were identified, together with strain-level associations between phages and bacterial members of the cheese microbiome [24]. Similarly, longitudinal analysis of smear-ripened cheeses demonstrated changes in viral populations accompanying microbial succession, with phages associated with *Lactococcus* decreasing during maturation and viruses predicted to infect ripening-associated bacteria becoming more prominent [25]. Comparable processing-dependent changes have been reported in Gouda-type cheese [48]. These findings support the ecological relevance of phage–host associations, although correlations and computational host predictions do not independently demonstrate infection or causal effects on microbial succession.

Selective bacterial lysis may alter the relative abundance of microbial hosts and consequently influence the distribution of resistance determinants within dairy communities. However, a reduction in ARG-associated bacterial populations does not necessarily indicate a corresponding reduction in genetic dissemination potential. Such interpretation requires information on the identity and viability of surviving hosts, the genomic location of resistance determinants, and their association with potentially mobile elements. Therefore, phage-induced changes in microbial community composition and changes in the resistome must be evaluated as related but distinct biological outcomes.

Temperate bacteriophages introduce an additional genetic dimension through lysogeny. Following infection, phage genomes may be maintained as prophages within bacterial chromosomes or, in some phage systems, as extrachromosomal elements. Prophage carriage can influence bacterial physiology, host susceptibility to subsequent phage infection, and interactions with other genetic elements. Under suitable conditions, prophage induction may initiate productive phage replication, potentially resulting in bacterial lysis and changes

in microbial community structure. Nevertheless, prophage identification alone does not establish induction, genetic mobilization, or horizontal transfer.

The relationship between the phageome and mobilome becomes particularly relevant through transduction. Generalized transduction involves the packaging and transfer of bacterial DNA during phage propagation, whereas specialized transduction can involve bacterial sequences associated with prophage excision. Additional phage-mediated mobilization mechanisms may also contribute to bacterial DNA transfer, depending on the phage–host system. These mechanisms provide biologically plausible pathways through which resistance-associated genetic material could move between bacterial hosts. However, their occurrence and quantitative contribution under dairy-processing conditions remain insufficiently established.

The distinction between genetic carriage and functional transfer is essential. ARGs have been detected in viral fractions recovered from dairy products, and selected ARG-associated phage particles have retained infectivity under experimental conditions [26]. Nevertheless, these observations do not establish that the detected ARGs were packaged in transferable phage genomes or successfully introduced and maintained in recipient bacteria. Demonstrating phage-mediated ARG transfer would require experimentally resolved donor–phage–recipient relationships, confirmation of ARG acquisition by the recipient, and appropriate controls excluding contamination or alternative transfer mechanisms.

Taken together, these interactions indicate that phages may influence dairy microbial quality and safety through two distinct but interconnected processes: ecological selection of bacterial populations and potential genetic exchange. Integrating phage–host interactions with ARG localization, MGE characterization, and experimental transfer evidence is therefore necessary to distinguish changes in microbial community structure from changes in resistance-dissemination potential. This distinction also provides a basis for evaluating the potential benefits and limitations of phage-based biocontrol interventions.

7.4. Phage-Based Biocontrol: Efficacy and Ecological Implications

The host specificity of bacteriophages offers opportunities for targeted control of pathogenic and spoilage-associated bacteria in dairy products. However, phage-based interventions should be evaluated not only in terms of pathogen reduction but also in relation to their effects on microbial community structure, host susceptibility, and genetic safety. Their efficacy depends on phage–host compatibility, bacterial physiological state, physicochemical properties of the dairy matrix, and processing or storage conditions.

Experimental studies have demonstrated the potential of lytic phages to control *Listeria monocytogenes*, *Salmonella* spp., and Shiga toxin-producing *Escherichia coli* in dairy matrices, although efficacy varies substantially among target strains, products, and experimental conditions [83–86]. Applications involving whey-protein-based edible coatings and biofilm-targeting phages further illustrate potential interventions at product surfaces and within dairy-associated contamination reservoirs [87,88]. Collectively, these studies demonstrate that phage efficacy cannot be extrapolated directly from laboratory cultures to complex dairy ecosystems and requires validation in the intended application matrix.

From an integrated microbial–genetic perspective, successful biocontrol should not be assessed exclusively through reductions in target bacterial counts. Phage-mediated selection may modify the relative abundance of susceptible and resistant bacterial populations, potentially affecting the composition of the residual microbiome and its associated resistance determinants. Such changes remain context-dependent and should not be assumed to increase or decrease ARG dissemination without supporting experimental evidence.

The selection of candidate biocontrol phages should therefore include confirmation of a suitable lytic infection profile, characterization of host range, genomic screening for undesirable genetic determinants, assessment of bacterial resistance development, and evaluation of stability and efficacy under relevant dairy-processing conditions. Where genetic mobility is a concern, additional investigation of phage-associated bacterial DNA and experimentally demonstrated transfer may be warranted. Integrating these criteria would support a more comprehensive evaluation of biocontrol performance, microbial ecological consequences, and genetic safety.

Thus, phage-based biocontrol represents an intervention operating within the same interconnected microbial ecosystem described throughout this review. Its technological value depends not only on targeted bacterial inactivation but also on understanding the interactions between phage populations, microbial hosts, resistance determinants, and mobile genetic elements.

7.5. Balancing Phage Control and Phage Application

Dairy phage management therefore has two apparently opposing objectives. Virulent phages infecting technologically valuable LAB must be restricted, whereas well-characterized lytic phages targeting pathogens or spoilage organisms may be intentionally introduced. The distinction is determined by the identity of the bacterial host, the phage lifecycle, the stage of manufacture, and the intended technological outcome.

For starter protection, no single intervention is likely to provide complete control. Factory-specific monitoring, culture rotation, resistant starter strains, separation of whey and fermentation areas, effective sanitation, and appropriately selected physical treatments act at different points of the phage cycle [16,70–74]. Recent work also shows that environmental decontamination remains highly phage-dependent. Jacob et al. [89] evaluated gaseous ozone against 16 dairy phages on stainless-steel and glass surfaces; most were reduced below the detection limit under the selected conditions, whereas several showed only partial inactivation, and efficacy was influenced by the suspending matrix, relative humidity, and ozone concentration [89].

For biocontrol, the objective is reversed: infective particles must remain stable until they encounter the target organism. Formulation, storage stability, spatial access to the host, and the physicochemical properties of the dairy matrix consequently become part of phage efficacy [83–88]. This contrast illustrates why phages cannot be classified as intrinsically detrimental or beneficial components of dairy systems. Their technological consequence depends on which phage interacts with which bacterial host, at which stage of production, and under which environmental conditions.

Effective phage management in dairy systems requires simultaneous consideration of microbial hosts, phage infection dynamics, resistance-associated genetic determinants, and mobile genetic elements. The same phage–host specificity that threatens starter-culture performance may support targeted pathogen control, whereas lysogeny and potential transduction introduce additional genetic considerations. Consequently, technological interventions should be evaluated through their effects on microbial community composition, fermentation performance, genetic mobility, and microbiological safety rather than through phage abundance or bacterial reduction alone.

8. From Detection to Biological Meaning: Analytical Resolution of the Dairy Microbial Network

The interpretation of dairy microbiome data is determined not only by the biological system under investigation but also by the analytical method used to characterize it. Culture-dependent approaches, amplicon sequencing, shotgun metagenomics, long-read

sequencing, metatranscriptomics, metaproteomics, metaviromics, and viability-resolved methods interrogate different components of the same microbial ecosystem and consequently support different biological inferences. In particular, detection of microbial DNA establishes molecular presence but does not necessarily demonstrate viability, metabolic activity, gene expression, infectivity, or genetic transfer. Analytical resolution must therefore be considered when microbiome, resistome, mobilome, and phageome datasets are compared or translated into conclusions concerning dairy quality and safety.

8.1. Culture-Based and Amplicon Approaches: Establishing Community Composition

Culture-dependent methods remain necessary when viable isolates are required for phenotypic characterization, antimicrobial susceptibility testing, phage–host assays, starter selection, or experimental gene-transfer studies. Their principal limitation is that recovery depends on the cultivation conditions applied; consequently, the culturable fraction represents only part of the viable microbial community. This limitation is particularly relevant in complex fermented products containing slow-growing, stressed, viable-but-nonculturable, or nutritionally dependent microorganisms.

Culture-independent amplicon sequencing substantially expands taxonomic coverage but introduces a different set of constraints. Targeted sequencing of bacterial 16S rRNA or fungal ITS regions can describe community composition and diversity without cultivation, but taxonomic resolution depends on the selected marker, amplified region, primers, sequencing platform, and reference database. Relative abundance is also affected by amplification bias and gene-copy-number variation, while DNA-based amplicon profiles do not distinguish living cells from residual DNA.

Direct comparison of analytical approaches illustrates these differences. Tsouggou et al. [90] investigated industrial PDO Sfela and artisanal cheese variants using culture-based identification, 16S rDNA amplicon sequencing, and shotgun metagenomics. The approaches provided complementary but non-identical descriptions of the microbiota, with shotgun analysis offering greater taxonomic and functional resolution than culture-dependent characterization alone [90]. Similar differences were observed in a white-cheese processing facility, where shotgun metagenomics detected substantially broader LAB diversity than culturomics [19].

Interpretation becomes particularly sensitive in milk because microbial biomass may be low relative to host DNA and environmental or reagent contamination. Dean et al. [28] demonstrated that extraction kits, laboratory environment, and sampling-related contamination can substantially influence the apparent bovine milk microbiome. Negative controls, extraction blanks, adequate biomass assessment, and appropriate contamination filtering are therefore integral components of experimental design rather than optional technical details.

8.2. Shotgun Metagenomics: From Taxonomy to Functional Potential

Shotgun metagenomics extends analysis beyond marker genes by sequencing the total recoverable DNA within a sample. It can provide species- and, under appropriate conditions, strain-level information, reconstruct metagenome-assembled genomes (MAGs), identify antimicrobial resistance determinants and metabolic pathways, and provide information on MGEs and other genomic features [9,35]. This makes shotgun sequencing particularly suitable for simultaneously interrogating several layers of the microbiome–resistome–mobilome network.

However, the functional information obtained from DNA is principally functional potential. Identification of a metabolic pathway, virulence determinant, ARG, or phage-related sequence does not establish that the corresponding gene is expressed, that its product

is functional, or that the microorganism carrying it is viable. In addition, relative abundance and detection sensitivity depend on sequencing depth, DNA extraction, host-DNA content, assembly procedures, databases, and bioinformatic thresholds.

These limitations are evident in milk resistome analysis. Urrutia-Angulo et al. [91] compared short-read Illumina and long-read Oxford Nanopore metagenomic sequencing in bovine milk following antimicrobial dry-cow therapy. The two approaches showed limited concordance in detected resistance determinants, while the high proportion of host DNA restricted microbial sequencing depth and sensitivity [91]. The study demonstrates that apparent differences in resistome composition can partly reflect platform and analytical-pipeline effects rather than biological differences alone.

Accordingly, shotgun metagenomic data are most informative when taxonomic profiles, gene content, and genomic context are interpreted together. Gene detection without reliable host assignment or genomic linkage provides weaker biological evidence than a determinant associated with a reconstructed bacterial genome or mobile element.

8.3. Long-Read Metagenomics: Resolving Strains, Plasmids, and Genetic Context

Long-read sequencing addresses several limitations associated with fragmented short-read assemblies. Longer sequences can improve reconstruction of complete or near-complete genomes, repetitive regions, plasmids, prophages, transposons, and other MGEs and can therefore strengthen linkage among ARGs, their genetic context, and potential microbial hosts.

This advantage is particularly relevant to the resistome–mobilome axis discussed in Section 6. Long-read WGS has resolved plasmid-associated resistance structures in dairy-associated enterococci [56] and raw-milk *E. coli* [61], while long-read metagenomic approaches have improved species- and strain-level resolution in cheese communities [20]. The method is therefore valuable when the biological question shifts from "Is this gene present?" toward "In which genome or mobile element is this gene located?"

Long reads should nevertheless not be regarded as intrinsically unbiased. DNA integrity, extraction procedures, sequencing depth, error profiles, host-DNA contamination, and downstream assembly can influence reconstruction. In the bovine-milk comparison by Urrutia-Angulo et al. [91], short- and long-read methods did not produce fully concordant resistome profiles. The additional structural information supplied by long reads therefore complements rather than automatically replaces short-read sequencing.

8.4. Metatranscriptomics and Metaproteomics: From Functional Potential to Activity

DNA-based sequencing identifies the genetic repertoire available within a community, whereas RNA- and protein-based methods provide information closer to biological activity. Metatranscriptomics characterizes transcripts present at the time of sampling and can therefore identify genes undergoing transcription within a microbial community. This distinction is particularly important during dairy fermentation and ripening, where microorganisms detected at similar abundance may contribute differently to acidification, proteolysis, lipolysis, amino-acid metabolism, or stress responses.

RNA analysis presents substantial technical challenges. Carbonne et al. [92] evaluated RNA extraction and mRNA enrichment across different cheese types and found large differences in RNA yield and integrity between matrices. In undepleted total-RNA libraries, approximately 99% of sequencing reads could correspond to rRNA, emphasizing the importance of effective rRNA depletion for metatranscriptomic analysis [92]. RNA is also less stable than DNA, making sample stabilization, extraction time, enrichment, and storage conditions especially important. A recent review of food metatranscriptomics

similarly identifies RNA stability, microbial enrichment, protocol standardization, database limitations, and bioinformatic processing as major sources of analytical variability [93].

Metaproteomics provides another level of functional information by identifying microbial proteins recovered from the sample. It can therefore connect microbial identity with expressed enzymatic machinery more directly than DNA-based predictions. Interpretation, however, depends strongly on the sequence database used for peptide identification. Da Silva Duarte et al. [94] showed that database selection materially affected shotgun metaproteomic analysis of low-diversity dairy microbiomes and that metagenome-derived databases produced particularly strong performance for taxonomic and functional assignment [94].

Application of metaproteomics to dairy fermentation also demonstrates its biological value. Porcellato et al. [95] showed that the initial microbial quality of milk affected proteome allocation in a mesophilic starter culture and altered metabolic pathways during subsequent fermentation, including carbohydrate- and citrate-associated metabolism [95]. Thus, similar starter compositions can display different functional states depending on the microbial and biochemical history of the milk.

Integration of metagenomics with metaproteomics and metabolite analysis can further connect genetic potential to expressed functions. Valentino et al. [96] combined microbiome profiling, metaproteomics, and volatilomics in Grana Padano, Trentingrana, and Parmigiano Reggiano cheeses and associated cheese-specific microbial populations with proteins and volatile compounds related to ripening metabolism [96]. Such integration provides stronger functional interpretation than genomic prediction alone, although correlations among genes, proteins, and metabolites should still not be interpreted automatically as causal relationships.

8.5. Metaviromics and Phage–Host Inference

Metaviromics provides access to viral populations that are poorly captured by bacterial marker-gene sequencing and allows the reconstruction of viral operational taxonomic units (vOTUs), assessment of viral diversity, and prediction of potential phage–host relationships. Its application to artisanal and ripened cheeses has revealed complex and dynamic viral communities associated with bacterial succession [24,25].

The interpretation of viral sequence data requires particular caution. Detection of viral DNA does not necessarily establish the presence of intact or infective particles. Likewise, prophage sequences integrated into bacterial chromosomes must be distinguished from extracellular virions when the objective is to characterize the active phage fraction. Host assignment may rely on CRISPR spacer matches, genomic similarity, prophage relationships, receptor information, or co-abundance patterns, but these predictions differ in confidence and remain distinct from experimentally demonstrated infection.

The same distinction applies to phage-associated ARGs. Detection of resistance-associated sequences in viral fractions [26] provides evidence of genetic carriage but does not demonstrate successful transduction, stable incorporation into a recipient genome, or expression of the transferred determinant. For phageome analysis, viral sequence detection, particle integrity, infectivity, host identity, and genetic transfer should therefore be treated as separate analytical endpoints.

8.6. Viability-Resolved Approaches: Separating DNA Detection from Living Cells

One of the principal limitations of DNA-based dairy microbiome studies is persistence of DNA after cellular death. This problem becomes particularly important following pasteurization, disinfection, fermentation stress, or prolonged storage, because sequencing can continue to detect organisms whose cells are no longer biologically active.

Yap et al. [97] directly examined this problem using a model microbial community containing viable and heat-inactivated cells in bovine milk. Shotgun metagenomics with and without propidium monoazide (PMA), RNA-based 16S sequencing, and metatranscriptomics were compared for their ability to distinguish viable from non-viable populations [97]. The results showed that viability interpretation depends on the analytical approach and demonstrated the value of combining conventional sequencing with viability-focused methods.

PMA and related dyes selectively reduce amplification of DNA from cells with severely compromised membranes and can therefore improve approximation of membrane-intact populations. In natural whey starters used for Comté cheese manufacture, Lutin et al. [98] combined culture-based enumeration, qPCR, viability-qPCR, fluorescence microscopy, and DNA metabarcoding to assess both microbial composition and viability [98]. Most cells within the investigated starter communities were viable, but the relative abundance of individual thermophilic species varied among factories and seasons.

Viability analysis can also be combined with cell-based and highly quantitative molecular techniques. Liu et al. [99] developed a PMA–antibiotic fluorescence in situ hybridization–flow cytometry method for quantifying viable *Bifidobacterium* in milk powder, allowing target-specific measurement within approximately two hours [99]. More recently, Wang et al. [100] combined strain-specific primers with PMA–droplet digital PCR to quantify viable *Lactiplantibacillus plantarum* YZH81 in fermented milk and distinguish the target strain from closely related *L. plantarum* strains [100].

These approaches improve interpretation but do not make "viability" a single universal measurement. PMA-based methods primarily reflect membrane integrity, culture methods measure the ability to reproduce under the selected growth conditions, and RNA-based methods provide evidence related to cellular activity. A membrane-intact cell is therefore not necessarily culturable or metabolically active, just as RNA detection is not equivalent to demonstrated growth. The analytical endpoint should be specified whenever viability claims are made.

8.7. Integrative Multi-Omics: Linking Composition, Function, and Phenotype

No individual method fully resolves the dairy microbial–genetic ecosystem. Amplicon sequencing describes community structure, metagenomics provides genomic and functional potential, metatranscriptomics measures transcriptional activity, metaproteomics identifies expressed proteins, metabolomics reflects accumulated biochemical outputs, and viability-resolved methods distinguish subsets of biologically intact cells. Integration of these data can therefore connect microbial identity with genetic capacity, activity, and product phenotype.

The value of multi-omics, however, depends on integration rather than simply on the number of analytical layers generated. Van Den Bossche et al. [101] emphasized that microbiome multi-omics should be evaluated according to the additional biological information generated by cross-layer integration and identified experimental standardization, metadata harmonization, benchmarking, and reproducible computational workflows as major requirements for interpretable analyses [101]. Dairy studies combining metagenomics, metaproteomics, and volatilomics already illustrate this principle by linking taxonomic differences to expressed proteins and product metabolites [96].

The analytical approaches discussed above therefore provide a progression from microbial detection toward increasingly specific biological interpretation. Their principal capabilities and limitations in the context of the microbiome–resistome–mobilome–phageome framework are summarized in Table 3.

Table 3. Analytical approaches for investigating the dairy microbiome–resistome–mobilome–phageome network and their principal interpretative limits.

Analytical Approach	Principal Question	Main Information Obtained	Viability Information	Functional/Activity Information	Principal Interpretative Limitation	Representative References
Culture-dependent methods	Which microorganisms can be recovered under the applied conditions?	Viable isolates, phenotypes, antimicrobial susceptibility, experimental material	Yes, for the culturable fraction	Phenotypic	Excludes viable organisms not growing under selected conditions	[19,98]
16S/ITS amplicon sequencing	Which taxa are detected?	Community composition and relative taxonomic structure	No	Limited; function usually inferred indirectly	Primer/amplification bias, limited strain resolution, DNA from non-viable cells	[28,90]
Shotgun metagenomics	Which organisms and genes are present?	Species/strain profiles, MAGs, ARGs, pathways, genomic content	No, unless combined with viability methods	Functional potential	Does not establish expression or viability; assembly/database dependence	[9,35,90,91]
Long-read sequencing/metagenomics	What is the genomic context of detected genes?	Improved strain resolution, plasmids, prophages, ARG–MGE linkage, repetitive regions	No	Functional potential	DNA quality, sequencing depth, error and assembly effects	[20,56,61,91]
Metatranscriptomics	Which genes are being transcribed?	Community-wide transcriptional profiles	Indirect	Transcriptional activity	RNA instability, rRNA dominance, extraction and enrichment bias	[92,93]
Metaproteomics	Which microbial proteins are detected?	Expressed proteins and associated pathways	Indirect	Protein-level functional evidence	Database dependence, protein dynamic range, incomplete taxonomic assignment	[94–96]
Metaviromics	Which viral populations and viral genes are detected?	vOTUs, viral diversity, prophage/viral sequences, predicted phage–host relationships	Limited	Limited unless infectivity/function is separately assessed	Viral DNA ≠ infective phage; host prediction may be indirect	[24–26]
PMA/viability-resolved molecular methods	Which detected cells retain membrane integrity?	Viability-adjusted abundance or target quantification	Yes, operationally defined	Limited	Membrane integrity ≠ culturability or metabolic activity; matrix-dependent performance	[97–100]
Integrated multi-omics	How are microbial composition, genetic potential, activity, and phenotype related?	Cross-layer associations among taxa, genes, transcripts, proteins, and metabolites	Depends on included methods	High when complementary layers are integrated	Complexity, standardization, data integration, and causality	[96,101]

A major consequence of these methodological differences is that findings derived from different analytical layers should not be treated as biologically equivalent. DNA detection establishes presence; viability-resolved analysis addresses whether cells remain biologically intact; RNA and protein measurements provide progressively stronger evidence of activity; genomic linkage can support mobility; and experimental assays are required to demonstrate transfer or infectivity. The interpretation of dairy microbial data should therefore match the biological endpoint that was actually measured.

This distinction becomes particularly important when analytical results are translated into food-safety conclusions. Detection of a microorganism, ARG, MGE, or phage sequence may identify a potential reservoir, but the implications for product quality, microbial transmission, AMR dissemination, or consumer exposure depend on viability, activity, host association, mobility, abundance, and processing context. These relationships form the basis of the risk-oriented framework developed in the following section.

9. From Presence to Risk: A Framework for Interpreting Dairy Microbial Data

High-resolution molecular methods increasingly reveal microorganisms, antimicrobial resistance genes (ARGs), mobile genetic elements (MGEs), and bacteriophages in milk, dairy products, and production environments. However, analytical sensitivity has progressed faster than the ability to assign biological or health significance to every detected signal. Microbiological risk assessment distinguishes the identification of a potential hazard from characterization of exposure and the probability of an adverse outcome [102,103]. Consequently, detection alone should not be interpreted as equivalent to viability, activity, transmission, exposure, or risk. In the present review, these relationships are organized as an evidence continuum extending from molecular detection to risk relevance, as illustrated in Figure 4. The framework is intended to guide biological interpretation of microbiome, resistome, mobilome, and phageome data and does not replace formal qualitative or quantitative microbiological risk assessment.

Molecular detection represents the initial evidence that a microbial taxon, antimicrobial resistance gene (ARG), mobile genetic element (MGE), or phage-associated sequence is present in a dairy sample. Increasing biological interpretation requires additional evidence concerning viability or structural integrity, functional activity, transfer potential, persistence and exposure, and ultimately the plausibility of an adverse outcome in a relevant host or system. The progression does not imply that every detected biological entity will advance toward risk; rather, each step requires additional experimental or epidemiological support. The framework applies across the microbiome, resistome, mobilome, and phageome and emphasizes that presence, activity, transfer, exposure, and risk represent distinct biological endpoints. The scheme is conceptual and should not be interpreted as a quantitative risk-scoring model.

9.1. Detection Identifies a Potential Hazard, Not Its Risk

The first step in interpretation is to distinguish presence from hazard relevance. Detection of a bacterial taxon indicates that its DNA or another analytical marker was recovered, but it does not establish viable-cell abundance, pathogenic phenotype, or the dose to which a consumer may be exposed. Similarly, detection of an ARG does not establish its microbial host, expression, location on a transferable element, or ability to reach a clinically relevant recipient. An MGE sequence does not demonstrate active mobility, and viral DNA does not necessarily represent an intact infectious phage.

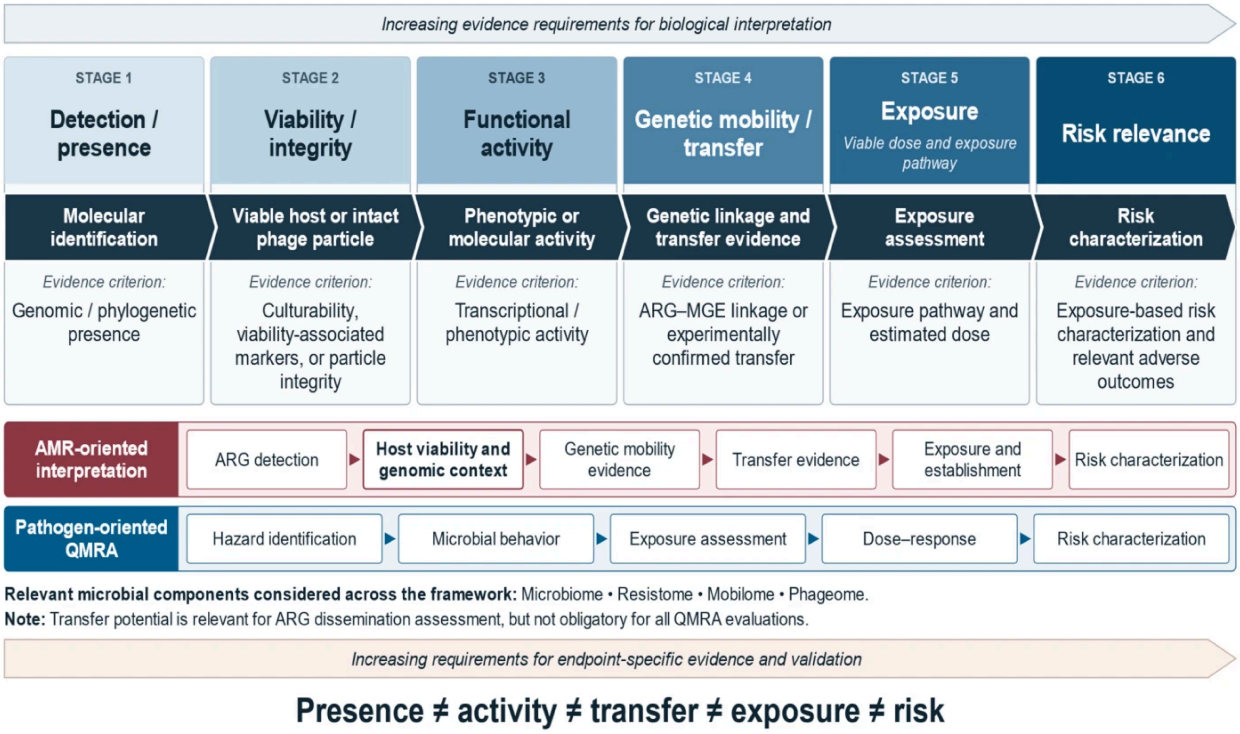


Figure 4. *Evidence-based interpretation of dairy microbial data and its interface with quantitative microbial risk assessment (QMRA).* The framework integrates six interpretive stages: molecular detection, viability or structural integrity, functional activity, genetic mobility and transfer, exposure, and risk relevance. The AMR-oriented pathway considers ARG–host associations, genetic mobility, transfer evidence, and exposure, whereas the pathogen-oriented QMRA pathway incorporates hazard identification, microbial behavior, exposure assessment, dose–response relationships, and risk characterization. Genetic linkage and experimentally demonstrated transfer represent distinct levels of biological evidence, and horizontal gene transfer is not a mandatory component of conventional pathogen-oriented QMRA. The stages are not necessarily sequential and require endpoint-specific supporting evidence. This framework supports the interpretation of molecular and microbiological data but does not constitute a validated quantitative risk-prediction model.

This distinction is consistent with established food-safety risk assessment, in which characteristics of the food, processing conditions, pathogen behavior, and consumer exposure determine whether the presence of a hazard becomes relevant to public health [102,103]. Dairy-specific models illustrate this difference particularly well. Basak et al. [104] developed a farm-to-fork multipathogen QMRA for raw-milk soft cheese that incorporated Listeria monocytogenes, Salmonella, and Shiga toxin-producing Escherichia coli (STEC), explicitly modeling contamination, processing, ripening, storage, consumption, and health outcomes rather than using pathogen detection alone as a proxy for risk.

A more recent multi-agent model for bovine raw-milk cheeses similarly represented farms, individual animals, cheese batches, processing steps, and consumers and showed that the effect of control measures depended on both the pathogen and the location of the intervention along the chain [105]. Product characteristics also influence interpretation. A cheese risk-ranking approach incorporating milk pasteurization, ripening method, and predicted pathogen growth classified cheeses differently according to their ability to support viable pathogens, while explicitly acknowledging that the model did not include subsequent cross-contamination or consumer handling [106]. These examples demonstrate why detection should be regarded as the beginning rather than the endpoint of microbiological interpretation.

9.2. Viability, Activity, and Transfer Represent Distinct Evidence Gates

The transition from molecular presence to biological significance requires evidence that the detected entity remains biologically relevant. For bacterial communities, this may involve demonstration of viable or membrane-intact cells, culturability, transcriptional activity, or expressed proteins. As discussed in Section 8, these endpoints are not interchangeable. PMA-based methods provide information related primarily to membrane integrity [97–100], whereas culture demonstrates growth under the selected conditions, and RNA- or protein-based analyses provide different forms of evidence for functional activity.

The same hierarchy applies to antimicrobial resistance. Detection of an ARG represents a lower level of biological evidence than reliable assignment to a bacterial host, localization on an MGE, identification of transfer-associated machinery, or experimental demonstration of horizontal transfer. Recent genomics-oriented approaches to AMR risk assessment therefore increasingly consider ARG–host–MGE combinations rather than ARG abundance alone. Cheng et al. [68] proposed that genomics can improve AMR risk assessment by incorporating the clinical importance of resistance determinants, genetic mobility, transmission pathways, evidence quality, and uncertainty.

Mobility is particularly important because two ARGs detected at comparable abundance may have very different dissemination potential. Klümper et al. [69] argued that ARG mobility should become an explicit component of AMR surveillance and risk assessment, together with viability, host association, and quantitative exposure information. This principle is directly relevant to dairy systems, where experimental conjugation has been demonstrated for selected resistance determinants [23,60,65], whereas many other reported ARG–MGE associations remain based on genomic inference.

Phage interpretation requires the same separation of analytical endpoints. Detection of phage DNA does not demonstrate particle integrity, host recognition, infectivity, or successful transduction. Conversely, demonstration of infectivity does not automatically establish transfer of an ARG carried within a viral fraction [26]. Thus, movement from presence → activity → transfer potential requires progressively stronger biological evidence for all four microbial layers represented in Figure 3.

9.3. Exposure Connects Biological Potential with the Dairy Production Chain

Even a viable and biologically active hazard cannot contribute to consumer risk without an exposure pathway. In dairy systems, exposure is determined by the concentration and distribution of the biological entity, its survival or growth during processing and storage, recontamination opportunities, serving size, frequency of consumption, and characteristics of the exposed population. Processing therefore modifies exposure continuously rather than acting as a binary elimination step.

For classical foodborne pathogens, these variables can be incorporated directly into QMRA models. Both the multipathogen model of Basak et al. [104] and the recent multi-agent raw-milk-cheese model [105] demonstrated that microbial behavior during cheese manufacture and storage must be combined with consumption scenarios to estimate health outcomes. The importance of this distinction is particularly evident for susceptible consumers, for whom the same microbial dose may not have the same health consequence as in the general population.

Exposure assessment becomes more complex for antimicrobial resistance because the relevant unit may be a resistant bacterium, an ARG-containing viable host, a transferable plasmid, or another genetic element rather than a single pathogen. A recent critical review of food-chain AMR-QMRA found that available models remain heterogeneous in how they represent resistance acquisition, horizontal gene transfer, transmission between One Health compartments, and the additional burden associated with resistant infections [107]. Major

limitations include insufficient empirical transfer rates, limited dose–response information for resistant organisms, and uncertainty regarding how genomic signals should be converted into model parameters.

Omics can improve this process when molecular information is linked to phenotypic or exposure parameters rather than used as a substitute for them. Lemos Junior et al. [108] showed that genomics, pangenomics, metagenomics, and transcriptomics can refine hazard characterization by identifying strain-level differences in growth, persistence, virulence, and stress responses and can thereby improve parameters used in predictive microbiology and QMRA. They also emphasized that translating molecular signals into quantitative growth, survival, or dose–response parameters remains a major methodological challenge.

A recent dairy study illustrates this interpretative problem directly. Tian et al. [109] found that pasteurization and other non-fermentation treatments substantially reduced culturable bacterial populations, whereas many ARG signals detected by sequencing changed comparatively little. In a mouse model, administration of an ARG-carrying E. coli strain altered the gut resistome, while consumption of pasteurized milk was associated with changes in selected gut ARG profiles; however, the authors did not establish a causal transfer of individual milk-derived ARGs to the gut microbiome [109]. The study therefore demonstrates why persistence of a molecular signal through processing should not be interpreted automatically as viable exposure or transfer to a consumer-associated microbiome.

9.4. From Exposure to Risk Relevance

The final step in the framework is risk relevance, not simply detection of a potential hazard. In formal microbiological risk assessment, exposure information is combined with hazard characterization and, when available, dose–response relationships to estimate the probability or burden of an adverse outcome [102–105]. For conventional pathogens, this relationship can often be expressed using established growth, inactivation, and dose–response models. For resistome, mobilome, and phageome data, equivalent quantitative relationships remain considerably less developed.

For the resistome, risk relevance depends on several interacting characteristics: the clinical importance of the resistance determinant, the identity and viability of its host, genomic location, mobility, expression, exposure level, opportunity for transfer, and relevance of the potential recipient. A highly abundant intrinsic resistance determinant in a non-pathogenic, non-transferable background does not necessarily have the same significance as a lower-abundance clinically important ARG located on a conjugative plasmid within a viable bacterium capable of reaching a relevant recipient [68,69,107]. This distinction supports the evidence hierarchy proposed in Section 6 and prevents ARG abundance from being used as a standalone surrogate for AMR risk.

For bacteriophages, risk relevance is context-dependent in a different manner. An infective phage directed against a starter culture can be technologically important because it may impair fermentation, whereas a well-characterized lytic phage directed against a foodborne pathogen may provide a desirable biocontrol function. The same analytical term, "phage detection," therefore has little interpretative value without information on the host, lifecycle, infectivity, concentration, and stage of production.

Uncertainty must also remain explicit. Biological variability, differences among bacterial strains and phages, sampling error, detection limits, incomplete databases, host-assignment uncertainty, sequencing and assembly biases, and limited empirical data on HGT can all propagate through interpretation [68,69,103,107,108]. Contemporary foodborne risk-assessment literature increasingly recognizes the value of genomic and multi-omics data but also emphasizes that greater analytical resolution does not automatically reduce

uncertainty unless the resulting measurements can be connected to biologically meaningful model parameters.

Accordingly, the framework presented in Figure 3 should be interpreted as a sequence of evidence requirements rather than an inevitable progression toward risk. A molecular signal may cease to be relevant at any stage if the corresponding organism is non-viable, the gene is not expressed, the genetic determinant is non-mobile, the phage particle is non-infective, or exposure is negligible. Conversely, convergence of evidence across viability, activity, mobility, persistence, and exposure increases confidence that a detected biological entity warrants further risk characterization. In practical terms, presence identifies what should be investigated; biological activity establishes what may function; mobility identifies what may disseminate; exposure establishes what may reach a relevant host; and risk characterization determines whether these events can plausibly contribute to an adverse outcome.

This evidence-based interpretation provides the link between microbial ecology and dairy food safety. It also explains why surveillance based exclusively on taxonomic abundance or gene counts may be insufficient for prioritizing interventions. The following section therefore considers how the integrated microbiome–resistome–mobilome–phageome perspective can inform dairy quality, process control, and microbiological safety.

10. Implications for Dairy Quality and Safety

An integrated microbiome–resistome–mobilome–phageome perspective changes the interpretation of microbial control in dairy production. The objective is not the indiscriminate elimination of microorganisms, because dairy systems depend on technologically desirable populations and, in many fermented products, on complex microbial succession. Instead, effective management requires differentiation among microorganisms contributing to fermentation and product identity, populations associated with spoilage or post-process contamination, microorganisms carrying transferable resistance determinants, and bacteriophages whose technological effect depends on their bacterial host. The practical value of high-resolution microbial analysis therefore lies in connecting microbial identity with function, persistence, genetic context, and the stage of production at which intervention is possible.

10.1. Technological Performance, Product Identity, and Sensory Quality

Dairy microbial communities contribute directly to acidification, proteolysis, lipolysis, amino-acid conversion, and formation of volatile metabolites that determine texture and sensory characteristics. Starter cultures provide controlled technological functions, while non-starter lactic acid bacteria, ripening organisms, and resident environmental populations can contribute additional metabolic activities during maturation [35,47,50,51,96]. Consequently, microorganisms detected in dairy environments should not automatically be regarded as undesirable contaminants.

Recent evidence shows that the processing environment can contribute to product-specific microbial and metabolic signatures. Ferrocino et al. [110] followed bacterial and fungal communities across environmental surfaces and different seasons during production of a traditional artisanal cheese and linked seasonal microbial shifts with differences in metabolite profiles, texture, and sensory characteristics. Several resident populations remained associated with the production environment, whereas season-dependent changes in both desirable and spoilage-associated microorganisms were reflected in final cheese quality [110]. These findings support the concept that product identity may partly emerge from controlled interactions among raw-material microbiota, starter organisms, processing conditions, and resident environmental communities.

This ecological contribution must nevertheless be separated from uncontrolled contamination. A resident microorganism may be technologically advantageous in one ecological context and detrimental in another, and the same taxon can contain strains with substantially different metabolic or safety-related characteristics. Management strategies should therefore move beyond species presence toward strain identity, functional phenotype, and technological context whenever the required analytical resolution is available.

10.2. Spoilage, Shelf Life, and Process Consistency

Shelf life is determined not only by the total number of microorganisms surviving processing but also by which populations persist or are reintroduced, their capacity to grow under storage conditions, and the stability of microbial enzymes and metabolites generated before or after processing. Refrigeration can strongly select psychrotrophic populations whose contribution to quality deterioration becomes disproportionate to their initial abundance.

Zhuang et al. [111] combined metagenomics and metabolomics to follow raw milk stored at 4 °C and reported succession from the initial community toward psychrotrophic populations, including *Flavobacterium* and *Pseudomonas*. The most pronounced metabolic changes occurred after approximately 3–4 days under the conditions investigated, coinciding with rapid changes in the dominant microbial community and lipid-associated metabolites [111]. These findings illustrate the potential value of linking microbial succession to measurable biochemical changes instead of relying on cell counts alone.

A similar principle applies after thermal processing. Zhang et al. [112] integrated metagenomic sequencing, LC-MS, and GC-MS across raw, pasteurized, and UHT milk and showed that microbial shifts and metabolite trajectories differed according to treatment and storage conditions. In pasteurized milk, specific microorganisms were strongly associated with differential metabolites, indicating that residual or selected populations can contribute to product-specific biochemical changes during storage [112].

Not all shelf-life deterioration is directly attributable to viable microorganisms at the time the defect becomes detectable. Thermostable microbial proteases and lipases produced before heating can remain technologically relevant after substantial reductions in bacterial populations, while chemical reactions occurring during storage can further modify flavor. Xi et al. [113], for example, combined volatile metabolomics, sensory evaluation, electronic-nose measurements, and chemometrics to discriminate UHT milk according to storage period and identify volatile compounds associated with stale flavor development [113]. This illustrates the advantage of combining microbial information with biochemical or sensory markers when the objective is quality prediction rather than microbial identification alone.

The range of microbial defects encountered in dairy products is correspondingly broad and includes proteolytic destabilization, gas production, discoloration, acidification, off-flavors, slime formation, and ropiness [114]. Post-pasteurization environments are particularly important because microorganisms persisting on equipment can bypass earlier lethality steps. Medjahdi et al. [115] recovered diverse spore-forming bacteria from equipment contacting pasteurized milk and demonstrated biofilm-forming and spoilage-enzyme phenotypes among the isolates. Almost one-third of the tested isolates were classified as strong biofilm producers under the experimental conditions, while subsets displayed marked proteolytic or lecithinase activity [115]. Control of shelf life should therefore address both microbial survival and environmental recontamination, together with the functional properties of the populations involved.

10.3. Environmental Monitoring and Process Hygiene

Environmental microbiota represents an important interface between dairy quality and food safety. Food-contact surfaces, drains, transfer equipment, brines, water, air, and non-food-contact niches can contain persistent microorganisms that may repeatedly enter production lines [8,11,34–36]. Longitudinal monitoring is particularly informative because a microorganism detected repeatedly at the same location has a different epidemiological and technological significance from a single transient detection.

High-throughput community profiling can extend conventional environmental monitoring by identifying ecological patterns and locations that warrant targeted investigation. Giagnoni et al. [116] applied 16S rRNA metabarcoding to 253 samples collected from 14 sheep-cheese plants and found clear differences between microbial communities associated with different technological areas. Sequences assigned to taxa containing potentially pathogenic species were concentrated in particular processing niches, with moulding areas and conveyors identified as important sites in the studied facilities [116]. Such observations are useful for identifying candidate hotspots, but sequence detection at genus or species level should not by itself be interpreted as evidence of viable pathogen contamination.

Environmental community structure may also affect pathogen behavior. Poswal et al. [117] characterized 167 environmental isolates from air and floor samples collected across three seasons in a commercial dairy-processing facility. *Pseudomonas* was consistently represented across sample types, and substantial seasonal and operational differences in background microflora were observed. In experimental co-culture assays, the investigated background communities did not provide consistent competitive exclusion of the *Listeria monocytogenes* test strain [117]. The result is relevant because environmental monitoring should consider microbial interactions and persistence rather than assuming that a diverse resident microbiota necessarily suppresses undesirable organisms.

Advanced detection technologies can improve the speed, sensitivity, and taxonomic resolution of dairy monitoring, but each method provides a different biological endpoint. Molecular, immunological, biosensor, and sequencing approaches are increasingly available for rapid detection of spoilage organisms and pathogens [118]. Their application should complement rather than replace validated culture-based verification, sanitation monitoring, Good Manufacturing Practices, and HACCP-based control. Current dairy-safety literature likewise supports combined use of conventional hygiene systems, rapid detection, biofilm-control strategies, and targeted physical, chemical, or biological interventions [119].

10.4. AMR- and Phage-Aware Food Safety Management

Integration of the resistome and mobilome adds another layer to dairy food-safety management. ARG abundance alone provides limited information for prioritization because resistance determinants differ in clinical relevance, microbial host, genomic location, mobility, expression, and likelihood of exposure. The more informative targets are therefore combinations involving a viable bacterial host, a resistance determinant of concern, association with a mobile genetic element, and a plausible opportunity for dissemination. The experimental transfer demonstrated for selected dairy-associated ARGs [23,60,65], together with plasmid-resolved genomic studies [56,61], supports this distinction.

This approach also avoids treating every detected resistance determinant as equivalent. An intrinsic resistance gene located chromosomally in a non-pathogenic organism has a different dissemination potential from a clinically important ARG associated with a conjugative plasmid in a viable bacterial host. As discussed in Sections 6 and 9, surveillance should therefore retain the distinction between ARG presence, host assignment, mobile genetic context, experimentally demonstrated transfer, and consumer exposure [68,69,107–109].

Phage management presents an apparently opposite technological problem. Virulent phages infecting starter bacteria can compromise acidification and fermentation performance, whereas strictly lytic phages directed against pathogens or spoilage organisms may serve as targeted biocontrol agents [70,76,81,83–89]. Phage management in dairy production is therefore inherently context dependent: the same biological property—host-specific lysis—represents a technological hazard when the host is a starter bacterium and a potential control tool when the host is an undesirable microorganism.

Practical phage control consequently requires information on host range, persistence within the processing environment, starter susceptibility, thermal and sanitation resistance, and the possibility of phage re-entry through whey or other process streams [16,70,74]. Conversely, biocontrol applications require genomic safety characterization, confirmation of a lytic lifecycle, stability in the intended dairy matrix, adequate contact with the target organism, and assessment of resistance development. Phage detection alone is therefore insufficient for either technological interpretation.

10.5. Toward Ecosystem-Informed Dairy Quality Management

Current evidence supports a transition from isolated microbial measurements toward ecosystem-informed monitoring, used here as a conceptual rather than regulatory framework. Such monitoring would combine baseline characterization of the resident microbiota with longitudinal environmental sampling, strain- or source-level tracking when required, identification of functional spoilage or fermentation markers, ARG–MGE characterization for selected resistance concerns, and targeted phage monitoring in starter-dependent processes.

Large-scale microbiome mapping has already shown that dairy facilities can harbor plant-specific microbial signatures and strains potentially traceable to particular production environments [35]. More recent work demonstrates that these resident populations are not necessarily static: seasonal changes and specific environmental niches can influence microbial transfer and final product quality [110]. The practical value of longitudinal monitoring is therefore not merely to expand the number of microorganisms detected but to distinguish persistent populations from transient signals and identify where ecological changes coincide with loss of process consistency, spoilage, or increased safety relevance.

Implementation should remain question-driven. A sequencing result identifying an unusual population can guide targeted culture or strain isolation; detection of an ARG can trigger investigation of its host and genomic context; recurring spoilage populations can direct intensified sanitation or source-tracking studies; and recurrent starter phages can inform culture rotation. In this model, omics provides additional resolution for decisions that still require phenotypic, technological, and food-safety verification.

These approaches should therefore complement, not replace, established dairy quality and food-safety systems. HACCP, prerequisite programs, sanitation verification, validated microbiological criteria, and process control remain the operational basis of safe manufacture. The added value of microbiome-, resistome-, mobilome-, and phageome-informed analysis is its capacity to reveal where microbial populations originate, how they persist, what functions they may perform, and which detected signals merit further investigation. Integrating these layers can support more targeted control of spoilage and contamination while preserving microbial functions that contribute to fermentation performance and product identity.

11. Research Gaps and Future Directions

The rapid expansion of sequencing and multi-omics technologies has substantially increased the resolution with which dairy microbial ecosystems can be characterized. How-

ever, greater analytical depth has not resolved several fundamental limitations concerning reproducibility, biological interpretation, microbial viability, host assignment of mobile genetic elements, phage–host validation, and translation of molecular information into industrial decisions. Future research should therefore focus less on generating increasingly large inventories of taxa and genes and more on establishing reproducible links among microbial identity, genetic context, biological activity, technological function, and experimentally demonstrated outcomes.

11.1. Standardization, Contamination Control, and Reproducibility

Methodological heterogeneity remains one of the main barriers to comparison among dairy microbiome studies. Differences in sample collection, storage, homogenization, DNA extraction, sequencing platform, library preparation, bioinformatic pipeline, reference database, and filtering criteria can generate substantial variation independently of the underlying biological system. This is particularly problematic in milk and other low-biomass samples, where background DNA and reagent contamination may represent a substantial proportion of the recovered microbial signal [28,91].

Recent interlaboratory work provides a basis for addressing this problem. Ferrocino et al. [120] evaluated standardized sample-preparation and DNA-extraction procedures across multiple laboratories and food and environmental matrices and demonstrated that harmonized standard operating procedures can improve interlaboratory comparability. For low-biomass microbiomes, Fierer et al. [121] further emphasized that contamination control must be incorporated throughout sampling, laboratory processing, sequencing, and data analysis. Their recommendations include appropriate negative controls, transparent reporting of contamination-removal procedures, and availability of sequence data from controls as well as biological samples.

Reporting standards are equally important. The STREAMS guidelines provide 67 recommendations covering study design, sample and environmental metadata, experimental procedures, bioinformatics, and data reporting for environmental and non-human host-associated microbiome research [122,123]. Although these recommendations were not developed specifically for dairy systems, their implementation would substantially improve reproducibility and facilitate meaningful comparison among studies.

Future dairy microbiome studies should therefore report, at minimum, biological and technical replicates, negative controls, extraction procedures, sequencing depth, database and software versions, quality-filtering thresholds, contamination-removal procedures, and sufficient production metadata to reconstruct the technological context. Where possible, mock communities and internal standards should also be incorporated. Greater sequencing depth cannot compensate for inadequate experimental design or insufficient control of contamination.

11.2. From Cross-Sectional Surveys to Longitudinal and Intervention Studies

Much of the available dairy microbiome literature remains cross-sectional. Such studies identify associations between microbial populations and particular farms, products, production stages, or environments but provide limited information on the direction of microbial transmission or the processes responsible for community change.

Longitudinal designs are better suited to distinguishing persistent microorganisms from transient contamination and to resolving succession through production. Existing studies have already demonstrated the value of following bacterial and viral communities during processing and ripening [25,48,51], tracking industrial phages over several years [70], and examining seasonal effects on cheese microbiota and product characteris-

tics [110]. The next step should be to link repeated sampling directly to individual batches and technological events.

Longitudinal multi-omics studies introduce specific statistical and experimental challenges because measurements obtained from the same system over time are not independent and because different omics layers may operate at different temporal scales. Ma et al. [124] highlighted the importance of experimental design, repeated-measurement structure, confounder control, temporal modeling, and cross-layer integration when longitudinal microbiome datasets are generated.

Future dairy studies should therefore follow defined production units from farm → raw milk → processing → fermentation/ripening → packaging → storage, ideally retaining sample identity across the chain. Intervention studies are particularly needed. Rather than observing that a microbial population changes after processing, controlled studies should determine whether changing storage temperature, sanitation procedure, starter composition, brining conditions, packaging atmosphere, or another technological parameter produces a reproducible change in microbial or genetic network structure. The key question should increasingly become not simply which microorganisms are present, but which populations persist, where they originate, what causes their transition, and whether intervention changes the outcome.

11.3. From Presence to Viability, Activity, and Causality

A second major gap is the continued reliance on DNA detection as a surrogate for biological relevance. Sections 8 and 9 showed that DNA-based sequencing cannot by itself establish whether a detected organism is viable, whether a gene is expressed, or whether the corresponding phenotype is active. This limitation becomes especially important after pasteurization, sanitation, fermentation stress, or long-term storage.

Future studies should increasingly combine metagenomic data with viability-resolved methods, RNA-based profiling, metatranscriptomics, metaproteomics, metabolomics, cultivation, and targeted functional assays [97–101]. The objective is not to apply every analytical platform to every experiment, but to select complementary methods according to the biological question. If the objective is to determine whether a microorganism survives heat treatment, viability must be measured. If the question concerns metabolic contribution during ripening, transcript, protein, or metabolite information is more informative than DNA abundance alone.

The same requirement applies to causal interpretation. Associations between microbial taxa and volatile compounds, spoilage metabolites, ARG abundance, or sensory characteristics are valuable for hypothesis generation but do not demonstrate that the organism detected is responsible for the observed phenotype. Isolation, defined-community experiments, targeted removal or addition of strains, controlled fermentation trials, and other perturbation approaches remain necessary to test causality.

The methodological priority should therefore shift from greater taxonomic resolution alone toward stronger linkage between molecular detection and biological function. This distinction is essential if microbiome data are to inform technological intervention rather than remain primarily descriptive.

11.4. Resolving ARG–MGE–Host Relationships and Transfer Under Dairy Conditions

AMR research in dairy systems still contains a substantial gap between detection of resistance determinants and demonstration of their dissemination. Short-read metagenomics can identify large ARG repertoires but frequently cannot establish whether a determinant is chromosomal or mobile, which microorganism carries it, or whether the element can transfer to another host. Long-read approaches and complete genome sequencing have substantially

improved ARG–MGE linkage [56,57,61], while conjugation experiments have demonstrated transfer for selected dairy-associated resistance determinants [23,60,65]. Nevertheless, such experimentally confirmed examples remain relatively uncommon.

Reliable host assignment of MGEs remains technically difficult even in well-characterized complex microbiomes. Bloemen et al. [125] compared approaches for recovering MGEs and assigning them to microbial hosts and showed that reconstruction and host linkage remain method-dependent, particularly for mobile sequences that differ in copy number and genomic composition from bacterial chromosomes. These limitations are directly relevant to dairy metagenomes, where plasmid or transposon detection should not automatically be interpreted as proof of a specific host association.

Future dairy AMR studies should therefore combine complementary approaches such as long-read sequencing, hybrid assemblies, plasmid reconstruction, and, where appropriate, proximity-linkage methods with cultivation and transfer assays. Particular priority should be given to clinically relevant ARGs associated with viable foodborne or opportunistic hosts and to elements detected repeatedly across farm, processing-environment, and product compartments.

Most importantly, experimental transfer should be evaluated under conditions that more closely reproduce dairy ecosystems. Current mating experiments are frequently conducted in optimized laboratory media, whereas transfer rates may differ considerably in raw milk, fermented milk, curd, brine, ripening cheese, biofilms, or refrigerated products. The highest evidence level proposed in Section 6—transfer demonstrated under representative dairy conditions—therefore remains one of the clearest research gaps.

11.5. From Phage Prediction to Experimentally Validated Host Interactions

Dairy phageome research faces a related gap between sequence-based prediction and biological interaction. Metaviromics can recover large numbers of vOTUs and infer potential hosts from CRISPR spacers, genomic similarity, prophage relationships, or co-abundance patterns [24,25]. These approaches substantially expand knowledge of viral diversity but do not necessarily demonstrate adsorption, infection, replication, or lysis.

Phage host range is determined by multiple interacting factors, including bacterial receptors, intracellular defense systems, phage counter-defense mechanisms, environmental conditions, and ongoing phage–host co-evolution. A recent synthesis by Leprince et al. [126] emphasizes that host range is dynamic and that experimental assessment of bacterial susceptibility remains fundamental for phage characterization and application. Computational host prediction is advancing rapidly, including protein-language-model approaches capable of extracting host-related information from viral sequences [127], but these predictions remain most useful when they guide rather than replace experimental validation.

Future dairy phage studies should therefore integrate metaviromics with phage isolation, host-range matrices, adsorption assays, receptor characterization, infectivity measurements, bacterial-defense profiling, and prophage-induction experiments. Longitudinal sampling should additionally determine whether predicted phage–host associations persist through processing or change with bacterial succession.

This requirement is relevant both to starter protection and to phage biocontrol. Industrial management requires information on which starter strains are susceptible to circulating phages, whereas biocontrol requires reliable characterization of target range, lytic behavior, genomic safety, matrix-dependent performance, and potential resistance development. Sequence prediction alone is insufficient for either application.

11.6. Integrative Multi-Omics and Translation into Industrial Decision-Making

The final research gap concerns translation. Dairy microbiology is increasingly capable of producing large taxonomic, genomic, transcriptomic, proteomic, metabolomic, and viromic datasets, but industrial value depends on whether these data lead to decisions that improve process consistency, shelf life, fermentation reliability, or microbiological safety. Multi-omics integration should therefore remain question-driven rather than data-driven. Studies combining metagenomics, metaproteomics, and metabolomics already demonstrate how microbial populations can be linked more directly with functional characteristics of ripening products [96]. However, future studies should extend this approach toward defined technological outcomes. Examples include identifying microbial signatures that predict spoilage before sensory failure becomes apparent, linking recurrent environmental strains to post-process contamination, determining whether specific ARG–MGE–host combinations persist after processing, or predicting starter-phage incompatibilities before fermentation failure occurs.

For such datasets to become useful beyond individual studies, methodological harmonization and interoperable metadata are necessary. The interlaboratory SOP work of Ferrocino et al. [120] and the STREAMS reporting framework [122] provide important foundations, while longitudinal analytical frameworks can assist in distinguishing correlations from temporally structured microbial transitions [123].

Computational and machine-learning approaches will be most valuable when they integrate microbial data with measurable processing variables and independently validated technological or safety outcomes. Predictive performance should be tested across factories, seasons, products, and production batches rather than assessed only within the dataset used for model development. Likewise, computational prediction of phage hosts, microbial sources, spoilage outcomes, or ARG mobility should be followed by experimental validation whenever the prediction is used to support a biological or technological conclusion.

The central research priority is therefore not to generate progressively larger inventories of microbial information, but to establish reproducible causal links from microbial identity to function, persistence, genetic mobility, technological outcome, and safety relevance. Achieving this transition will require standardized experimental designs, longitudinal sampling, complementary culture-dependent and culture-independent approaches, functional validation, and closer integration between dairy microbiology, food technology, bioinformatics, and risk assessment. Such studies would allow the microbiome–resistome–mobilome–phageome framework to move from descriptive ecosystem characterization toward experimentally supported tools for dairy quality and safety management.

12. Conclusions

This review critically integrates the microbiome, resistome, mobilome, and phageome within a process-oriented framework for interpreting dairy microbial ecosystems. Its principal contribution lies not in identifying previously unknown microbial interactions, but in connecting established biological mechanisms with the technological stages of dairy production and the strength of the evidence supporting their interpretation. By considering microbial host identity, antimicrobial resistance determinants, genetic mobility, and phage–host relationships together, the proposed framework helps distinguish changes in microbial community composition from changes in functional potential, persistence, and possible genetic dissemination. Importantly, it emphasizes that molecular detection, biological activity, transfer potential, and demonstrated risk represent distinct endpoints that cannot be considered interchangeable.

From a practical perspective, this integrated approach can inform more targeted microbiological investigations and quality-management decisions throughout the dairy pro-

duction chain. Longitudinal sampling and strain-level characterization can help identify potential microbial reservoirs associated with raw milk, processing equipment, production environments, and finished products. Combined microbiological and genomic analyses can support the investigation of persistent spoilage-associated populations, the characterization of starter cultures, and the assessment of bacteriophage-related fermentation disturbances. Similarly, identifying antimicrobial resistance determinants together with their bacterial hosts and genetic contexts can help prioritize isolates, genes, or processing environments requiring further investigation. These applications are intended to complement, rather than replace, established hygiene monitoring, culture-based testing, process validation, and food-safety management systems.

The reviewed evidence also reveals important limitations that currently restrict practical implementation. Many studies remain predominantly descriptive, and the detection of antimicrobial resistance genes or mobile genetic elements frequently lacks sufficient information on bacterial viability, gene expression, physical ARG–MGE linkage, and experimentally confirmed transfer. Likewise, the presence of phage-associated sequences does not necessarily establish infectivity, host range, or transduction capability. Differences in sampling design, sequencing approaches, bioinformatic pipelines, and reporting criteria further limit comparisons among dairy products and production systems. Consequently, the proposed evidence hierarchy and interpretive framework should not be regarded as validated quantitative risk-scoring or predictive tools.

Future investigations should prioritize longitudinal, multi-site studies that follow microbial populations and genetic determinants from primary production through transportation, processing, and storage, while explicitly distinguishing raw-milk products from those manufactured using thermized or pasteurized milk. Improved strain-resolved metagenomics, long-read sequencing, and complementary culture-dependent approaches are needed to strengthen ARG–host–MGE associations and distinguish genetic transfer potential from experimentally demonstrated transfer. Controlled studies under representative dairy-processing conditions should investigate the effects of temperature, fermentation, microbial competition, and matrix composition on horizontal gene transfer and phage–host interactions. Standardized experimental protocols and reporting criteria, together with validation across different facilities, products, and production batches, will be essential for translating omics-derived findings into reproducible technological and microbiological indicators.

An additional research priority is to establish whether integrated microbial and genetic measurements improve the prediction of spoilage, starter-culture failure, microbial persistence, or safety-relevant outcomes compared with conventional monitoring approaches. Such validation will require defined biological endpoints, independent datasets, and, where appropriate, integration with exposure assessment and quantitative microbial risk assessment. Until these relationships are demonstrated, the practical value of multi-omics integration lies primarily in improving the interpretation of available evidence and identifying where targeted experimental investigation is justified.

Ultimately, advancing dairy microbiology beyond taxonomic description requires a transition from identifying which microorganisms and genetic determinants are present to establishing which are viable, functionally active, persistent, transferable, and relevant to technological performance or food safety. The integrated perspective developed in this review provides a structured basis for this transition while highlighting the experimental evidence still needed before its broader application in dairy quality and safety management.

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