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Biofilm Formation by Foodborne Pathogens in Meat Processing Facilities: Challenges and Control Strategies

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Abstract

Biofilm formation by foodborne pathogens in meat-processing facilities represents a major challenge to food safety, product quality, and public health worldwide. In these environments, microorganisms attach to food-contact surfaces and develop structured microbial communities embedded within extracellular polymeric substances (EPS), which enhance resistance to disinfectants, environmental stress, and conventional sanitation procedures. This review examines the mechanisms of biofilm formation, including microbial attachment, colonization, maturation, and dispersion, as well as the environmental and operational factors that promote biofilm persistence in meat-processing systems. The review further discusses major biofilm-forming foodborne pathogens associated with meat-processing environments, including Salmonella enterica, Listeria monocytogenes, Escherichia coli O157:H7, Campylobacter spp., and Pseudomonas spp., alongside critical contamination sites such as slaughter lines, cutting equipment, storage areas, and drainage systems. The public health significance of biofilms is highlighted through their contribution to pathogen persistence, foodborne disease outbreaks, antimicrobial resistance, and reduced meat shelf life. Additionally, current detection and characterization techniques, including microbiological, molecular, and imaging-based approaches, are evaluated.

Finally, the review explores conventional and emerging biofilm control strategies, with particular focus on natural antimicrobials, bacteriophage applications, enzymatic treatments, surface modification technologies, and integrated HACCP-based hygiene systems. Understanding the ecology and persistence of biofilms within meat-processing facilities is essential for developing effective prevention and control strategies capable of improving meat safety, reducing contamination risks, and supporting sustainable food production practices.

Keywords: *Biofilms; Meat processing facilities; Foodborne pathogens; Antimicrobial resistance; Food safety management*

Introduction

The meat production sector stands out as one of the major and most economically important segments of the agri-food production industry (Suychinov et al., 2024). Meats provide a rich source of proteins, amino acids, and various essential nutrients; at the same time, they are highly vulnerable to microbial colonization and subsequent spoilage processes due to the presence of moisture, abundant nitrogen-containing compounds, neutral pH levels, and nutrient-rich body tissues (Rathod et al., 2025). The growing number of contacts between animals, their body parts and the processing equipment creates ideal conditions (High moisture content, abundant nitrogenous compounds, neutral pH, and nutrient-rich tissues) for pathogen multiplication in the meat processing facilities (Sokra et al., 2026). The industrialization process involved in the meat processing has brought about great improvements in processing efficiency and product availability; however, increased contamination risks have also been created in a complex and highly interlinked chain of meat processing stages (Zwirzitz et al., 2020). Modern slaughterhouse plants and meat production companies operate through intensive contacts of the carcass with industrial equipment, water lines, transport belts, cutting devices, people, packaging containers, etc. (Zwirzitz et al., 2020).

All these processes increase contamination risks and contribute to the need for microbial protection of meat production and processing facilities, which has become the key challenge worldwide (Belk, 2025). Every year, millions of Americans suffer from foodborne illnesses with meat products serving as a primary source of deadly infections related primarily to Salmonella and Listeria monocytogenes infections (Megahed et al., 2020). In addition to these issues, the high incidence of zoonotic agents in animal hides and intestines requires regular development of anti-microbial strategies in slaughterhouses (Yang et al., 2024). Microbial contamination during meat processing is especially dangerous due to the ability of the pathogens to persist not only as planktonic microorganisms but also in the form of biofilms adhered to the equipment surface and providing resistance against cleaning agents and disinfection procedures as well as desiccation (Bhusal et al., 2026). Such biofilms enable survival of the pathogens within meat processing facilities for long periods of time.

Biofilms as an Emerging Challenge in Meat Processing Environments

Biofilm formation is one of the many microbial threats in meat processing settings that have proved to be extremely difficult to manage technologically. Biofilms refer to organized microorganisms encased in self-secreted EPS, firmly adhering to both biotic and abiotic surfaces (Lynch & Robertson, 2008). Unlike free-floating planktonic bacteria, biofilm-associated microbes demonstrate greater resistance to sanitizers, stress, lack of nutrients, and antibiotics (Lynch & Robertson, 2008). Biofilms can form on a variety of surfaces in meat processing plants, such as stainless steel walls, conveyor belts, cutting boards, drain pipes, refrigeration units, rubber gaskets, knives, and pipelines supplying water for washing purposes. Moisture, organic debris, fat, protein, and temperature variations provide ideal conditions for bacterial attachment and biofilm development. These microbial films act as sources of contamination, sporadically releasing pathogenic cells into the processing area and infecting meat products during subsequent production. Such biofilms provide a protective shield against disinfectants while allowing microbial interactions via quorum sensing (Melander et al., 2020).

Thus, the aim of this paper is to presents an analysis of the role of food pathogen biofilms on meat processing facilities and the risk of health hazards, concentrating on mechanisms of biofilm formation, sustainability of pathogens, and related health hazards. This work further considers the role played by processing methods in creating contamination that is resistant to sanitation and causes shortened shelf life.

Biofilm Biology and Development Mechanisms

Biofilms are organized microbial communities irreversibly attached to biotic or abiotic surfaces and embedded within a self-produced extracellular polymeric substance (EPS) matrix. Unlike planktonic cells, biofilm-associated microorganisms function collectively within coordinated communities that exhibit enhanced adaptability and environmental persistence (Flemming et al., 2016). Biofilm formation is recognized as a major microbial survival strategy in meat-processing environments. Biofilms commonly develop on stainless steel equipment, conveyor belts, cutting tools, drains, refrigeration systems, plastics, rubber seals, and water pipelines, where pathogenic bacteria, spoilage organisms, yeasts, and fungi coexist within a shared matrix, thereby increasing resistance to cleaning and sanitation procedures (Bridier et al., 2015).

Biofilms possess heterogeneous three-dimensional structures in which variations in oxygen, pH, nutrient availability, and metabolic activity promote physiological diversification, including the formation of dormant persister cells capable of surviving antimicrobial treatments (Hall et al., 2004). The EPS matrix, composed mainly of polysaccharides, proteins, extracellular DNA (eDNA), and lipids, anchors cells to surfaces while protecting them from environmental stressors (Flemming & Wingender, 2010). The matrix also acts as a diffusion barrier that limits disinfectant penetration and enhances biofilm resistance. Biofilm formation occurs through sequential stages of attachment, colonization, maturation, and dispersion regulated by microbial genetics, environmental conditions, surface properties, and quorum sensing mechanisms.

Biofilms in Meat Processing Facilities: Major Foodborne Pathogens and Contamination Ecology

Biofilms in meat-processing facilities are typically composed of diverse microbial communities containing both pathogenic and spoilage-associated organisms embedded within extracellular polymeric substances (EPS). Microorganisms originating from animal hides, intestinal contents, contaminated water, aerosols, equipment surfaces, and worker handling can become persistent colonizers capable of surviving routine sanitation procedures (Giaouris et al., 2014). Major biofilm-associated microorganisms in meat industries include Salmonella spp., Listeria monocytogenes, Escherichia coli O157:H7, Pseudomonas spp., and Campylobacter spp., all of which possess adaptive mechanisms that enhance persistence and foodborne disease transmission (Muhammad et al., 2020).

Salmonella spp. are among the most common pathogens associated with meat-related foodborne outbreaks worldwide. Contamination frequently originates from animal feces, intestinal leakage during slaughter, contaminated feed, transport equipment, and poorly sanitized processing surfaces (Carrasco et al., 2012). In meat and poultry-processing environments, Salmonella enterica adheres effectively to stainless steel, polyethylene, rubber, and glass surfaces through fimbriae, flagella, cellulose, and curli-mediated attachment mechanisms that promote biofilm formation (Vestby et al., 2009). Once incorporated into mature biofilms, Salmonella becomes highly tolerant to desiccation, disinfectants, and nutrient limitation, enabling long-term persistence in slaughter lines, conveyor systems, drainage areas, and cutting equipment. Persistent strains have repeatedly been isolated from meat-processing facilities despite routine sanitation, demonstrating the resistance of biofilms to conventional cleaning methods (Giaouris et al., 2015). Infections caused by Salmonella may result in gastroenteritis, septicemia, and severe systemic complications, particularly in vulnerable individuals.

Listeria monocytogenes is especially problematic because of its psychrotrophic nature and ability to survive under refrigeration conditions. The organism can persist and proliferate at temperatures below 4°C, allowing colonization of refrigeration units, cold rooms, storage areas, and chilled processing equipment (Carpentier & Cerf, 2011). Biofilm formation increases resistance to sanitizers and promotes long-term persistence in ready-to-eat (RTE) meat-processing environments, where contamination risks are particularly serious because products are consumed without additional cooking (Ferreira et al., 2014). Recurrent contamination by genetically identical Listeria strains has been documented in many facilities, indicating that biofilm-associated populations may survive for months or years. Clinically, L. monocytogenes causes listeriosis, a severe disease associated with septicemia, meningitis, fetal loss, and high hospitalization rates, particularly among pregnant women, neonates, elderly individuals, and immunocompromised patients (Finn et al., 2023).

Escherichia coli O157:H7, Pseudomonas spp., and Campylobacter spp. also contribute significantly to biofilm-associated contamination in meat industries. E. coli O157:H7 is particularly important in beef-processing systems because cattle are major reservoirs of Shiga toxin-producing E. coli (STEC). The pathogen adheres effectively to food-contact surfaces through curli fibers, fimbriae, and extracellular polysaccharides, enabling stable biofilm formation on industrial equipment (Wang et al., 2013). Biofilm-associated populations exhibit increased resistance to disinfectants and environmental stress, increasing the risk of carcass contamination and severe outbreaks involving hemorrhagic colitis and hemolytic uremic syndrome (HUS). In contrast, Pseudomonas spp. are mainly spoilage-associated psychrotrophic organisms that dominate refrigerated meat environments. Their strong EPS production and surface adhesion capabilities accelerate meat spoilage through slime formation, discoloration, off-odors, and shelf-life reduction (Doulgeraki et al., 2012). Although not always pathogenic, Pseudomonas biofilms may facilitate pathogen persistence within mixed-species communities. Campylobacter spp., particularly Campylobacter jejuni, are strongly associated with poultry-processing environments where they survive through biofilm formation and viable-but-nonculturable (VBNC) states. Although relatively sensitive under laboratory conditions, Campylobacter can persist in water systems, equipment surfaces, and poultry-processing lines when incorporated into multispecies biofilms (Teh et al., 2014).

Detection and Characterization of Biofilms in Meat Processing Systems

There are two factors which are considered most important when dealing with Effective control of biofilm-associated contamination in meat-processing facilities, and these are accurate detection and monitoring of microbial communities. This is because unlike planktonic microorganisms, biofilm-associated cells are embedded within extracellular polymeric substance (EPS) matrices and often exist in heterogeneous physiological states, making detection more difficult (Flemming et al., 2016). Conventional culture-based methods, including swab sampling and microbial enumeration, remain widely used for monitoring pathogens on food-contact surfaces (Chmielewski & Frank, 2003). The problem though is that these techniques are not only limited by long incubation periods and reduced sensitivity for stressed or injured cells, but they often underestimate contamination because attached microorganisms can evade routine sampling, while viable but non-culturable (VBNC) cells may persist undetected (Oliver, 2010).

To address these limitations, advanced microscopic and molecular techniques are now being used. Scanning electron microscopy (SEM) enables detailed visualization of biofilm architecture and microbial attachment on industrial surfaces, while confocal laser scanning microscopy (CLSM) provides three-dimensional imaging of intact biofilms and differentiates viable from damaged cells (Shi & Zhu, 2009; Neu & Lawrence, 2015). Then there are the molecular approaches such as polymerase chain reaction (PCR) and quantitative PCR (qPCR) improve detection sensitivity by targeting species-specific genes and biofilm-associated markers (Law et al., 2015). More recently, metagenomic and transcriptomic analyses have enhanced understanding of biofilm ecology by identifying microbial diversity, gene expression, and interspecies interactions without reliance on culture-based isolation.

Rapid detection systems and biosensor technologies are also gaining importance for real-time industrial monitoring. ATP bioluminescence assays are commonly used for hygiene verification because of their speed and simplicity, although they cannot distinguish microbial contamination from residual organic matter (Velusamy et al., 2010). Electrochemical and optical biosensors show strong potential for detecting biofilm-associated pathogens through identification of microbial metabolites, nucleic acids, and surface antigens (Dwevedi et al., 2016). Nevertheless, biofilm detection remains challenging due to surface heterogeneity, inaccessible equipment areas, intermittent microbial shedding, and the presence of VBNC populations. Consequently, integrated monitoring approaches combining microbiological, molecular, imaging, and biosensor-based techniques are increasingly recommended for effective surveillance in meat-processing environments.

Control and Prevention Strategies Against Biofilms in Meat Processing Facilities

Because mature biofilms exhibit substantial resistance to disinfectants, environmental stress, and mechanical removal, effective control requires integrated interventions targeting microbial attachment, EPS stability, metabolic activity, and recolonization processes (Bridier et al., 2011). Conventional cleaning-in-place (CIP) systems remain central components of industrial hygiene programs and involve automated circulation of detergents, alkaline solutions, acids, and disinfectants through processing equipment without disassembly. These systems are designed to remove organic residues and reduce microbial loads while minimizing production downtime (Simões et al., 2010). Chemical sanitizers such as chlorine compounds, quaternary ammonium compounds, peracetic acid, and hydrogen peroxide are widely employed due to their broad-spectrum antimicrobial activity; however, their effectiveness against mature biofilms is often limited by inadequate penetration through EPS matrices and the presence of metabolically dormant persister cells (Bridier et al., 2011). Repeated exposure to sub-lethal sanitizer concentrations may additionally select for increasingly tolerant microbial populations and contribute to adaptive resistance mechanisms. These limitations have intensified interest in alternative and sustainable antimicrobial approaches capable of disrupting biofilm integrity while reducing chemical dependence within food-processing systems.

Among emerging interventions, natural antimicrobials have attracted substantial scientific and industrial attention due to their biodegradability, consumer acceptance, and broad-spectrum anti-biofilm properties. Plant-derived essential oils obtained from thyme, oregano, clove, cinnamon, rosemary, and garlic contain bioactive compounds such as thymol, carvacrol, eugenol, cinnamaldehyde, and allicin that disrupt microbial membranes, interfere with quorum sensing pathways, and inhibit EPS synthesis. These compounds have demonstrated inhibitory activity against biofilm-forming pathogens including Listeria monocytogenes, Salmonella enterica, and E. coli O157:H7 under meat-processing conditions. Organic acids such as lactic, acetic, and citric acid additionally contribute to microbial control by reducing intracellular pH and destabilizing membrane integrity, while bacteriocins such as nisin and pediocin exhibit targeted antimicrobial activity against Gram-positive pathogens, particularly Listeria monocytogenes (Gálvez et al., 2007). Phytochemicals including flavonoids, tannins, and phenolic acids further inhibit microbial adhesion and quorum sensing, thereby reducing biofilm maturation and structural stability. Importantly, synergistic combinations involving essential oils, bacteriocins, organic acids, and conventional sanitizers frequently demonstrate enhanced anti-biofilm efficacy compared with single-agent applications. Nanoencapsulation and controlled-release delivery systems have also emerged as promising technologies for improving the stability, solubility, and sustained antimicrobial activity of natural compounds within industrial environments (López-Romero et al., 2015). Nevertheless, industrial implementation remains constrained by factors including sensory alterations in meat products, volatility of essential oils, regulatory considerations, and variability in antimicrobial performance under complex processing conditions.

Additional strategies for controlling industrial biofilms include bacteriophage therapy, surface engineering technologies, enzymatic treatments, and integrated hygiene management systems. Bacteriophages are highly specific bacterial viruses capable of penetrating biofilm matrices, lysing target pathogens, and disrupting microbial communities without significantly affecting beneficial microbiota (Sillankorva et al., 2012). Phage cocktails targeting Salmonella, Listeria, and E. coli have shown promising efficacy in food-processing environments, although regulatory limitations, host specificity, and resistance development remain challenges. Surface engineering approaches, including antimicrobial coatings, nanoparticle-functionalized materials, superhydrophobic surfaces, and photocatalytic systems, are also being developed to reduce microbial adhesion and improve self-cleaning properties (Hasan & Chatterjee, 2015).

Enzymatic treatments targeting extracellular polymeric substance (EPS) components such as polysaccharides, proteins, and extracellular DNA can weaken biofilm structure and improve sanitizer penetration. Competitive exclusion strategies using beneficial microorganisms may also suppress pathogen colonization through nutrient competition and antimicrobial metabolite production. Despite advances in these technologies, long-term biofilm control still depends on effective implementation of Hazard Analysis and Critical Control Point (HACCP) systems, environmental monitoring, sanitation verification, and staff training. Strong hygiene management remains essential because advanced antimicrobial technologies alone cannot compensate for poor sanitation practices or inadequate equipment design.

Conclusion

Biofilms formed by foodborne pathogens in meat-processing facilities represent a major challenge to both food safety and public health. Their ability to adhere to industrial surfaces, resist sanitation procedures, and persist under harsh environmental conditions enables continuous contamination of meat products and increases the risk of foodborne outbreaks. Pathogens such as Listeria monocytogenes, Salmonella enterica, and Escherichia coli O157:H7 are particularly concerning due to their strong biofilm-forming capacity and clinical significance. Environmental factors including moisture, organic residues, poor sanitation, and surface irregularities strongly promote biofilm development within processing systems. Consequently, conventional cleaning procedures alone are often insufficient for complete eradication. Effective control therefore requires integrated strategies involving advanced detection methods, improved hygienic design, natural antimicrobials, bacteriophage applications, and strict HACCP-based sanitation management. A deeper understanding of biofilm ecology and microbial persistence will remain essential for improving meat safety, reducing industrial contamination, and protecting public health in modern food-processing environments.

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