Review Article

Veterinary Probiotics in the Era of Antimicrobial Resistance: Therapeutic Promise, Resistance Gene Dissemination Risk, and One Health Implications: A Critical Review

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Abstract | This critical review aimed to synthesise the available evidence on the dual role of veterinary probiotics in the context of antimicrobial resistance (AMR), evaluate the safety risks posed by antibiotic resistance gene (ARG) carriage in commercial and experimental probiotic strains, and identify research and regulatory gaps within a One Health framework. A systematic literature search was conducted in PubMed/MEDLINE, Web of Science, and Scopus/Frontiers covering January 2012 to April 2026, following PRISMA 2020 guidelines. Search terms combined 'veterinary probiotics', 'antimicrobial resistance', 'antibiotic resistance genes', 'horizontal gene transfer', 'One Health', and livestock species. From 4,034 initially identified records, 29 studies met eligibility criteria following deduplication, title/abstract screening, full-text assessment, and quality evaluation. Probiotic genera including *Lactobacillus, Bacillus, Enterococcus*, and *Pediococcus* demonstrated significant benefits- pathogen exclusion, immune modulation, gut barrier reinforcement, and gut resistome suppression- across multiple livestock species. However, whole-genome sequencing and minimum inhibitory concentration (MIC) testing revealed that commercial and experimental probiotic strains harbour clinically significant ARGs, including plasmid-borne tetS, efmA, and APH(3')-Ia determinants. The gastrointestinal tract creates favourable conditions for horizontal gene transfer (HGT) via conjugative plasmids and mobile genetic elements (MGEs). Concurrent probiotic-antibiotic use was found to amplify macrolide resistance gene carriage in porcine microbiota. Regulatory frameworks remain inadequate, particularly for companion animal probiotic products, where mandatory ARG screening is absent in most jurisdictions. Veterinary probiotics represent valuable, evidence-based alternatives to antibiotic growth promoters, but their application must be guided by mandatory whole-genome sequencing, strain-specific safety profiling, and One Health-integrated surveillance to prevent inadvertent acceleration of the global AMR crisis.

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Introduction

Antimicrobial resistance (AMR) has emerged as a defining public health crisis of the modern era. In 2019, approximately 1.27 million human deaths were directly attributable to bacterial AMR, with projections suggesting this figure could reach 10 million annually by 2050 if current trajectories remain unchecked (Antimicrobial Resistance Collaborators, 2022; Naghavi *et al*., 2024). The burden of AMR is not confined to human medicine- it extends deeply into veterinary medicine and food-producing animal systems. Animal agriculture accounts for a disproportionately large share of global antibiotic consumption, and antimicrobial use in food animals is projected to increase by up to 67% in low- and middle-income countries by 2030 (Van Boeckel *et al*., 2015).

In response to growing recognition of veterinary antibiotic use as a driver of AMR, the European Union enacted a landmark ban on antibiotic growth promoters (AGPs) in 2006, and EU Regulation 2019/6 further curtailed their routine use (European Parliament and Council 2018). These regulatory shifts have accelerated the search for effective alternatives, with veterinary probiotics- defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill *et al*., 2014), among the most extensively investigated. The global animal feed probiotics market is projected to approach USD 76 billion by 2026 (Global Market Insights, 2023).

However, a critical and underappreciated paradox has emerged: Probiotic strains- despite widely regarded safety profiles- may themselves harbour transferable ARGs, positioning them as potential vectors for the very resistance they are intended to mitigate (Kerek *et al*., 2025a, b). This concern is amplified by the dense microbial milieu of the gastrointestinal tract, which provides ideal conditions for HGT via conjugation, transformation, and transduction (Kim and Cha, 2021). The simultaneous use of probiotics and antibiotics- a common clinical practice- may further exacerbate ARG dissemination (Monger *et al*., 2024).

Despite the growing evidence base, several critical research gaps persist. Regulatory frameworks in many jurisdictions do not mandate comprehensive ARG profiling of commercial probiotic products (Kerek *et al*., 2024c), and strain-specific data on ARG carriage, dose-response relationships, and long-term ecological consequences remain scarce (Idowu *et al*., 2025). This review aims to critically synthesise the available evidence on the dual nature of veterinary probiotics in the AMR context, evaluate methodological strengths and limitations of the underlying studies, identify specific research gaps, and propose directions for future investigation within the One Health framework.

Materials and Methods

This narrative critical review was conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 reporting guidelines (Page *et al*., 2021). All methodological steps are detailed below to ensure transparency and reproducibility.

Databases searched

A systematic literature search was performed across three major academic databases: (1) PubMed/MEDLINE, (2) Web of Science (Core Collection), and (3) Scopus/Frontiers in Medicine platform. These databases were selected for their broad coverage of veterinary science, microbiology, and AMR-related literature.

Search terms and strategy

The following Boolean search string was applied consistently across all databases: ("veterinary probiotics" OR "animal feed probiotics" OR "livestock probiotics") AND ("antimicrobial resistance" OR "antibiotic resistance genes" OR "horizontal gene transfer" OR "One Health" OR "ARG" OR "resistome" OR "Lactobacillus" OR "Bacillus" OR "Enterococcus") AND ("livestock" OR "poultry" OR "swine" OR "cattle" OR "companion animals").

Inclusion and exclusion criteria

Inclusion criteria: (a) peer-reviewed articles published in English; (b) publication date between January 2012 to April 2026; (c) studies directly investigating probiotic efficacy against AMR, ARG carriage in probiotic strains, HGT from probiotic bacteria, or regulatory/safety evaluation of veterinary probiotic products; (d) in vitro, *In vivo*, and review articles with primary data components.

Exclusion criteria: (a) articles not available in full text; (b) studies restricted to human medicine with no veterinary relevance; (c) conference abstracts, book

chapters, and grey literature; (d) articles focusing exclusively on non-probiotic microbiota interventions (e.g., fecal transplantation); (e) studies with no data on AMR-related outcomes.

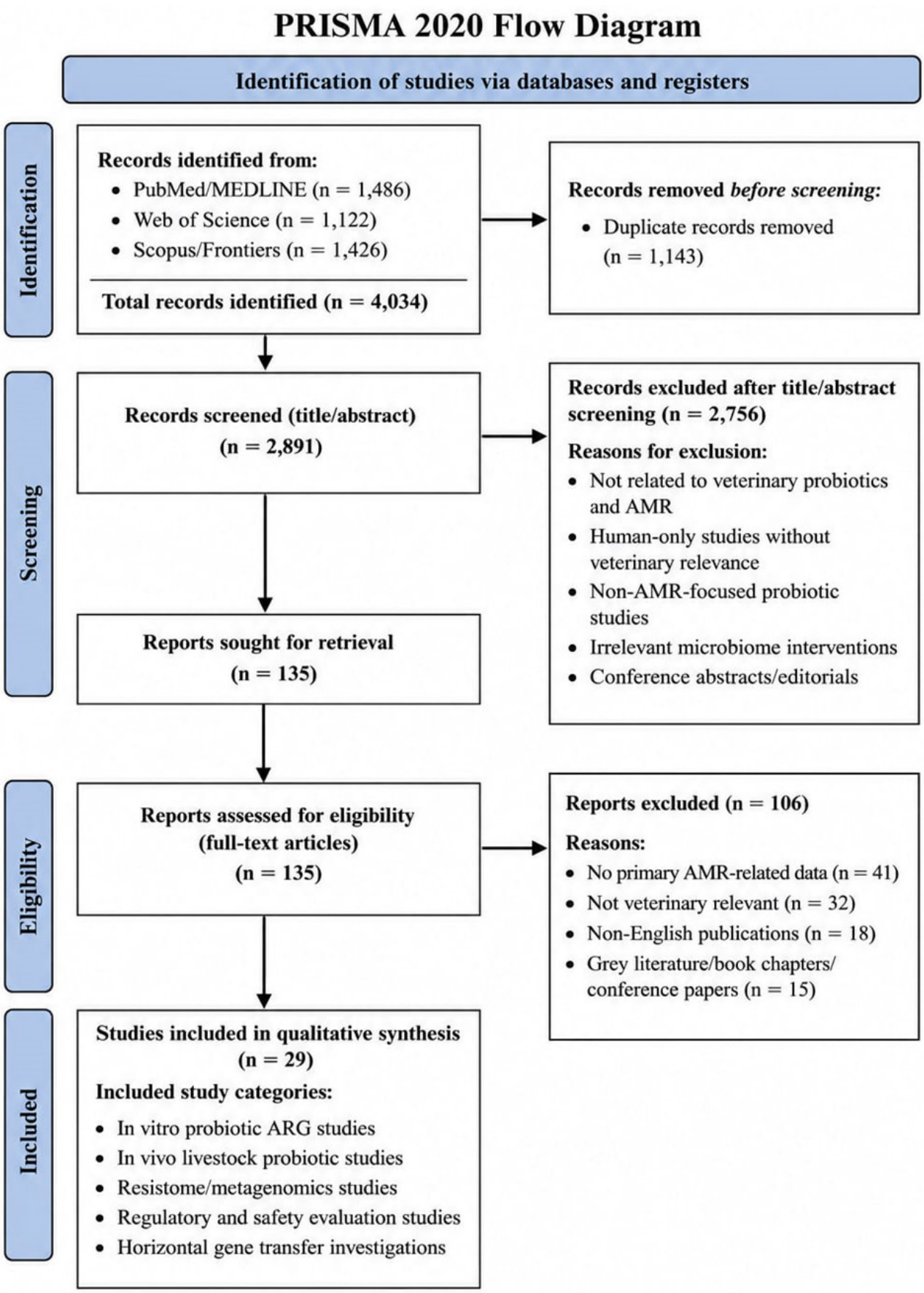

Figure 1: *PRISMA 2020 flow diagram illustrating the study selection process for this review on veterinary probiotics, antimicrobial resistance, and One Health implications.*

Time period

The search covered publications from January 2012 to April 2026, spanning 14 years.. This period was chosen to capture modern regulatory developments (e.g., EU AGP ban impact, PRISMA, 2020 publication) and the emergence of whole-genome sequencing as a routine ARG screening tool in veterinary contexts.

Screening strategy and article selection

A total of 4,034 records were initially identified across all databases. Following automated deduplication, 2,891 unique records remained. Title and abstract screening excluded 2,756 records that did not meet inclusion criteria. Full-text assessment was performed on 135 articles, of which 106 were excluded (no primary AMR data: n=41; not veterinary-relevant: n=32; non-English: n=18; grey literature: n=15). A total of 29 studies met all eligibility criteria and were included in the final qualitative synthesis. The complete selection process is illustrated in Figure 1 (PRISMA, 2020 flow diagram).

Quality assessment

Included studies were evaluated for methodological quality using domain-based criteria adapted for narrative reviews: (a) clarity of study design; (b) adequacy of sample size; (c) specificity of strain characterisation (phenotypic vs. genotypic); (d) use of standardised susceptibility testing (MIC, EUCAST/CLSI breakpoints); (e) statistical reporting; and (f) conflict of interest disclosure. Studies were classified as high, moderate, or low quality, and quality-specific limitations are discussed in the relevant sections.

The Global AMR Crisis: Veterinary Medicine at the Nexus

Scope and scale of AMR in animal agriculture

The global burden of AMR in veterinary contexts is both substantial and complex. Antimicrobials are routinely deployed in livestock for therapeutic, prophylactic, metaphylactic, and historically growth-promotion purposes (Van Boeckel *et al.*, 2015). The selective pressure exerted by sub-therapeutic antibiotic use drives the emergence of resistance not only in target pathogens but also in commensal bacteria that serve as ARG reservoirs (Pandey *et al.*, 2024). Critically, ARGs disseminate beyond farm boundaries through manure application to agricultural land, contaminated water bodies, aerosol transmission, and the food chain, creating complex environmental resistomes that intersect with human clinical settings (González Zorn and Escudero García-Calderón, 2012).

The livestock gut microbiome serves as a major reservoir of ARGs. Metagenomics and whole-genome sequencing (WGS) have revealed that enteric bacteria in food animals harbour diverse ARG classes-encoding resistance to tetracyclines, aminoglycosides, beta-lactams, macrolides, and fluoroquinolones-many of which are medically important in human therapeutics (Young *et al.*, 2022). Mobile genetic elements (MGEs), including conjugative plasmids, integrons, and transposons, facilitate the intra- and inter-species transfer of these ARGs, accelerating their dissemination across ecological compartments (Kim and Cha, 2021).

Table 1: *Key AMR burden estimates and One Health indicators relevant to veterinary medicine.*

Parameter	Key Finding	Reference	Year
Human deaths attributable to AMR (2019)	1.27 million deaths directly attributable; 4.95 million associated	Antimicrobial Resistance Collaborators (2022)	2022
Projected AMR deaths by 2050	Up to 10 million deaths/year if unchecked; largest burden in Sub-Saharan Africa and Asia	Naghavi *et al.* (2024)	2024
Veterinary AMU projections	Global antimicrobial use in food animals projected to increase by 67% by 2030, predominantly in LMICs	Van Boeckel *et al.* (2015)	2015
EU AGP ban impact	AGP ban demonstrated feasibility of maintaining productivity without antibiotics for growth promotion	Laxminarayan et al. (2015)	2019
Global probiotic market	Animal feed probiotics market projected to approach USD 76 billion by 2026	Global Market Insights (2023)	2023
One Health surveillance gap	AST/ART surveillance practices must be harmonised; veterinary diagnostic laboratory stewardship urgently needed	Maddock *et al.* (2024)	2024

AMR, antimicrobial resistance; AMU, antimicrobial use; AGP, antibiotic growth promoter; LMICs, low- and middle-income countries; AST, antimicrobial susceptibility testing.

The one health imperative

AMR is fundamentally a One Health issue, operating at the interface of human, animal, and environmental health (González Zorn and Escudero García-Calderón, 2012; Young *et al.*, 2022). The gut microbiomes of livestock workers have been shown to resemble those of the animals they tend, carrying ARGs characteristic of farm environments. Agricultural runoff carries resistant bacteria and resistance determinants into water systems, soil, and ultimately food products consumed by humans. An eco-evolutionary approach to AMR- recognising the role of selection, HGT, and ecological interactions across One Health sectors- is essential for developing effective mitigation strategies (Bustamante *et al.*, 2025).

Despite this recognition, integrated One Health surveillance systems that unify data from veterinary, environmental, and human medicine remain underdeveloped in many regions. Harmonisation of antimicrobial susceptibility testing (AST) and surveillance practices across these sectors is critical but has not yet been achieved at a global scale (Maddock *et al.*, 2024). This context makes evaluation of probiotic safety as potential ARG vectors all the more urgent (Table 1).

Beneficial mechanisms of veterinary probiotics against AMR

The following sub-sections critically evaluate the evidence base for each mechanism. Where studies present limitations in sample size, experimental design, or generalisability, these are explicitly noted.

Competitive exclusion and pathogen suppression

One of the most well-characterised mechanisms by which probiotics combat AMR is competitive exclusion- the ability of probiotic organisms to outcompete pathogenic bacteria for nutrients, adhesion sites, and ecological niches within the gastrointestinal tract (Ibeagha-Awemu *et al.*, 2025). In poultry, *Lacticaseibacillus rhamnosus* GG reduced *Salmonella Typhimurium* caecal colonisation by approximately 1.9 log CFU (Closs Jr *et al.*, 2025). However, this study was conducted in a controlled broiler challenge model (n= 120 birds), and the single-strain design limits conclusions about multi-strain commercial products. Similarly, *Lactobacillus plantarum* produces plantaricin with antagonistic activity against *Salmonella* and *Escherichia coli* in broiler chickens (Sachdeva *et al.*, 2025), though most supporting data derive from in vitro agar diffusion assays rather than confirmed *In vivo* colonisation data.

In swine, *Bifidobacterium strains* reduced *Clostridium perfringens* prevalence, while *Lactobacillus acidophilus* and *Enterococcus faecium* combinations reduced *E. coli* abundance in dairy cattle (Sachdeva *et al.*, 2025). In ruminants, *Pediococcus acidilactici* pediocin demonstrated activity against *Listeria monocytogenes* (Sachdeva *et al.*, 2025). A critical limitation common to these studies is the absence of dose-response data and standardised outcome metrics across species, making direct cross-study comparison difficult.

Bacteriocin production and antimicrobial peptides

Probiotics produce diverse antimicrobial compounds including organic acids, hydrogen peroxide, bacteriocins, and antimicrobial peptides that create a hostile environment for pathogens (Ibeagha-Awemu *et al.*, 2025; Mârza *et al.*, 2025). Bacteriocins- ribosomally synthesised antimicrobial peptides- exhibit broad-spectrum activity against clinically significant pathogens including drug-resistant strains (Khalid *et al.*, 2025). *Lactobacillus reuteri* produces reuterin, which inhibits *Staphylococcus aureus* in swine, while *Bacillus subtilis* produces subtilin with activity against *S. aureus* in cattle (Sachdeva *et al.*, 2025). Methodologically, most bacteriocin studies rely on minimum inhibitory concentration (MIC) assays and disc diffusion in controlled laboratory settings. *In vivo* confirmation of clinical efficacy in field conditions remains limited, and resistance to bacteriocins- an understudied phenomenon- warrants further investigation.

Gut barrier enhancement and immune modulation

Probiotics reinforce the intestinal epithelial barrier- a critical line of defence against pathogen invasion. *Lactobacillus rhamnosus* increases mucus production in poultry, while *Bifidobacterium* lactis strengthens tight junction integrity in calves, reducing susceptibility to *S. aureus* intramammary infections (Sachdeva *et al.*, 2025). *Bacillus subtilis* up-regulates tight-junction proteins, cytokines, and immunoglobulins, with dual-strain formulations demonstrating consistent immunostimulatory effects (Mârza *et al.*, 2025). In poultry challenge models, a multi-species *Lactobacillus-Bacillus* probiotic achieved *Salmonella Enteritidis* clearance comparable

Table 2: *Summary of beneficial mechanisms of veterinary probiotics and their anti-AMR implications.*

Mechanism	Probiotic strains	Target/Effect	Species	Reference
Competitive exclusion	*L. rhamnosus* GG	Reduced *Salmonella typhimurium* ~1.9 log CFU in caecum	Poultry	Closs Jr *et al.* (2025)
Competitive exclusion	*Bifidobacterium* spp.	Reduced *C. perfringens* prevalence; GI disorder control	Swine	Sachdeva *et al.* (2025)
Bacteriocin production	*L. plantarum* (plantaricin)	Inhibits *Salmonella* and *E. coli*; reduces pathogen load	Poultry	Sachdeva *et al.* (2025)
Bacteriocin production	*B. subtilis* (subtilin)	Inhibits *S. aureus*; reduces mastitis incidence	Cattle	Sachdeva *et al.* (2025)
Gut barrier enhancement	*B. subtilis* (multi-strain)	Upregulates tight-junction proteins, cytokines, IgA	Poultry/Cattle	Mârza *et al.* (2025)
Immune modulation	*L. rhamnosus* GG	Hastened *S. enteritidis* clearance; comparable to oxytetracycline	Poultry	Mârza *et al.* (2025)
Resistome modulation	*C. butyricum*/ LAB	Reduced tet and sul ARG abundance; downregulates HGT genes	Poultry	Jian *et al.* (2026)
Synbiotic effect	*L. plantarum* + Oligosaccharides	Stable ARG reduction; enhanced SCFA; pH-mediated ARB suppression	Poultry/Swine	Jian *et al.* (2026)

LAB, lactic acid bacteria; ARG, antibiotic resistance gene; ARB, antibiotic-resistant bacteria; SCFA, short-chain fatty acid; HGT, horizontal gene transfer; IgA, immunoglobulin A.

to an oxytetracycline reference group (Mârza *et al.*, 2025), a finding of particular translational value. However, the small sample sizes in several immunological studies (frequently n= 20-30 animals per group) and the absence of blinding protocols in some trials introduce risk of bias that should be acknowledged.

Reduction of AMR genes in the gut resistome

Beyond direct pathogen suppression, probiotics may alter gut resistome composition. Studies in chickens demonstrated that *Clostridium butyricum* and lactic acid bacteria reduced the abundance of tetracycline (tet) and sulfonamide (sul) resistance genes, while downregulating HGT-facilitating genes (Jian *et al.*, 2026). Synbiotic formulations further stabilised these reductions by promoting short-chain fatty acid (SCFA) production, which lowers gut pH and suppresses resistant bacterial growth (Jian *et al.*, 2026). Notably, these findings derive primarily from 16S rRNA and qPCR-based ARG quantification; metagenomics-based confirmation of functional ARG suppression remains limited, representing a methodological gap (Table 2).

The Hidden Risk: ARGs in Veterinary Probiotic Strains

This section critically examines the evidence for ARG carriage in probiotic strains, with explicit discussion of the experimental techniques used to detect and characterise such genes. Understanding the methodological basis for ARG detection is essential for evaluating the strength and limitations of published findings.

Key experimental methodologies for arg detection

The detection of ARGs in probiotic strains has relied on several complementary methodological approaches, each with distinct strengths and limitations According to (Kerek *et al.*, 2025a; Zhu *et al.*, 2016).

Whole-Genome Sequencing (WGS): WGS provides comprehensive genomic characterisation, enabling identification of all ARGs present in a strain, determination of their chromosomal versus plasmid location, and mapping of mobile genetic elements (MGEs) such as integrons, insertion sequences, and transposons. Short-read platforms (Illumina) are widely used for ARG identification, while long-read technologies (Oxford Nanopore, PacBio) are increasingly employed to resolve complete plasmid architectures. The primary limitation is that WGS identifies genetic presence but does not confirm phenotypic expression.

Minimum Inhibitory Concentration (MIC) Testing: MIC testing, conducted according to EUCAST or CLSI breakpoints, provides phenotypic evidence of resistance. Antibiotics tested in veterinary probiotic safety studies typically include gentamicin, amoxicillin,

tetracycline, chloramphenicol, florfenicol, tylosin, trimethoprim-sulphamethoxazole, streptomycin, and erythromycin. The limitation is that MIC testing detects the net phenotypic outcome but does not identify the specific resistance mechanism.

PCR-Based ARG Detection: Targeted polymerase chain reaction (PCR) assays amplify known ARG sequences (e.g., tet(S), erm(B), aph(3')) from extracted genomic DNA. Multiplex PCR panels allow simultaneous screening of multiple ARG families. While rapid and cost-effective, PCR is limited to known ARG sequences and cannot detect novel or divergent resistance determinants.

Conjugation Assays: To assess actual HGT potential, conjugation assays involve co-culture of probiotic donor strains with suitable recipient bacteria (e.g., *Enterococcus faecalis* JH2-2 or *Escherichia coli* K-12 derivatives) on selective media, followed by confirmation of ARG transfer in transconjugants by PCR and WGS. This provides direct experimental evidence of transferability, though in vitro conjugation frequencies may not reflect *In vivo* gut conditions.

Metagenomics: Metagenomic sequencing of gut or environmental DNA allows culture-independent characterisation of the resistome in complex microbial communities. Shotgun metagenomics provides functional ARG profiles and can detect HGT events *In vivo*. Bioinformatic analysis using databases such as CARD (Comprehensive Antibiotic Resistance Database) or ResFinder enables standardised ARG annotation. Current limitations include high cost, incomplete database coverage of novel ARGs, and difficulty distinguishing mobile from chromosomally-fixed resistance.

Plasmid Profiling: Alkaline lysis-based plasmid extraction followed by agarose gel electrophoresis, restriction mapping, and replicon typing (PCR-based PBRT) identifies the number, size, and incompatibility group of plasmids in probiotic strains. Combined with WGS, this allows complete characterisation of plasmid-borne ARG content. The limitation is that some large plasmids are poorly recovered by standard extraction protocols.

Evidence of ARG carriage in common probiotic genera

A landmark study by Kerek *et al.* (2025a) characterised the ARG profiles of the most commonly used veterinary probiotic genera using combined WGS and MIC testing. Even strains with established safety records were found to carry clinically significant resistance determinants. In *Lactobacillus* species, *L. rhamnosus* and *L. plantarum* harboured oxacillin and cephalosporin resistance genes, with multiple isolates additionally carrying aminoglycoside, glycopeptide, and folate synthesis inhibitor resistance genes (Kerek *et al.*, 2024c). *Bacillus licheniformis* demonstrated intrinsic chloramphenicol and clindamycin resistance, attributed to species-specific chromosomally encoded efflux pumps (blt, bmr) (Tran *et al.*, 2024) (Table 3).

In a phenotypic and genotypic characterisation study of industrially applied veterinary probiotic strains, MIC testing of *Enterococcus faecium, Bacillus licheniformis*, *Bacillus subtilis*, *L. rhamnosus*, and *Pediococcus acidilactici* revealed resistance to clinically relevant antibiotics including gentamicin (MIC >32 µg/mL for *E. faecium*), amoxicillin, tylosin, and florfenicol. WGS identified 27 distinct ARGs in these strains, primarily associated with efflux pump mechanisms and target protection or modification (Kerek *et al.*, 2025b). A limitation of this study is that conjugation assays to confirm *in vitro* transferability were not performed for all identified ARGs.

ARGs in commercial veterinary probiotic products

The presence of ARGs extends beyond individual laboratory strains to commercially available veterinary probiotic products. A study of ARG profiles in probiotic preparations for companion animals identified plasmid-borne ARGs in two products- including the tetS gene and APH(3')-Ia gene- on MGEs with significant transfer potential (Kerek *et al.*, 2024c). Critically, a novel *L. plantarum*-derived *efmA* resistance gene, previously undescribed in this species, was detected, highlighting the ongoing discovery of novel resistance determinants in probiotic strains (Kerek *et al.*, 2024c). Of all ARGs identified, 57.9% were located on plasmids- inherently transferable elements.

A large-scale Chinese study of 33 commercial veterinary probiotic products across 13 provinces used antimicrobial susceptibility testing and next-generation sequencing (NGS), revealing widespread ARG carriage in *Bacillus* spp. isolates across multiple antibiotic classes (Guan *et al.*, 2025). A Thai study similarly found broad antibiotic resistance in *Lactobacillus* and *Bacillus* isolates from commercial

probiotic products for food-producing animals (Tran *et al*., 2024). A shared limitation across both studies is that they did not perform conjugation assays to confirm transferability; ARG transfer risk was inferred from MGE location alone.

Horizontal gene transfer: From probiotic to pathogen

The critical risk posed by ARG-harbouring probiotics is their potential to facilitate HGT to co-residing pathogenic or commensal bacteria within the gut ecosystem. The gastrointestinal tract, with its high microbial density, abundant MGEs, and optimal biochemical conditions, represents one of the most active environments for bacterial gene exchange (Kim and Cha, 2021; Lerner *et al*., 2017). HGT occurs through three primary mechanisms: conjugation (plasmid-mediated direct cell-to-cell contact), transformation (uptake of free DNA), and transduction (phage-mediated transfer) (Kim and Cha, 2021).

A pivotal study by Monger *et al*. (2024) examined the effect of probiotics and antibiotics on the porcine gut mobilome using a randomised *in vivo* swine model. Macrolide resistance genes were detected in significantly higher proportions in the microbiome of pigs treated with antibiotics or the combination of probiotics and antibiotics, compared to controls. Resistance-carrying conjugative plasmids from *E. coli* and *Klebsiella pneumoniae* were amplified in fecal samples from antibiotic-treated and combination-treated animals, providing direct evidence that concurrent probiotic-antibiotic use does not mitigate- and may enhance- plasmid-mediated ARG spread. The study's limitation is a relatively short observation period (8 weeks), and long-term resistome dynamics following combined treatment remain unknown.

The GRAS status paradox

Many probiotic strains carry 'Generally Recognised as Safe' (GRAS) or Qualified Presumption of Safety (QPS) designations. However, these classifications were developed to assess pathogenicity and toxin production- not ARG carriage and transferability. As a result, GRAS/QPS-designated strains may still serve as ARG reservoirs when introduced into the food chain (Kerek *et al*., 2025b). This represents a critical regulatory gap. The integration of mandatory WGS-based ARG screening into safety evaluation frameworks is now technically feasible and has been recommended by multiple expert groups (Kerek *et al*., 2025a, b).

Regulatory Landscape and Critical Gaps

Current regulatory frameworks

The regulatory oversight of veterinary probiotics varies markedly across jurisdictions. In the European Union, the European Food Safety Authority (EFSA) evaluates probiotic microorganisms for feed use, assessing pathogenicity and antimicrobial susceptibility. EU regulations for livestock probiotic products prohibit inclusion of resistance genes of public health concern; however, these requirements are substantially less stringent for companion animal products, and ARG assessment methods are not yet standardised (Kerek et al., 2024c). In the United States, probiotic microorganisms used in animal feed fall under GRAS designation overseen by the FDA, which does not currently mandate systematic ARG profiling by WGS. In many low- and middle-income

Table 3: *ARG profiles detected in commonly used veterinary probiotic genera.*

Species	ARGs Detected	Mechanism	Transfer Risk	Reference	Year
L. rhamnosus	Oxacillin, cephalosporin resistance; efmA	Intrinsic; target modification; efflux pump	Moderate (plasmid-borne in some strains)	Kerek *et al*. (2024c)	2024
L. plantarum	Penicillin, cephalosporin resistance; novel efmA gene	Beta-lactamase; efflux; novel gene	High (plasmid-borne novel ARG)	Kerek *et al*. (2024c)	2024
B. licheniformis	Chloramphenicol, clindamycin; efflux genes (blt, bmr)	Intrinsic; chromosomal efflux	Low-moderate (primarily chromosomal)	Kerek *et al*. (2025a, 2025b)	2025
E. faecium	Gentamicin resistance (MIC >32 µg/mL); trimethoprim-sulfa resistance	Aminoglycoside-modifying enzymes; DHFR alteration	High (plasmid-borne conjugative ARGs)	Kerek *et al*. (2025a; 2025b)	2024-2025
P. acidilactici	tetS (plasmid-borne); variable resistance	Ribosomal protection protein; efflux	High (tetS on transferable MGE)	Kerek *et al*. (2024c)	2024

ARG, antibiotic resistance gene; MGE, mobile genetic element; NGS, next-generation sequencing; MIC, minimum inhibitory concentration; DHFR, dihydrofolate reductase. Transfer risk based on ARG location (plasmid vs. chromosomal) and confirmed or inferred transferability.

countries, regulatory oversight is minimal or absent, and commercial probiotic products may reach livestock farms without any AMR assessment (Idowu *et al.*, 2025). This regulatory heterogeneity creates significant public health risks in an era of globalised food trade.

Critical research and policy gaps

Based on synthesis of the current evidence base, five critical research and policy gaps are identified and prioritised below (Table 4):

- First, standardised ARG screening protocols for veterinary probiotic registration are lacking globally. Mandatory WGS combined with phenotypic MIC testing according to EUCAST/CLSI breakpoints should be required as a condition for commercial approval across all jurisdictions and animal species (Kerek *et al.*, 2025a, c).
- Second, strain-specific safety data remain insufficient. Despite extensive literature on probiotic efficacy, comprehensive data on strain-specific ARG profiles, dose-response relationships for ARG transfer risk, and standardised application protocols across diverse livestock species are scarce (Idowu *et al.*, 2025). Strains with non-transferable intrinsic ARGs may present acceptable risk profiles, while those with plasmid-borne transferable ARGs require far more stringent scrutiny.
- Third, the ecological and epidemiological consequences of long-term probiotic use on farm-level resistomes have not been adequately characterised. Long-term longitudinal studies employing metagenomics and metatranscriptomics are needed to assess whether regular probiotic administration results in net ARG enrichment or depletion within livestock gut microbiomes and surrounding farm environments (Jian *et al.*, 2026).
- Fourth, the interaction between concurrent probiotic and antibiotic use- a common scenario in clinical veterinary practice- requires dedicated investigation. Randomised controlled trials examining ARG dynamics with concurrent versus sequential probiotic-antibiotic regimens are urgently needed. The porcine mobilome study demonstrated that combining probiotics with antibiotics did not reduce ARG burden and may have facilitated plasmid spread (Monger *et al.*, 2024), with direct implications for clinical management protocols.
- Fifth, regulatory equity between livestock and companion animal products must be addressed. The current disparity- whereby livestock probiotic products face stricter ARG regulation than companion animal products- creates a critical blind spot given the close human-animal bond and frequent physical contact between pets and owners (Kerek et al., 2024c).

One Health Implications and Future Directions

The dual nature of veterinary probiotics- as both AMR mitigation tools and potential ARG vectors- exemplifies the complexity of managing resistance

Table 4: *Critical research gaps in veterinary probiotic safety and AMR risk- prioritisation framework.*

S.	Research gap	Current limitation	Recommended action	Priority	Reference
1	Standardised ARG screening protocols	No mandatory WGS + phenotypic AST required for commercial probiotic registration globally	Require WGS + AST as condition for approval in all jurisdictions and species categories	Critical	Kerek *et al.* (2025a; 2024c)
2	Strain-specific safety data	Paucity of strain-specific ARG profiles, dose-response data, and standardised protocols across livestock species	Mandate strain-level WGS and MGE characterisation; publish strain passport databases	High	Idowu *et al.* (2025)
3	Long-term ecological consequences	No longitudinal metagenomics studies assessing net ARG enrichment/depletion in farm environments	Conduct 12–24 month farm-level metagenomics studies monitoring resistome and mobilome dynamics	High	Jian *et al.* (2026)
4	Probiotic-antibiotic interaction effects	Concurrent probiotic + antibiotic use shown to facilitate plasmid spread; clinical implications poorly characterised	Randomised controlled trials examining ARG dynamics with concurrent vs. sequential regimens	High	Monger *et al.* (2024)
5	Regulatory equity: livestock vs. companion animals	EU restricts ARGs in livestock probiotics but not companion animal products a blind spot given human-pet proximity	Extend livestock-level ARG restrictions to companion animal probiotic products globally	Moderate	Kerek *et al.* (2024c)

WGS, whole-genome sequencing; AST, antimicrobial susceptibility testing; MGE, mobile genetic element; ARG, antibiotic resistance gene. Priority levels assigned based on immediacy of public health risk, availability of feasible interventions, and evidence strength.

Table 5: *Emerging technologies and strategies for safer veterinary probiotic development.*

Technology/ Strategy	Mechanism/Description	Current Status	Key Limitation	Reference
CRISPR-Cas ARG deletion	Precise genomic removal of ARGs from probiotic strains while preserving beneficial functional properties	Pre-clinical/early development phase	Regulatory GMO classification ambiguity; off-target effects	Talat and Khan (2024)
Postbiotics	Heat-inactivated probiotic cells/cell-wall fragments retaining immunostimulatory activity without live organism HGT risk	Regulatory framework being established (ISAPP 2022 definition)	Less data on *In vivo* veterinary efficacy vs. live probiotics	Vinderola *et al.* (2022)
WGS strain libraries	Curated databases of veterinary probiotic strains with complete ARG profiles, plasmid content, and MGE mapping	Emerging; some EU member state initiatives underway	No international standardisation; incomplete coverage of commercial strains	Kerek *et al.* (2025a; 2025b)
Synbiotics (ARG-screened)	ARG-screened probiotic strains combined with prebiotics; SCFA production suppresses ARB and downregulates HGT genes	Promising preclinical and some clinical evidence in poultry and swine	Strain selection complexity; variable host microbiota interactions	Jian *et al.* (2026)
One Health resistome surveillance	Integrated metagenomics monitoring of human, animal, and environmental resistomes linked to probiotic usage data	Conceptual framework established; few operational systems	High cost; bioinformatics standardisation needed	Bustamante *et al.* (2025); Maddock *et al.* (2024)

CRISPR-Cas, clustered regularly interspaced short palindromic repeats-associated protein; GMO, genetically modified organism; HGT, horizontal gene transfer; ARG, antibiotic resistance gene; ISAPP, International Scientific Association for Probiotics and Prebiotics; SCFA, short-chain fatty acid; ARB, antibiotic-resistant bacteria; MGE, mobile genetic element.

within a One Health framework. Probiotic-derived ARGs that transfer to pathogenic bacteria in the livestock gut may ultimately reach human populations through the food chain, direct animal contact, or environmental routes (González Zorn and Escudero García-Calderón, 2012; Lerner *et al.*, 2017). This transmission pathway represents a previously underappreciated link in AMR dissemination.

From a One Health perspective, an integrated surveillance approach is needed- one that tracks not only AMR in clinical pathogens but also the ARG content of commercial probiotic products, the resistome composition of probiotic-supplemented livestock populations, and the downstream environmental ARG burden from probiotic-supplemented farm systems (Bustamante *et al.*, 2025; Maddock *et al.*, 2024). Environmental resistome monitoring- including wastewater, surface water, and soil- is feasible and cost-effective when combined with modern metagenomics platforms (Bustamante *et al.*, 2025).

Promising technological developments offer pathways to safer probiotic formulations (Table 5). Next-generation probiotics engineered using CRISPR-Cas systems offer the possibility of precise ARG deletion while preserving beneficial functional properties (Talat and Khan, 2024). Postbiotics- preparations of inanimate microorganisms or their components- circumvent HGT risk while retaining immunomodulatory and antimicrobial properties (Vinderola *et al.*, 2022). However, both approaches require substantial further research and regulatory clarity before widespread veterinary deployment.

Ultimately, responsible probiotic stewardship in veterinary medicine must be founded on the same evidence-based principles as antibiotic stewardship: rigorous strain characterisation, minimum necessary dosing, species-appropriate formulation, avoidance of resistance-selecting combinations, and continuous post-market surveillance. The probiotic industry's trajectory toward a multi-billion-dollar global market makes the establishment of robust safety frameworks an urgent scientific and regulatory priority (Global Market Insights, 2023; Kerek *et al.*, 2025a).

Conclusion

Veterinary probiotics have emerged as a promising component of sustainable livestock production systems, particularly in the era of increasing antimicrobial resistance. The evidence synthesised in this review demonstrates that well-characterised probiotic strains can effectively reduce pathogen colonisation, enhance gut barrier integrity, modulate immune responses, and contribute to measurable

reductions in gut resistome burden. These multifaceted benefits position probiotics as valuable alternatives or adjuncts to antibiotics in animal agriculture, strongly aligning with global One Health strategies.

However, this review also highlights a critical and underrecognised paradox: veterinary probiotics may simultaneously function as reservoirs and vectors of antibiotic resistance genes, particularly when strains harbour plasmid-borne or mobile genetic elements capable of horizontal gene transfer. Experimental methodologies including WGS, MIC testing, conjugation assays, metagenomics, and plasmid profiling collectively provide evidence of this dual role, though important methodological limitations- including small sample sizes, limited conjugation data, and short observation periods- constrain the strength of conclusions that can be drawn from individual studies.

Therefore, while probiotics remain an important tool for AMR mitigation, their application must be guided by rigorous strain-level genomic screening, standardised regulatory frameworks incorporating mandatory WGS and phenotypic susceptibility testing, and continuous post-market surveillance integrated within a One Health approach. Future research must prioritise long-term longitudinal resistome studies, randomised trials of probiotic-antibiotic combination regimens, and harmonisation of companion animal probiotic regulations with existing livestock standards. Only through such evidence-based stewardship can the therapeutic benefits of veterinary probiotics be maximised while minimising their potential contribution to the global AMR crisis.

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Novelty Statement

This review uniquely examines veterinary probiotics as both antimicrobial resistance (AMR) mitigation tools and potential sources of antibiotic resistance gene (ARG) dissemination. By integrating recent evidence on ARG carriage, horizontal gene transfer, gut resistome dynamics, and regulatory gaps, it provides a balanced risk-benefit assessment within a One Health framework. The review further highlights the need for mandatory whole-genome sequencing-based safety screening and harmonized probiotic stewardship strategies in veterinary medicine.

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