Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.329

REVIEW ARTICLE

Applications of Beneficial Microbes and Natural Antimicrobials in Poultry with Integrated Strategies for Food Safety: A Review

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ARTICLE HISTORY (25-1041)

Received: October 26, 2025
Revised: December 25, 2025
Accepted: December 27, 2025
Published online: December 29, 2025

Key words:
Food Safety
Multi-Mechanistic Pathogen Control
Natural Antimicrobials in Poultry
Prebiotics
Probiotics

ABSTRACT

The world poultry sector is facing some dire problems in balancing the production efficiency against food safety, especially as regulatory pressures are mounting and usage of antibiotic growth promoters is being restricted. This review provides a synthesis of the current findings on the application of beneficial microbes and natural antimicrobials as viable options to control pathogens throughout the farm-to-fork continuum. Probiotics, particularly *Lactobacillus* and *Bacillus* species, along with combinations like synbiotics and bacteriophages, have shown significant efficacy by competitive exclusion, host immunity regulation and antimicrobial compound synthesis leading to 1.5-4.5 log reductions of major pathogens including but not limited to *Salmonella, Campylobacter* and pathogenic *Escherichia coli*. The combined probiotic-prebiotic, essential oil, organic acid and bacteriophage formulations offer multifactorial intervention models that address each step of production, such as hatchery procedures to post-harvest interventions. Despite the current challenges of standardization, regulatory regulation, and cost-efficiency, the unified use of these natural options is a viable way to continue the production efficiency and at the same time reduce the risk of global antimicrobial resistance and guarantee the microbiological safety of poultry products to consumers on a global scale.

To Cite This Article: Aljasir SF, 2025. Applications of beneficial microbes and natural antimicrobials in poultry with integrated strategies for food safety: a review. Pak Vet J, 45(4): 1463-1476. http://dx.doi.org/10.29261/pakvetj/2025.329

INTRODUCTION

Poultry meat is the largest form of animal protein in the world, and the world produces more than 130 million metric tons per year, which is expected to rise to 150 million metric tons by the year 2030 (van der Laan *et al*., 2024). However, such massive growth has been coupled with increased food safety issues, with large-scale production systems that create environments that support the growth of pathogens throughout the farm-to-fork continuum (Elbehiry & Marzouk, 2025). Contaminated poultry products contribute substantially to foodborne illness, leading to significant medical costs, productivity losses, and public health burdens, and are responsible for 20–30% of all foodborne disease outbreaks worldwide (Ashpalia *et al*., 2024). The poultry production system is a reservoir of numerous zoonotic pathogens of great public health concern. *Salmonella* species, especially *S. Enteritidis* and *S. Typhimurium,* remain the major causative agents with a prevalence rate ranging between 5-40% in commercial flocks (Mkangara, 2023). These pathogens are highly adaptable and carry various virulence factors, such as the invasion of intestinal epithelial cells, as well as the development of biofilms on the processing equipment (Jahan *et al*., 2022). The most common bacterial etiology of gastroenteritis across all countries in the world is represented by *Campylobacter* species, most commonly *C. jejuni* and *C. coli*, and poultry is the primary reservoir of the bacteria that facilitates human infection (Amjad, 2023). The rates of colonization of broiler flocks are often more than 80% at slaughter with a bacterial load of 10^7-10^9 colony-forming units/ gram cecal content. The small infective dose (500-800 bacterial cells) of *Campylobacter* is unique in the application of control measures (Abdulazeez, 2022). Enteropathogenic EPEC, enterohemorrhagic EHEC, and avian pathogenic *E. coli* (APEC) strains have a wide repertoire of virulence determinants that are involved in disease in birds and humans (Hu *et al*., 2022). *E. coli* genomic plasticity and its ability to exchange genes horizontally make it extremely difficult to control and help to generate multidrug-resistant strains (Saini *et al*., 2024). This long history of using subtherapeutic levels of antibiotics as growth-promoters has been a significant contributor to the worldwide epidemic of antibiotic resistance. The extensive use of antibiotics has been going on since the 1950s to improve feed and growth rates in animals by putting them into feed at sub-inhibitory levels (Goes, 2024). This has placed a lot of selective pressure on gut microbiota, leading to the development of antibiotic-resistant microorganisms and subsequent expansion of resistance genes. Antimicrobial resistance (AMR) has been ranked among the ten leading global health issues of significance by the World Health Organization and it is estimated that an estimated 700,000 people die each year with antibiotic-resistant infections, a figure that is set to rise to 10 million by 2050 (Fatima *et al*., 2023). Rates of resistance to critically important antimicrobials in poultry systems have become increasingly alarming, with multi-drug-resistant *Salmonella* and *Campylobacter* isolates reported at prevenance exceeding 60% in some surveillance studies (Khatun, 2025). The transfer of AMR determinants from poultry-associated microbes to human pathogens via mobile genetic elements obtained horizontally through horizontal gene transfer represents a significant public health concern (Vinayamohan *et al*., 2022).

The regulatory response to the use of antibiotic growth promoters has been built up over time in different jurisdictions. The European Union, South Korea, and the United States had banned them in 2006 and 2011, respectively, and in the Veterinary Feed Directive of 2017 (Torok *et al*., 2022). This type of legislative intervention has compelled the drastic transformations in the livestock production system, which places an immediate need for viable solutions to attain the same level of production efficiencies and at the same time retain high standards of food safety. The use of antibiotic growth promoters has had mixed impacts on the important measures of production; empirical research often records a decrease in weight gains up to 3-5% as well as increases in feed conversion ratios up to 2-4% (Canibe *et al*., 2022). However, reduced use of antibiotics has also been associated with increased susceptibility to enteric diseases under some conditions of operation, thus underlining the urgent need for sound replacement measures. This review aims to synthesize current evidence on the application of beneficial microbes and natural antimicrobials across the poultry production chain, discussing their mechanisms, efficacy, implementation strategies, and impact on food safety and public health.

Beneficial Microbes in Poultry Production

Probiotics: Probiotics are live microorganisms that confer health benefits to the host when administered in adequate amounts and are now regarded as effective alternatives to antibiotic growth promoters in poultry (Krysiak *et al*., 2021). Food safety in poultry production begins with controlling intestinal colonization by zoonotic pathogens that can be transferred to meat and eggs during processing. Beneficial microbes are effective in reducing the load of *Salmonella*, *Campylobacter*, *E. coli*, and *Listeria monocytogenes* in both experimental and field studies. Lactic acid bacteria are the most widely studied and used probiotics in poultry. Species such as *Lactobacillus acidophilus*, *Lactiplantibacillus plantarum*, *Limosilactobacillus reuteri*, *Ligilactobacillus salivarius*, *Lacticaseibacillus casei*, and *Lacticaseibacillus rhamnosus* improve growth, immunity, and gut health as shown in Table 1 (Fathima *et al*., 2022). These species tolerate gastric acidity (pH 3.0 for up to four hours) and bile salt concentrations of 0.3–1.0% (Thuy & Trai, 2024). They enhance nutrient digestibility by increasing intestinal amylase activity (Leal, 2022), regulate mucosal immunity through cytokine expression (Tian *et al*., 2021), and produce bacteriocins effective against *Salmonella*, *Campylobacter*, and *Clostridium perfringens* (Mokoena *et al*., 2021). *Bifidobacterium* spp. (*B. bifidum*, *B. longum*, and *B. animalis*) also contribute to gut health by fermenting oligosaccharides into short-chain fatty acids, lowering intestinal pH, and enhancing immunoglobulin levels (Asadpoor *et al*., 2021; Rousseaux *et al*., 2023). Multi-strain formulations combining *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* show greater efficacy than single-strain products (McFarland, 2021). Spore-forming *Bacillus* spp. (*B. subtilis*, *B. amyloliquefaciens*, *B. licheniformis*, *B. coagulans*, *B. clausii*) are notable for their resilience and stability during feed processing (Elleithy *et al*., 2023; Pawar *et al*., 2025). They produce lipopeptides, bacteriocins, and enzymes (proteases, lipases, cellulases, xylanases, phytases) that inhibit pathogens, degrade anti-nutritional factors, and enhance nutrient bioavailability (Luise *et al*., 2022; Xu *et al*., 2025). Dietary supplementation with *B. subtilis* (10^5 CFU/g feed) has been shown to improve body weight gain, feed conversion, and survival in broilers (Mohamed *et al*., 2022).

Prebiotics: Prebiotics are non-digestible food components that selectively enhance the growth and action of commensal microorganisms in the gastrointestinal tract, and offer health benefits to the host organism (Ballini *et al*., 2023). Oligosaccharides are the most researched type of prebiotics in the sphere of poultry nutrition. Mannan-oligosaccharides (MOS) extracted cell wall of the *Saccharomyces cerevisiae* have been proven to be effective in increasing production parameters and immune competency (Baek *et al*., 2024). Broiler diets supplemented with MOS at a dietary level of 0.1-0.5 percent have a significant effect on body weight gain, feed conversion ratio, and lower mortality rates compared to control diets that were not supplemented (Baker *et al*., 2021). Fructo-oligosaccharides (FOS) and inulin specifically stimulate the growth of species of the genus *Bifidobacterium* and *Lactobacillus* in the cecum, resulting in the rise of short-chain fatty acids, including butyrate, propionate, and acetate, as shown in Table 1. Galacto-oligosaccharides (GOS) and xylo-oligosaccharides (XOS) are potential prebiotics that are effective, but their effectiveness depends on the degree of polymerization and dosage level (Morgan, 2023). In the lower gastrointestinal tract, inulin, a polydisperse carbohydrate made up of fructose units connected by β (2→1) bonds, acts as a substrate for the fermentation of beneficial bacteria (Popoola-Akinola *et al*., 2022). Adding inulin to the diets of poultry at 0.5-2.0 percent has been shown to regulate the composition of cecal microbiota, expand populations of beneficial bacteria, and boost the generation of metabolites that have anti-inflammatory and barrier-protective properties (Fotschki *et al*., 2023). Another class of prebiotics with immunomodulatory qualities is β-glucans, which are obtained from yeast cell walls, fungi, and cereal grains. These substances activate innate immune responses and improve resistance to infectious diseases by interacting with immune cell receptors, especially Dectin-1 (Singh & Bhardwaj, 2023). The complete mechanism is shown in Fig. 1.

Synbiotics: Synbiotics are clever blends of prebiotics and probiotics that are intended to improve the activity, colonization, and survival of good bacteria in the gastrointestinal system (Yue *et al*., 2025). The synergistic effect between probiotic strains and their preferentially used prebiotic substrates ideally provides better benefits than the use of either component individually. The in vitro screening research indicates a high degree of variability in use of prebiotics amongst different strains of probiotics. A combination of *Enterococcus faecium* with galacto-oligosaccharides shows better growth rates as compared to mannan-oligosaccharides, oligofructose or xylo-oligosaccharides (Jaswal, 2025). The combination of *Ligilactobacillus salivarius* with GOS and combined with raffinose-family oligosaccharides and *Lactiplantibacillus plantarum* is an optimized symbiotic (Sasi *et al*., 2025). Field experiments of synbiotic supplementation in broiler chickens indicate that there is a consistent increase in productive performance. MOS (0.5) + probiotic mixture (0.1) showed significant body weight gain at the starter, grower, and finisher stages with gain of 3-7 percent compared to controls (Charandas, 2024). Feed conversion ratios are increased by 25-5%, which is very economical. Synbiotic supplementation leads to improvement of immune parameters such as high antibody titers to Newcastle disease and infectious bursal disease viruses, high serum levels of immunoglobulin, and improved intestinal morphology (Hossain *et al*., 2025). Synbiotics have protective effects, which also include stress mitigation. Chronic heat-stressed broilers (35±2°C for 8 h/day) supplemented with synbiotic combinations have lower serum corticosterone levels and better intestinal microarchitecture and retain growth performance in contrast with heat stressed, unsupplemented controls (Du *et al*., 2023).

Mechanism of Action

Competitive Exclusion and Colonization Resistance: Competitive exclusion is one of the core ecological processes where commensal microorganisms prevent the establishment of pathogens through direct competition to access limiting nutrients, adhesion sites, and ecological niches in the gastrointestinal tract (Horrocks *et al*., 2023). Nutrient competition is a major competitive exclusion strategy whereby useful microbes obtain the necessary substrates needed in pathogen growth and metabolic processes, represented in Fig. 2 (Wang & Kuzyakov, 2024). Competition over carbohydrates has been well-studied; commensal microbes are quick to absorb available monosaccharides, oligosaccharides, and polysaccharides through a wide variety of glycolytic pathways and thus restrict substrate availability to pathogenic microbes such

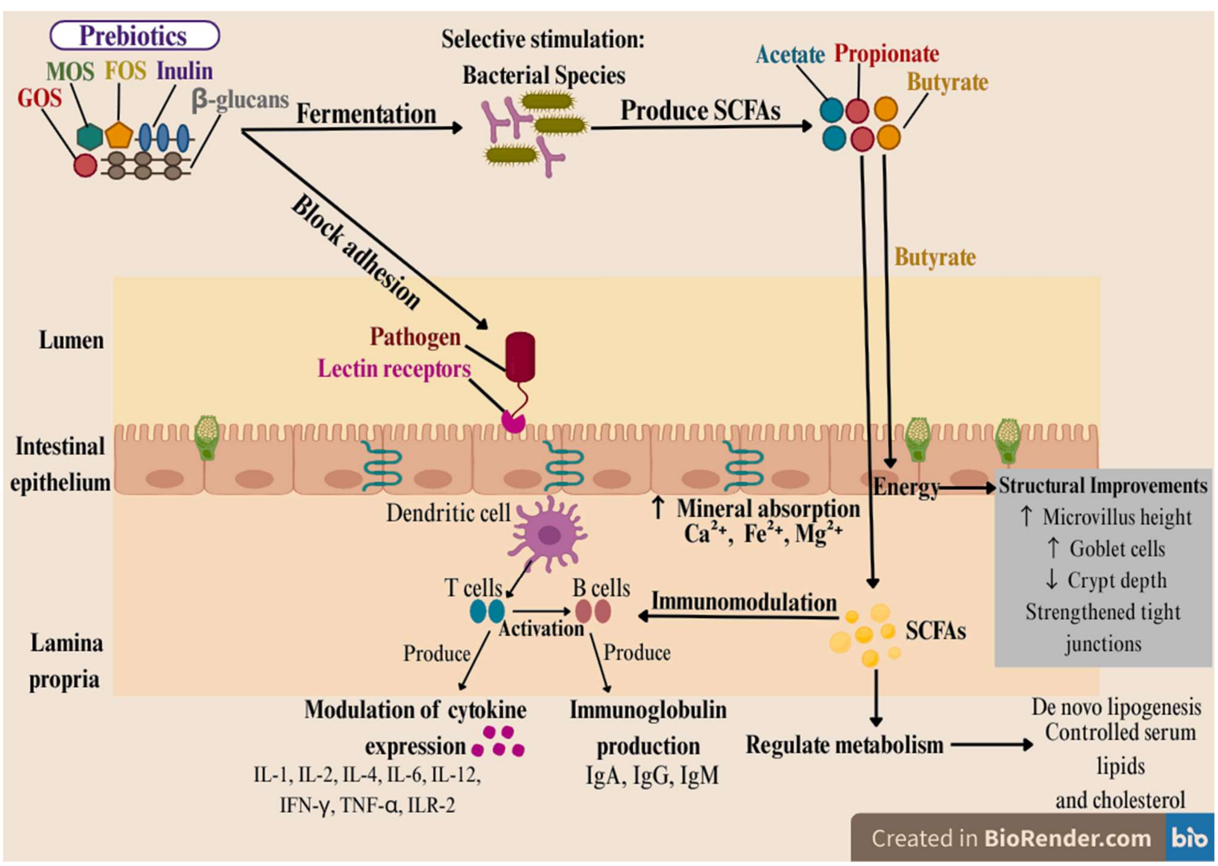

Fig. 1: Mechanisms of prebiotic action in the poultry gut: MOS, FOS, and inulin enhance gut health by stimulating SCFA production, modulating immune responses, improving intestinal morphology, and increasing mineral absorption.

Table 1: Useful microorganisms that exhibited efficacy on pathogens in the poultry production systems

Category	Agent/Strain	Target Pathogen(s)	Primary Mechanism	Application Method	Efficacy (Log Reduction)	References
Lactobacillus	*Lactobacillus acidophilus*	*S. Typhimurium*, *E. coli* O157:H7	Competitive exclusion, lactic acid production, bacteriocin	Feed supplement (10^8 CFU/g feed)	2.1-2.8	(Shapwa, 2022)
	Lactiplantibacillus plantarum	*Salmonella* spp., *C. perfringens*	Plantaricin production, competitive exclusion, biofilm inhibition	Feed/Water additive (10^9 CFU/mL)	2.5-3.2	(Chuwatthanakhajorn, 2025)
	Limosilactobacillus reuteri	*Salmonella enteritidis, C. perfringens*	Reuterin production, immune modulation, gut barrier enhancement	Feed supplement (10^8 CFU/g)	1.9-2.6	(Shi *et al.*, 2022)
	Ligilactobacillus salivarius	*Campylobacter* spp., *E. coli*	Bacteriocin (Salivaricin), adhesion competition	Water treatment (10^8 CFU/mL)	1.8-2.4	(Chiba *et al.*, 2024)
	L. fermentum	*Salmonella* spp., *E. coli*	H_2O_2 production, lactic acid, immune stimulation	Feed additive (10^8 CFU/g)	1.7-2.3	(Guo *et al.*, 2021)
Bacillus	*B. subtilis*	*C. perfringens*, *E. coli*, *Salmonella*	Surfactin, fengycin production, spore stability	Spore form in feed (10^8-10^9 CFU/g)	2.3-3.1	(Cheng *et al.*, 2018)
	B. licheniformis	*Salmonella* spp., *E. coli*	Lichenicidin production, competitive exclusion	Feed supplement (10^8 CFU/g)	1.8-2.4	(Shleeva *et al.*, 2023)
	B. coagulans	*S.Typhimurium*, *C. perfringens*	Lactic acid, coagulin production, spore resilience	Feed additive (10^8 CFU/g)	2.0-2.7	(Guo *et al.*, 2021)
	B. amyloliquefaciens	*Salmonella spp., E. coli, molds*	Bacillomycin, macrolactin synthesis	Feed supplement (10^8 CFU/g)	2.1-2.8	(Ngalimat *et al.*, 2021)
Bifidobacterium	*B. animalis subsp. lactis*	*E. coli*, *S. enteritidis*	Acetic acid production, immune modulation, barrier function	Feed supplement (10^8 CFU/g)	1.6-2.2	(Cheng *et al.*, 2021)
Enterococcus	*E. faecium*	*Salmonella spp., C. perfringens*	Enterocin production, colonization resistance	Feed additive (10^8 CFU/g)	1.9-2.5	(Vela & Logrono, 2023)
Pediococcus	*P. acidilactici*	*Salmonella spp., Listeria monocytogenes*	Pediocin production, lactic acid	Feed supplement (10^8 CFU/g)	2.0-2.6	(Khorshidian *et al.*, 2021)
Next-Gen Probiotic	*Akkermansia muciniphila*	*C. perfringens, Salmonella*	Mucin layer enhancement, Amuc_1100 protein, barrier integrity	Water additive (research phase)	1.8-2.4	(Mo *et al.*, 2024)
	Faecalibacterium prausnitzii	*E. coli*, inflammatory pathogens	Butyrate production, anti-inflammatory	Feed supplement (experimental)	1.5-2.1	(Ali *et al.*, 2022)
Bacteriocin-Producing Probiotic	*Lactococcus lactis* subsp. *lactis* (nisin producer)	*C. perfringens*, *Listeria*, Gram-positive pathogens	In situ nisin production, competitive exclusion, lactic acid	Feed supplement (10^8 CFU/g)	2.2-3.0	(Hassan *et al.*, 2021)
Yeast	*S. cerevisiae* (active/inactive)	*Salmonella* spp., *E. coli*, mycotoxins	Pathogen binding (cell wall), immune stimulation, mycotoxin adsorption	Feed additive (0.1-0.2%)	1.4-2.0	(Davis, 2022)
Multi-Strain Consortium	6-strain probiotic mix (*Lactobacillus* + *Bacillus* + *Bifidobacterium*)	Multi-pathogen (*Salmonella*, *E. coli*, *Campylobacter*)	Synergistic mechanisms, metabolic cooperation	Feed/in-ovo (10^9 CFU/g or egg)	3.5-4.1	(Afsharnia *et al.*, 2025)
Prebiotic	Fructooligosaccharides (FOS)	Indirect (supports beneficial microbes)	Selective fermentation, SCFA production, bifidogenic	Feed inclusion (0.2-0.5%)	Supportive (enhances probiotics)	(Hotchkiss *et al.*, 2022)
	Mannan-oligosaccharides (MOS)	*Salmonella* spp., *E. coli*	Pathogen agglutination (Type-1 fimbriae binding), immune modulation	Feed additive (0.1-0.2%)	1.2-1.8	(Davis, 2022)
	Inulin	Indirect (bifidogenic effect)	SCFA production, beneficial bacteria proliferation	Feed inclusion (0.3-0.5%)	Supportive	(Klostermann, 2023)
Postbiotic	*Lactobacillus* cell-free supernatant (CFS)	*Salmonella* spp., *E. coli*, *Campylobacter*	Antimicrobial metabolites (organic acids, bacteriocins, peptides), no live cells	Water additive or feed spray (5-10% v/v)	1.8-2.6	(Ozturk & Sengun, 2025)

as *Salmonella* and *E. coli* (Muramatsu & Winter, 2024). The iron sequestration by siderophore synthesis by the commensals creates an environment lacking iron, which hinders the colonization of the pathogens as the pathogenic bacteria need iron to carry out vital metabolic activities, including DNA replication and the electron transport chain (Marchetti *et al.*, 2020). Adhesion site competition entails physical occupation of intestinal epithelial binding sites by useful microorganisms, thus preventing the pathogenic attachment and eventual colonization (Lin *et al.*, 2024). Surface layer proteins (S-layer proteins) and exopolysaccharides produced by *Lactobacillus* species contribute to strong adhesion to mucin glycoproteins and intestinal epithelial cells through lectin-carbohydrate binding and hydrophobic forces (Muscariello *et al*., 2020). These competitive exclusion strategies together form a first line of defense, which averts pathogenic colonization in poultry, and hence minimizes the chances of contamination along the food chain.

Production of Antimicrobial Compounds: Bacteriocins are ribosomally synthesized antimicrobial peptides that are produced mainly by lactic acid bacteria, and they are active against closely related bacterial species (Darbandi *et al*., 2022). Class I bacteriocins (lantibiotics), such as nisin, subtilin, or mersacidin, also have post-translationally modified amino acids, including lanthionine and methyl-anthionine, which provide structural stability and antimicrobial activity based on forming membrane pore and bind lipid II (Antoshina *et al*., 2022). Nisin attaches to lipid II, a peptidoglycan precursor molecule, which results in complexes of pore that cause depolarization of the membrane, leakage of ions, and cell death of vulnerable bacteria (Sharma *et al*., 2021). Class II bacteriocins, such as pediocin PA-1, leucocin A and sakacin P, are non-modified heat-resistant peptides that identify mannose phosphotransferase systems and cause membrane permeabilisation by pore formation mechanisms (Goswami *et al*., 2021). Production of organic acids is a widespread antimicrobial response by the beneficial microorganisms, especially lactic acid bacteria, that prevent growth of pathogenic microorganisms and shown in Fig. 2. The antimicrobial activity of acetic acid produced by species of the genera *Bifidobacterium* and some strains of *Lactobacillus* is better than that of lactic acid because of a higher degree of membrane permeability and a larger degree of intracellular acidification effect (Cizeikiene & Jagelaviciute, 2021). The cyclic lipopeptide biosurfactant, Surfactin produced by the Gram-positive bacterium, *B. subtilis*, exhibits a high level of activity against Gram-positive and Gram-negative bacteria by solubilizing the membrane and forming pores on the bacteria (Chen *et al*., 2022). The variety of antimicrobial repertoire generated by useful microbes offers direct and potent method of managing foodborne pathogens in the avian GIT, which makes poultry products safer.

Immune System Modulation: The pattern recognition receptor (PRR) signaling is the major pathway by which the commensals interact with the host immune system. Toll-like receptors (TLRs) specifically TLR2, TLR4, TLR5 and TLR9, detect microbial-associated molecular patterns (MAMPs) such as lipoteichoic acid, lipopolysaccharide, flagellin, and unmethylated CpG DNA patterns (Al-Abdulwahid, 2021). TLR activation by probiotics activates intracellular signaling pathways that include MyD88, TRIF, and downstream protein kinases to ultimately activate NF- 0 B and AP-1 transcription factors and induce expression of pro-inflammatory cytokines (Surai *et al*., 2021). Cytokine modulation is an essential process in which commensals can influence the development of an immune response against resistance to

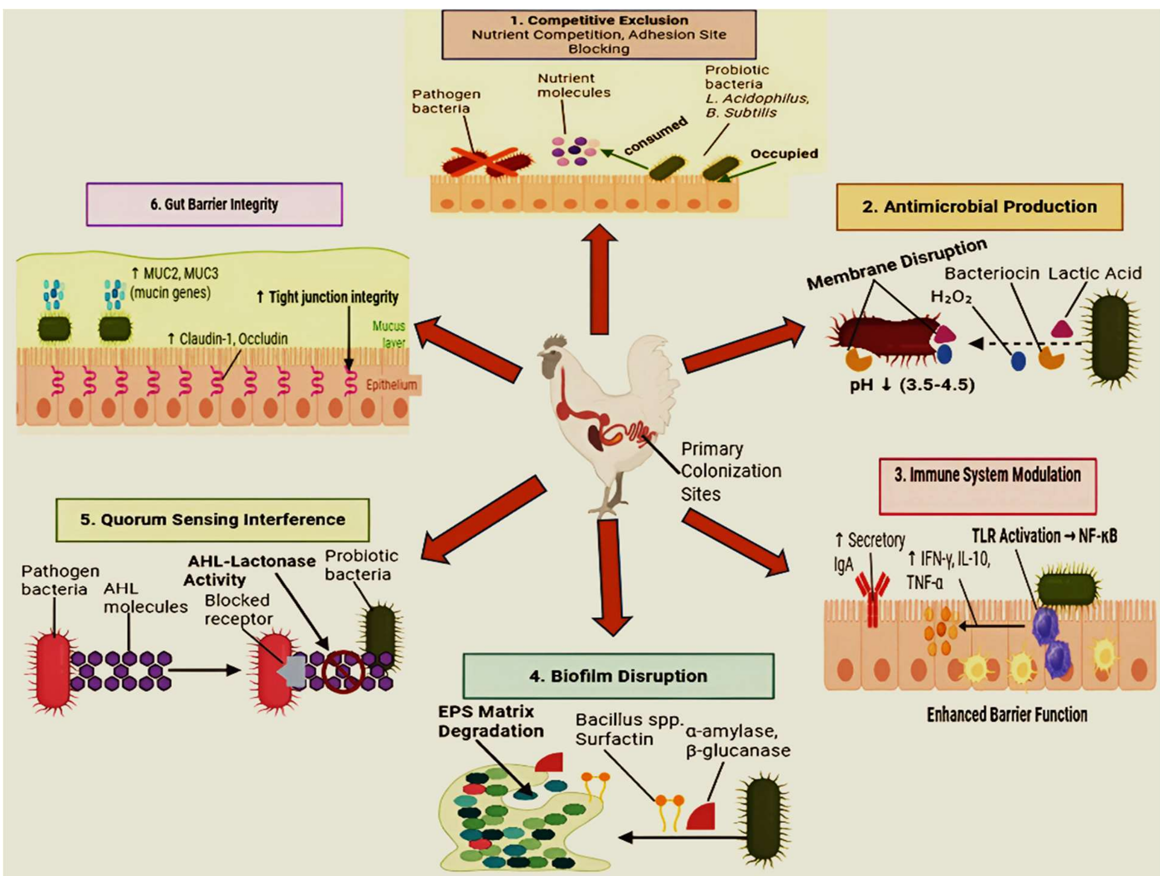

Fig. 2: Multifactorial mechanisms of beneficial microorganisms in poultry for pathogen control and enhanced food safety.

pathogens and resistance to immunopathology. The administration of probiotics affects the ratio between the pro-inflammatory cytokines (IL-1β, IL-6, IL-8, IL-12, TNF-α, IFN-γ) and the anti-inflammatory cytokines (IL-10, TGF-β), in general favoring the phenomenon of controlled pro-inflammatory reaction which strengthens the ability of the organism to eliminate pathogens (Bilal *et al*., 2022). The production of secretory IgA in the intestinal mucosa is also enhanced by the stimulation of B cells and plasma cells in the intestinal mucosa through the action of probiotics and TLR-dependent or cytokine-mediated pathways (Walrath *et al*., 2021). Through regulation of homeostatic immune reactions, commensal microbial communities contribute to the overall ability of the host to withstand pathogenic infections and reduce tissue destruction due to inflammation, and in the end, promote the health and food safety goals of animals.

Biofilm Disruption Mechanisms: The first protective step in preventing biofilm formation is the inhibition of the initial adhesion. Biosurfactants produced by the species of Bacillus and lactobacilli lower the surface tension and the hydrophobicity of the substrates and provide adverse environments where the pathogen cannot adhere, as shown in Fig. 2 (Patel *et al*., 2021). The degradation of the extracellular polymeric substance interferes with the structure of the formed biofilms. The microorganisms that are beneficial release various enzymes that degrade the components of the matrix, such as polysaccharides, proteins and extracellular DNA (Kim *et al*., 2023). Glycosidic bonds of biofilm exopolysaccharides are broken by the action of polysaccharide-degrading enzymes including α-amylase, β-glucanase, and alginate lyase, which weaken the cohesiveness and stability of the matrix (Anso *et al*., 2024). Food safety relies, in part, on the ability to prevent and disrupt microbial biofilms, as biofilm-associated bacteria exhibit increased resistance to antimicrobials and environmental stresses, and act as persistent sources of contamination in poultry production systems.

Quorum-Sensing Interference: Quorum sensing (QS) is a bacterial cell-to-cell communication system that regulates the expression of genes that depend on population density, which includes the regulation of virulence factors, biofilm formation, and cooperation in metabolism. Pathogen QS interference by natural antimicrobials and commensal microorganisms is also an original anti-virulence approach that does not eliminate bacterial populations, but rather reduces the selection pressures on resistance evolution (Nag *et al*., 2021). QS systems based on acyl-homoserine lactone (AHL) are more commonly found in Gram-negative pathogens, including *Salmonella*, *Campylobacter,* and pathogenic strains of *E. coli* (Koley *et al*., 2023). Mechanisms of quorum-quenching, which disrupt AHL signaling, are enzyme breakage of signal molecules and signal-receptor interactions. The hydrolysis or modification of AHL molecules catalyzed by AHL-lactonases, AHL-acylases, and oxidoreductases expressed by *Bacillus* species and some lactic acid bacteria make them unable to activate receptors as illustrated in Fig. 2 (Raya *et al*., 2022). This strategy represents a novel antivirulence approach that attenuates pathogenicity without exerting bactericidal pressure, thereby offering a sustainable method for pathogen control that minimizes selective pressure for AMR in food production systems.

Gut Barrier Integrity Enhancement: The intestinal barrier is a combination of various elements, which include the layer of mucus, the monolayer of the epithelial cells with tight junctions between cells, and the immune cell populations present underneath (Duangnumsawang *et al*., 2021). Stabilization of tight junction proteins and upregulation is one of the major mechanisms of barrier improvement. Paracellular permeability is regulated by tight junction complexes, which are assemblies of transmembrane proteins including claudins, occludin, and junctional adhesion molecules, and cytoplasmic scaffold proteins including the zonula occludens isoforms, ZO-1, ZO-2, and ZO-3 (Horowitz *et al*., 2023). Probiotics increase the expression of barrier-forming claudins (claudin-1, claudin-3, and claudin-4) and also suppress pore-forming claudins (claudin-2), which enhance epithelial permeability as in Fig. 2. Enhancement of the mucus layer gives a physical and biochemical barrier to avoid direct contact with pathogens and the epithelial surfaces. Probiotic bacteria increase the proliferation and expression of the mucin gene (MUC2, MUC3, MUC4) via the activation of transcription factors, such as SPDEF, GFI1, and KLF4 (Duangnumsawang, 2023). Butyrate is a microbial metabolite that prevents histone deacetylase (HDAC) thereby promoting the increase in the MUC2 gene transcription (Yang *et al*., 2021). The intestinal epithelial cells also help the host defense system by the production of antimicrobial peptides, which create another form of defense barrier against pathogen colonization. Avian 2-defensin (AvBD), cathelicidin, and RegIII proteins are the products synthesized by epithelial cells in response to microbial challenges and collectively form a chemical barrier that can neutralize invading pathogens (Awad *et al*., 2017). Enhancement of intestinal barrier function is fundamental to preventing pathogen translocation from the small and large intestines into the systemic circulation and poultry tissues, thereby directly reducing microbiological contamination of carcasses and poultry products reaching the end consumer.

Natural Antimicrobials in Poultry: Plant-based antimicrobials are a heterogeneous group of bioactive molecules that have been demonstrated to warrant significant potential as an alternative in the form of substituting traditional antibiotics in poultry production (Acharya & Barsila, 2025). Plants are rich sources of a plethora of bioactive components with antimicrobial properties against bacteria, yeasts, and molds. Antimicrobial peptides are a heterogeneous category of extremely conserved molecules that form part and parcel of innate immune defense mechanisms in all life. These peptides are usually 12-50 amino acids long, have a net positive charge at physiological pH and have amphipathic structures that allow them to interact with negatively charged bacterial membranes (Wang, 2023). Antimicrobial peptides are reported to kill bacteria more quickly (have bactericidal kinetics), have a broad-spectrum effect, and are unlikely to cause resistance (have a low resistance development) because of their membrane-directed action (Simonson *et al*., 2021). The use of organic acids and their salts as preservatives in livestock and poultry production as antimicrobial additives dates back several decades. Short-chain organic acids (C1-C7) are antimicrobial agents with several effects on gastrointestinal physiology, including the reduction of pH, direct antimicrobial action, energy supply, and intestinal architecture (Ebeid & Al-Homidan, 2022). Bacteriophages, viruses that infect and lyse bacterial cells specifically, are very specific biological antimicrobials, and they promise significant potential in eliminating foodborne pathogens in poultry production. Their great host specificity allows them to selectively eliminate pathogenic bacteria but protection of commensal microbiota, avoiding one of the main disadvantages of non-selective antimicrobial agents (Rebenaque & Orenga, 2022). Table 2 shows all these processes in detail with practical examples.

Table 2: Natural antimicrobial agents that exhibited efficacy on pathogens in the poultry production systems

Category	Agent/Strain	Target Pathogen(s)	Primary Mechanism	Application Method	Efficacy (Log Reduction)	References
Essential Oil	Carvacrol (Oregano oil)	*Salmonella* spp., *C. perfringens*, *E. coli*, *C. perfringens*	Membrane disruption, efflux pump inhibition, QS interference	Feed supplement (100-200ppm)	2.2-3.0	(Al-Mnaser, 2019)
	Thymol (Thyme oil)	*Salmonella enteritidis*, *C. perfringens*, *E. coli*	Membrane permeabilization, ATPase inhibition, oxidative stress	Feed additive (100-150ppm)	2.0-2.7	(CHAURASIA, 2024)
	Cinnamaldehyde (Cinnamon oil)	*E. coli, S. typhimurium, Campylobacter*	Membrane integrity disruption, cell wall synthesis inhibition	Feed inclusion (75-150ppm)	1.8-2.5	(CHAURASIA, 2024)
	Eugenol (Clove oil)	*Salmonella spp., E. coli, S. aureus*	Membrane permeabiliza-tion, protein denaturation, enzyme inhibition	Feed additive (50-100ppm)	1.6-2.3	(Alagawany *et al*., 2022)
	Limonene (Citrus oil)	*Salmonella spp., Campylobacter*	Membrane fluidity alteration, respiratory chain disruption	Feed supplement (80-120ppm)	1.5-2.1	(Sinche Ambrosio, 2022)
Plant Extract	Garlic extract (*Allicin, organosulfuic* compounds)	*C. perfringens, Salmonella spp., E. coli*	Sulfhydryl group interaction, QS inhibition, membrane damage	Feed/Water (0.5-1.0%)	1.9-2.6	(AbdAl-Rudha & AL-Nasiry, 2023)
	Green tea polyphenols (EGCG, catechins)	*E. coli*, *Salmonella* spp., *C. perfringens*	Antioxidant activity, membrane disruption, protein binding	Feed additive (200-400ppm)	1.7-2.4	(Zhang *et al*., 2021)
	Turmeric extract (Curcumin)	*C. perfringens*, *Salmonella* spp., *E. coli*	Anti-inflammatory, membrane disruption, FtsZ inhibition	Feed supplement (100-300ppm)	1.5-2.2	(Orimaye *et al*., 2024)
	Grape seed extract (Proanthocyanidins)	*S. enteritidis*, *E. coli*	Antioxidant, membrane damage, enzyme inhibition	Feed additive (150-300ppm)	1.6-2.3	(Kovács, 2022)
Organic Acid	Butyric acid (sodium/calcium butyrate)	*Salmonella* spp., *E. coli*, *C. perfringens*	pH reduction, gut barrier enhancement, histone deacetylase inhibition	Feed/Water (0.1-0.3%)	1.8-2.5	(Melaku *et al*., 2021)
	Propionic acid	*Salmonella Typhimurium*, molds, *E. coli*	pH reduction, metabolic disruption, feed preservation	Feed preservation (0.2-0.5%)	1.6-2.3	(Ben Braïek, O., & Smaoui, 2021)
	Lactic acid	Multi-pathogen (*Salmonella*, *Campylobacte*, *E. coli*)	pH reduction, membrane disruption, acidification	Carcass wash/spray (2-3% solution)	2.0-2.8	(Wong, 2023)
	Formic acid	*Salmonella* spp., *E. coli*	Undissociated acid penetration, intracellular pH disruption	Feed acidification (0.5-1.0%)	1.7-2.4	(Taylor & Doores, 2020)
Antimicrobial Peptide	Nisin (from *L. lactis*)	*C. perfringens*, *Listeria monocytogene*, *Staphylococcus*	Pore formation (Lipid II binding), membrane permeabilization	Feed additive (25-50ppm)	2.1-2.9	(Anumudu *et al*., 2021)
	Pediocin PA-1	*Listeria monocytogene*, *E. coli*	Membrane permeabilization, pore formation	Processing application/feed	1.9-2.6	(Khorshidian *et al*., 2021)
Medium-Chain Fatty Acids	Lauric acid (C12:0) and Monolaurin	*S. typhimurium*, *E. coli*, *C. perfringens*, enveloped viruses	Membrane disruption, viral envelope solubilization, biofilm inhibition	Feed additive (0.2-0.5%)	2.0-2.8	(Çenesiz & Çiftci, 2020)
Antimicrobial Enzyme	Lysozyme (hen egg-white derived or recombinant)	Gram-positive bacteria (*C. perfringens*, *Staphylococcu*, *Listeria*)	Peptidoglycan hydrolysis (β-1,4-glycosidic bonds), cell wall lysis	Feed additive or processing spray (50-200ppm)	1.5-2.3 (mainly Gram-positive)	(Nawaz *et al*., 2022)
Herbal Immunomodulator	*Echinacea purpurea* extract (polysaccharides, alkylamides)	Indirect pathogen control via immune enhancement	Immune stimulation (macrophage activation, cytokine modulation), phagocytosis enhancement	Feed supplement (0.1-0.5%)	1.2-1.9 (immune-mediated reduction)	(Magnavacca *et al*., 2022)
Bacteriophage	Anti-*Salmonella* phage cocktail (multiple phages)	*S. enteritidis*, *S. Typhimurium* (serovar-specific)	Bacterial lysis, biofilm disruption, host-specific infection	Spray/feed (10^7-10^9 PFU/mL or g)	3.2-4.5	(Shaji *et al*., 2021)
	Anti-*Campylobacter* phage cocktail	*C. perfringens*, *C. coli*	Targeted bacterial lysis, reducing colonization	Pre-harvest/processing spray (10^8-10^9 PFU/mL)	2.8-3.8	(Abd El-Hack *et al*., 2021)

Application Strategies Across the Poultry Production Chain

Hatchery Interventions (In Ovo and Early Colonization): The hatchery-based interventions are important control areas in the development of the beneficial microbial communities and prevention of pathogen colonization at the susceptible early stages of poultry development (Oliveira *et al*., 2024). Embryonic and immediate post-hatch phases offer opportunities of manipulating the microbiome because the gastrointestinal tracts are initially sterile and are colonized by environmental microorganisms within a short time after hatching (Shehata *et al*., 2021). Probiotics and prebiotics *in ovo* administration are beneficial agents that are directly injected into hatching embryos, usually at 17-18 days of incubation through the air cell, amnion, or the yolk sac (Fig. 3) (Das *et al*., 2021). The amnion is the most preferred site for injecting probiotics because embryos instinctively consume amniotic fluid that contains the inserted microorganisms as they are pipped, which facilitates gastrointestinal colonization (Castañeda Bustillo, 2020). When prebiotic oligosaccharides such as galacto-, fructo-, and inulin-type fructans, are administered *in ovo*, they stimulate the proliferation of indigenous beneficial bacteria and improve intestinal morphological parameters, including villus height, crypt depth, and absorptive surface area (Reube *et al*., 2021). Interventions of post-hatch colonization target the timely absence of resident microbiota in the first 24h compared to 72h of life in order to develop advantageous microbial consortia with the ability to provide colonization resistance (Shehata *et al*., 2021). The form of the application regimes is usually 10^7-10^9 CFU/bird, which is sprayed by automated spray cabinets built in commercial chicken-processing lines (Kang, 2020).

Feed Supplementation Strategies: Introduction of useful microorganisms and natural antimicrobials through the feed matrix is the most viable and scalable approach to commercial poultry farms and enables prolonged exposure throughout the poultry rearing period (Mak *et al*., 2022). Another approach to preserve the heat-sensitive probiotics during the pelletizing step is the use of microencapsulation technology that does not depend only on the sporogenic species. Alginate, chitosan, or lipid compounds are used to form encapsulation matrices that protect *Lactobacillus* and *Bifidobacterium* strains against thermal stress to achieve the survival rates of 80-90% at pelleting temperatures of

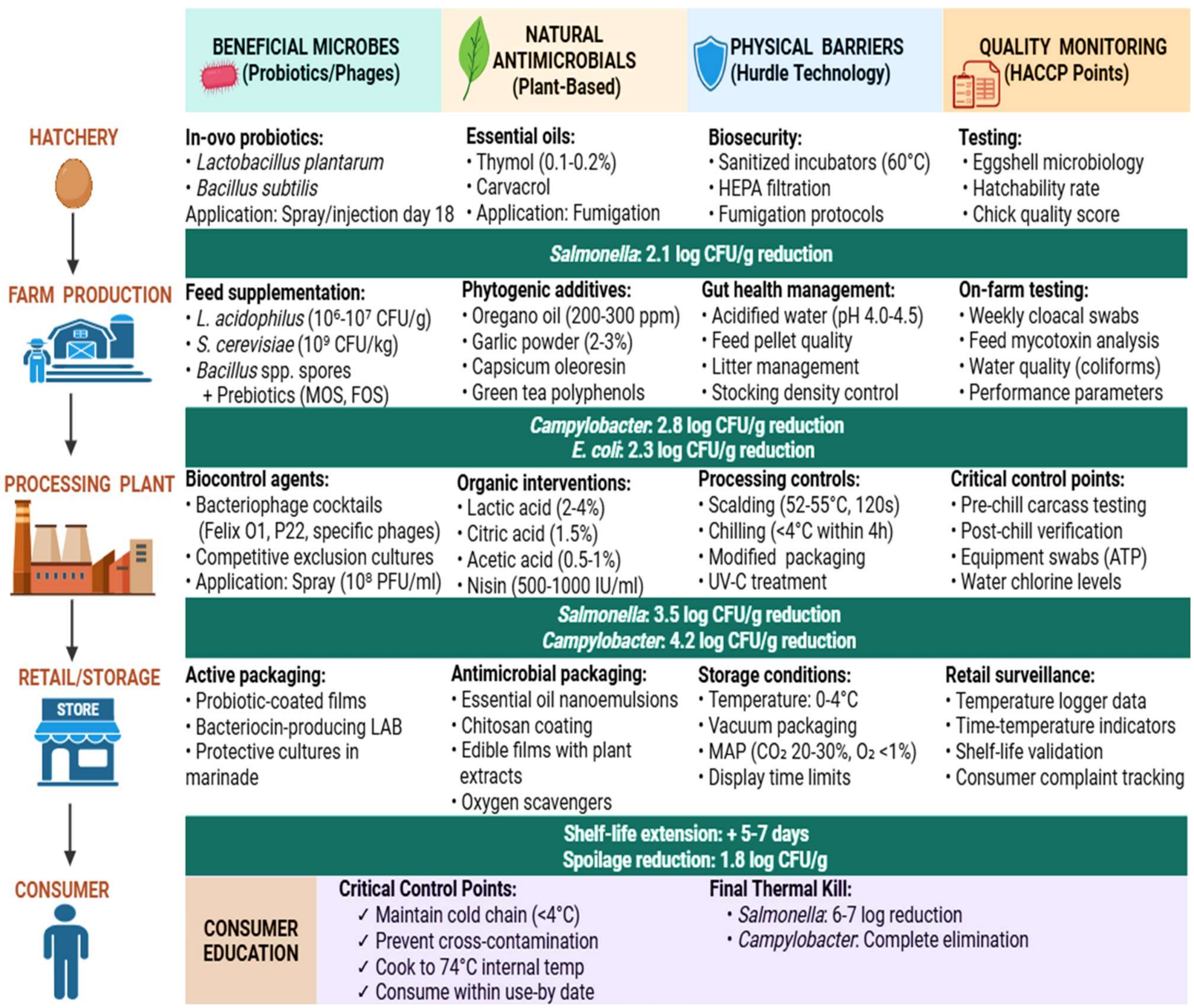

Fig. 3: Integrated multi-hurdle approach for pathogen reduction across the poultry production chain, from hatchery to processing. Cumulative log reductions in pathogens (e.g., *Salmonella*, *Campylobacter*) are depicted at each stage.

85-90°C and less than 10% for unprotected cells. The technology expands the range of potential probiotics in the use of pellet feeds, where specific targets can be delivered and released into a specific section of the intestines. The common range of probiotics added to feed is 10^6 to 10^9 CFU/kg of final feed, with the specific dosage varying depending on animal species, strain properties, age, and environmental stress factors (Arsène *et al*., 2021). Extraction of Mannan-oligosaccharides from yeast wall matrices are not only used as fermentable source of carbon by probiotic bacteria but also as binding agents to type-1 fimbriae of Gram-negative pathogens, thus interfering with epithelial colonization. Inulin-derived fructans and fructo-oligosaccharides, at a dietary concentration of 0.5-1.0 stimulates the growth of *Bifidobacterium* spp., and *Lactobacillus* spp. and increase short-chain fatty acid production, especially butyrate, which is the energy source of choice of the colonic epithelial cell (Panwar *et al*., 2022). Normal levels of inclusion are 50 to 300mg/kg of single essential oils in feed and 100-500mg/kg of blended extracts. The short and medium fatty acids, including butyric, propionic acid, caprylic acid, and capric acid, have been shown to have bacteriostatic effects with Gram-negative pathogens and simultaneously anabolic effects with commensal anaerobes (Gomez-Osorio *et al*., 2021).

Processing Plant Interventions (Carcass Decontamination): Post-harvest operations in processing facilities are considered to be the key control points of pathogen contamination reduction or elimination in carcass surfaces, prevention of cross-contamination during processing, and microbiological safety of the final poultry products (Morshdy *et al*., 2025). Application of lactic acid solutions (2-4% concentrations) by immersion, spray, or foam results in log reductions of one to two in *Salmonella*, *Campylobacter* (Figure 3), and generic *E. coli* population on carcass surfaces (Bueno López *et al*., 2022). Peroxyacetic acid (0.01-0.1%) is a potent antimicrobial agent that exhibits oxidation to cause antimicrobial effects, and it breaks down to acetic acid and water, leaving behind slight residues (Duarte *et al*., 2022). Antimicrobial solutions containing carvacrol, thymol, and eugenol, at concentrations of 0.1-0.5%, appear to demonstrate antimicrobial activity against surface-associated pathogens when solubilized with appropriate surfactants or solubilizing agents increasing aqueous solubility and accessibility to the substrate. *Campylobacter* and *Salmonella* bacteriophage cocktails used as spray systems with concentration of 10^7-10^9 PFU/mL will generate one to three log reductions on targeted pathogens (Cole, 2024). Lightly acidic electrolyzed water (pH 5.065 oxidation-reduction potential +600 to +900mV) has antimicrobial activity similar to traditional chlorine treatments, and a nearly neutral pH that reduces equipment corrosion and the effects of meat quality (Roobab *et al*., 2023).

Packaging and Post-Harvest Applications: Interventions that are done post-processing on the packaged poultry products are the last chance of improving the microbiological safety and shelf-life of the product by treatment using beneficial microorganisms and natural antimicrobials (Papadochristopoulos *et al*., 2021). Active systems involve the inclusion of antimicrobial substances into the packaging materials or as additives that deactivate into active form during the storage period (Deshmukh, R. K., & Gaikwad, 2024). Essential oil impregnated sachets of oregano oil, thyme oil or cinnamon oil release volatile terpenes and phenolic compounds which spread throughout the package headspace, condense on product surfaces, and inhibit the growth of bacteria on their surfaces (Bibow & Oleszek, 2024). Chitosan-based coatings exhibit inherent antimicrobial activity mediated by electrostatic interactions with negatively charged bacterial cell membranes, resulting in membrane destabilization and the release of intracellular contents (Yilmaz Atay, 2020). Coating of poultry surfaces with chitosan solutions (0.5-2.0% w/v) at pH 4-6 forms transparent films which inhibit the rate of microbial growth and extended refrigerated shelf-life three to seven days over uncoated controls (Giatrakou *et al*., 2023). Applied on concentrations of 106-108 CFU/g product surface, lactic acid bacteria cultures (especially *Lactobacillus sakei*, *L. lactis*, and *Carnobacterium maltaromaticum*) exhibit anti-listerial effects and increase refrigerated shelf-life (Pellegrini, 2024).

Impact on Food Safety and Public Health: The growing demand for safe and sustainable poultry production highlights the importance of non-antibiotic interventions. By combining beneficial microbes with natural antimicrobials, producers can mitigate foodborne pathogens, slow the emergence of AMR, and align with One Health objectives that connect animals, human, and environmental well-being.

Reduction of *Salmonella*, *Campylobacter*, and *E. coli* Contamination: Among major agents of foodborne disease worldwide are *Salmonella*, *Campylobacter*, and pathogenic *E. coli*, which are frequently associated with contaminated poultry meat and eggs. The established methods of control, such as the use of chemical disinfectants and antibiotics, are being undermined by the rising cases of antibiotic resistance and antibiotic residues (Davies & Wales, 2019). The use of probiotics in combination with plant-based biotics helps to counteract these pathogens in a number of production phases. Probiotics block colonization by producing organic acids and bacteriocins in the gastrointestinal tract, and antimicrobials produced by plants destroy cell membranes and biofilm frameworks in pathogen cells. This twofold action reduces the bacterial content of feces and cecal material and, thus, minimizes the chances of cross-contamination in the slaughterhouse. Natural antimicrobials, including lactic acid, acetic acid, and essential-oil rinses, may also be used at the processing stage and reduce surface contamination by an additional 23 log cycles (Chakraborty & Dutta, 2022). The net effect is a quantifiable decrease in the prevalence of the pathogens in the retail poultry products, which will directly increase food safety and positively affect the health of consumers.

Influence on AMR Development: Abuse of antibiotics in poultry farming greatly accelerates the development of AMR strains of bacteria. Such resistant strains can spread resistance determinants to the human pathogens through the food supply chain. A sustainable solution that can help in checking this problem is provided by natural antimicrobials and probiotics. In contrast to the traditional antibiotics, natural antimicrobials can act upon multiple cellular structures- causing membrane disruption, enzymatic activity suppression, and creation of oxidative stress- and making the emergence of resistance relatively less likely (Nourbakhsh *et al*., 2022). Probiotics also overcome AMR by hindering colonization of resistant taxa and degrading remaining antibiotics in the gastrointestinal tract. In addition, other studies have reported the downregulation of resistance genes, including the tetA and blaTEM genes, in poultry fed with probiotic-phytobiotic mixes. Replacement of prophylactic antibiotics with these natural products, therefore, lowers the level of antibiotic residues in poultry meat and eggs, curbs horizontal gene transfer among the microbiota, and enhances the worldwide AMR containment strategies (Abreu *et al*., 2023).

Role in Sustainable and Antibiotic-Free Poultry Production: The ultimate goal of sustainable poultry production is to have high productivity, animal welfare, environmental protection, and food safety. The combination of useful microbes and natural antimicrobials is one of the foundations of this strategy. These natural approaches promote sustainability through increased gut integrity and the immune system, which will reduce the frequency of diseases and the need to perform therapeutic interventions, and thus the general health and well-being of animals. The decrease in the use of antibiotics also leads to the reduction of the release of drug residues into the soil and water, thus eliminating environmental pollution (Yang *et al*., 2021). From a consumer perspective, products labeled as antibiotic-free or raised under natural production systems align with the growing demand for safe, ethical, and high-quality food. At the production level, such interventions improve feed conversion efficiency, reduce mortality rates, and enhance economic profitability for producers. At a broader policy level, the incorporation of probiotics, prebiotics, essential oils, and organic acids into national poultry production strategies is consistent with the One Health paradigm, which recognizes the interconnectedness of human, animal, and environmental health (Zheng *et al*., 2025). Encouraging the adoption of these approaches can reduce reliance on antibiotics, limit the progression of AMR, and promote a more sustainable and safe future for poultry production.

Challenges Future prospective

Heterogeneity of Efficacy: Despite strong evidence supporting the effectiveness of beneficial microorganisms and natural antimicrobial agents in poultry production systems, their large-scale adoption remains constrained by several challenges. One of the most significant barriers is the heterogeneity of efficacy, which is influenced by multiple interacting factors, including strain specificity, host age, diet composition, and environmental conditions such as temperature and humidity. Comparative studies demonstrate that not all *Lactobacillus* or *Bacillus* strains exhibit equivalent acid–bile tolerance, colonization efficiency, or persistence within the avian gastrointestinal tract. Similarly, the bioactivity of phytochemical-derived compounds is strongly affected by plant genotype, harvesting time, extraction procedures, and storage conditions (Ngurube, 2022).

Furthermore, feed formulation, gastrointestinal pH, and water quality critically influence probiotic viability and the release of bioactive constituents, emphasizing the need for standardized and optimized delivery systems. Commercial formulations frequently display wide variability in microbial load, strain composition, and additive concentration, resulting in inconsistent product performance. The absence of universally accepted in vitro and in vivo validation protocols further limits inter-study comparability, while the predominance of controlled-environment experiments restricts extrapolation to heterogeneous farm conditions (Vashishat et al., 2024).

Economic and Scaling Challenges: Beyond biological variability, economic and scaling constraints represent major obstacles to the widespread implementation of beneficial microbes, botanicals, and bacteriophages in poultry production. High-quality probiotic formulations, essential oils, phage cocktails, and nano-encapsulated phytochemicals often incur costs that exceed those of conventional antibiotics, limiting adoption, particularly among small- and medium-scale farmers. In many cases, comprehensive cost–benefit analyses are lacking, making it difficult for producers to justify higher upfront investments despite potential long-term gains in flock health, productivity, and food safety.

Additional challenges include limited supply chains, inconsistent product availability, inadequate distribution networks, and insufficient extension services, especially in developing countries. Scaling up the production of consistent-quality botanicals and phages remains technically demanding due to batch-to-batch variability, stability concerns, and regulatory requirements. Moreover, global regulatory frameworks for safety and efficacy assessment remain fragmented, prolonging product development timelines and market entry. Addressing these challenges will require economic incentives, such as subsidies, public–private partnerships, and supportive policy frameworks, alongside the standardization of industrial manufacturing processes. Such measures could facilitate large-scale production of uniform, affordable bio-based alternatives while ensuring farmer-level feasibility and sustained adoption.

Future prospective: Recent advances in high-throughput sequencing, metagenomics, and microbiome profiling have significantly enhanced understanding of the poultry gastrointestinal ecosystem, enabling the identification of stress-tolerant probiotic lineages with improved metabolic and immunomodulatory functions (Wang *et al.,* 2024). Next-generation probiotics, including *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and *Clostridium butyricum*, show strong potential for enhancing gut health and suppressing pathogenic colonization (Al-Fakhrany & Elekhnawy, 2024). Technological innovations such as microencapsulation, lyophilization, and nanotechnology-based delivery systems (e.g., nanoemulsions, liposomes, and polymeric nanoparticles) offer improved stability, bioavailability, and targeted release of microbial and phytochemical agents, protecting them from gastrointestinal stressors (Ani *et al.,* 2024).

Importantly, the integration of machine learning and artificial intelligence (AI) represents a promising frontier in this field. AI-driven models can support strain selection, microbiome response prediction, and optimization of synergistic combinations of probiotics, botanicals, and phages tailored to specific production systems. These tools may enable precision nutrition strategies that enhance efficacy, reduce variability, and improve economic returns. Within a One Health framework, such interdisciplinary approaches can contribute to mitigating AMR while simultaneously improving poultry health, food safety, and farm profitability.

Conclusions: The transition of poultry farming from a production that is not reliant on antibiotics to the application of natural antimicrobials is a required development and a promising trend towards the creation of sustainable food systems. The empirical evidence shows that the application of useful microorganisms and plant-based antimicrobials, when appropriately implemented in the production chain, may positively affect the process of foodborne pathogen suppression, leaving or even raising the parameters of production. The optimization of these interventions is provided with a scientific basis of a mechanistic concept of competition elimination, immune regulation, biofilm disintegration, and quorum-sensing disruption. The important issues to tackle in the future are to determine the efficacy of the strain used, devise effective delivery methods, and standardized quality-control parameters, and conduct economic viability studies. Multi-hurdle strategies, which include probiotics, prebiotics, essential oils, organic acids, and bacteriophages, have synergies that outperform what each of them delivers. The poultry industry needs to use these evidence-based substitutes as regulatory systems keep changing and the consumers are increasingly interested in antibiotic-free products; more research on microbiome engineering, next-generation probiotics and precision application technologies should improve the efficacy and acceptance of natural antibiotics in the global poultry production systems.

Acknowledgments: The Researcher would like to thank the Deanship of Graduate Studies and Scientific Research at Qassim University for financial support (2025-QU-APC).

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