Int J Adv Life Sci Res. Volume 9(4) 19-41
https://doi.org/10.31632/ijalsr.2026.v09i04.002

International Journal of Advancement in Life Sciences Research

Online ISSN: 2581-4877

Journal homepage http://ijalsr.org



Review Article

Bacterial Biofilms in Livestock Diseases: Mechanisms of Pathogenesis, Persistence, and Therapeutic Challenges

Sahana Ghosh[1], Dipankar Das[2], Ankan Mitra[2], Anushree Ghosh[2], Sriparna De[2*]

[1]*Department of Microbiology, Vijaygarh Jyotish Ray College, Bijoygarh, Jadavpur, Kolkata, 700032 West Bengal, India*

[2]*Department of Allied Health Sciences, Brainware University, Barasat, 700125 West Bengal, India*

Corresponding Author's Email: sd.ah@brainwareuniversity.ac.in

Abstract

Bacterial biofilms pose a perilous challenge in livestock health, contributing to chronic infections, treatment failures, and economic losses across livestock. These designed microbial communities render an extracellular matrix, conferring improved resistance to antimicrobials and host immune defences in contrast to their planktonic counterparts. This review emphasizes the basic structural biology of bacterial biofilms, illustrating the molecular mechanisms controlling their growth and proliferation with significant correlation with the quorum sensing (QS) pathway and cyclic-di-GMP signalling scheme. QS initiates cell populations via autoinducers, while cyclic GMP acts as a ubiquitous second messenger that controls phase switching via modulating exopolysaccharide production, adhesion expression and flagellar motility. Biofilms display pivotal role in major livestock diseases, including bovine mastitis (primarily *Staphylococcus aureus*), bovine respiratory disease complex, endometritis, and enteric diseases across livestock. The maturation of biofilm exacerbates antimicrobial resistance (AMR), impeding therapeutic interventions. Current therapeutics comprising conventional antibiotics, antimicrobial peptides and matrix-degrading enzymes limit functional impairment in livestock disease management. Also, emerging technological advancements leverage CRISPR-Cas gene editing tools to target biofilm-associated pathogens, restrict QS and c-di-GMP signalling cascades, and eradicate AMR. Finally, this current review proposes the therapeutic potential of amalgamating CRISPR-based antimicrobials with conventional approaches to mature next-generation biofilm management protocols for sustainable livestock disease management.

Keywords: *AMR; Biofilm; CRISPR; Livestock; Quorum Sensing; Therapeutics*

Introduction

Biofilm is a consortium of bacterial cells that are adhere to one another, enclosed within a slime matrix comprised of extracellular polymeric substances (EPS), composed of eDNA, and proteins (e.g., fibrin). Under adverse conditions, most microorganisms develop biofilms to protect themselves (Chatterjee *et al.*, 2025; Jacques & Malouin, 2022). Research studies have revealed that microorganisms producing biofilms not only colonize different organs of humans, causing pathogenic infections, but also colonize different organs of animals, which indirectly affect human health. Studies on biofilm-related infections (mastitis, endometritis, respiratory infections, etc.) in livestock have been going on since the 1980s (Bjarnsholt *et al.*, 2013). Cells in biofilms provide shielding from the host immune system by hiding within the extracellular matrix. When the immune system identifies a foreign object, both innate and adaptive immunity are activated, leading to long-lasting inflammation and the production of polymorphonuclear neutrophils (PMNs), which may damage tissues without affecting the biofilm (Bjarnsholt *et al.*, 2013; Rybtke *et al.*, 2015; Zare *et al*., 2022). Bacterial cells remain protected within biofilms against bactericides and antibiotics (Uruén *et al*., 2020; Høiby *et al*., 2010; Stewart, 2002). In the dairy sector, bovine mastitis is the most significant and prevalent animal disease that lowers milk output (Stewart, 2002). Multidrug-resistant staphylococci are the most potent pathogenic bacteria causing bovine mastitis, thereby becoming a problem of concern to the animal industry. The most

Received on :15th March 2026; Revised version received on :9th August 2026; Accepted:23rd September 2026

predominant species of staphylococci are *Staphylococcus aureus* and *Staphylococcus epidermidis*, which are responsible for biofilm formation, causing pathogenesis (Bouari *et al*., 2026).

However, the most common cause of bovine mastitis is coagulase-negative staphylococci (CNS), found in many countries. Besides staphylococci, coliforms, enterococci and are also recurrently isolated from infected cows (Schwarz *et al*., 2010). Apart from these, other biofilm-associated diseases include bovine myocarditis (caused by *Histophilus somni*), endometritis in cows, Bovine Respiratory Disease Complex (BRDC), Porcine Enzootic Pneumonia caused by *Mycoplasma hyopneumoniae* and Porcine pleuropneumonia, caused by *Actinobacillus pleuropneumoniaes* (Nesse *et al*., 2023; Andrés-Lasheras *et al*., 2022). Bacterial biofilms in livestock settings are not exclusively associated with animal health problems. Subsequently, the crosstalk between human health, animal health, and environmental integrity is a prime concern in accordance with the "One Health Concept." [Figure 1]. This framework identifies that pathogens and antimicrobial resistance (AMR) determinants now conglomerate with occupational settings and ecosystems. In this plethora, the One Health Concept explores a synergistic approach to mitigate the human-animal-environmental interface (Food and Agriculture Organization of the United Nations (FAO), (Centers for Disease Control and Prevention 2022, One Health Basics. CDC, Atlanta), World Organization for Animal Health, World Health Organization (WHO) (2019) Taking a Multisectoral One Health Approach: A Tripartite Guide to Addressing Zoonotic Diseases in Countries. WHO, Geneva). The successful implementation of this concept relies on the removal of constraints derived from veterinary medicine and human populations from various aspects, such as environmental, ecological, evolutionary, and socioeconomic perspectives (Jacques & Malouin, 2022). Specifically, zoonotic transmission of biofilm integrated pathogens such as *Campylobacter* spp., *Staphylococcus aureus*, *Salmonella* spp. including livestock mediated methicillin-resistant *S. aureus* (MRSA), and *Listeria monocytogenes* is accelerated by biofilm-mediated survival on animal mucosa, hides, and farm equipment. Biofilm maturation increases tolerance to antimicrobials and biocides, thereby enhancing fomite survival and maintaining the transmission chain.

Another public health priority is to evaluate the significant impact of biofilms in the spread of AMR. Environmental biofilms can serve as secondary reservoirs, thereby enabling downstream transmission. The evolution of biofilms renders inhibition of antimicrobial penetration and horizontal gene transfer of resistance determinants among pathogens. The "One Biofilm Concept" could be the precise keystone fundamental to the "One Health Concept." To address this, a "One Health"-aligned scheme integrates monitoring of biofilm-associated pathogens and AMR determinants among animal, human, and environmental samples. Considering biofilm biology within the "One Health" framework, interventions can synergistically minimize animal disease burden, zoonotic transmission, foodborne risk, environmental threats, and the global consequences of AMR. This review focuses on the detailed study of various microorganisms affecting diverse organ systems in animals and the impact of bacterial biofilms in the livestock diseases pathogenesis (Figure 1). It also highlights the current therapeutic strategies to inhibit antimicrobial resistance (AMR) and various genomic editing tools with a regulatory framework to combat the therapeutic challenges in biofilm management (Andrés-Lasheras *et al*., 2021).

The primary aims of this review basically rely on providing an overview of the mechanisms involved in bacterial biofilms that directly contribute to pathogenesis and prolonged persistence in infections affecting livestock, with special highlights on clinically significant veterinary pathogens. Additionally, we scrutinize how biofilm formation instigates antimicrobial resistance and substantiates clinical therapeutic outcomes. Furthermore, we critically point out mechanistic insights into in vivo pathogen genome targeting in disease management. It also elaborates on different organ systems of livestock influenced by bacterial biofilms. In summary, detailed biofilm formation and its associated pathogenic disease consequences are also described. By conglomerating all these perspectives, this review leverages future research endeavours intended to mitigate the implications of biofilm-driven infections in livestock.

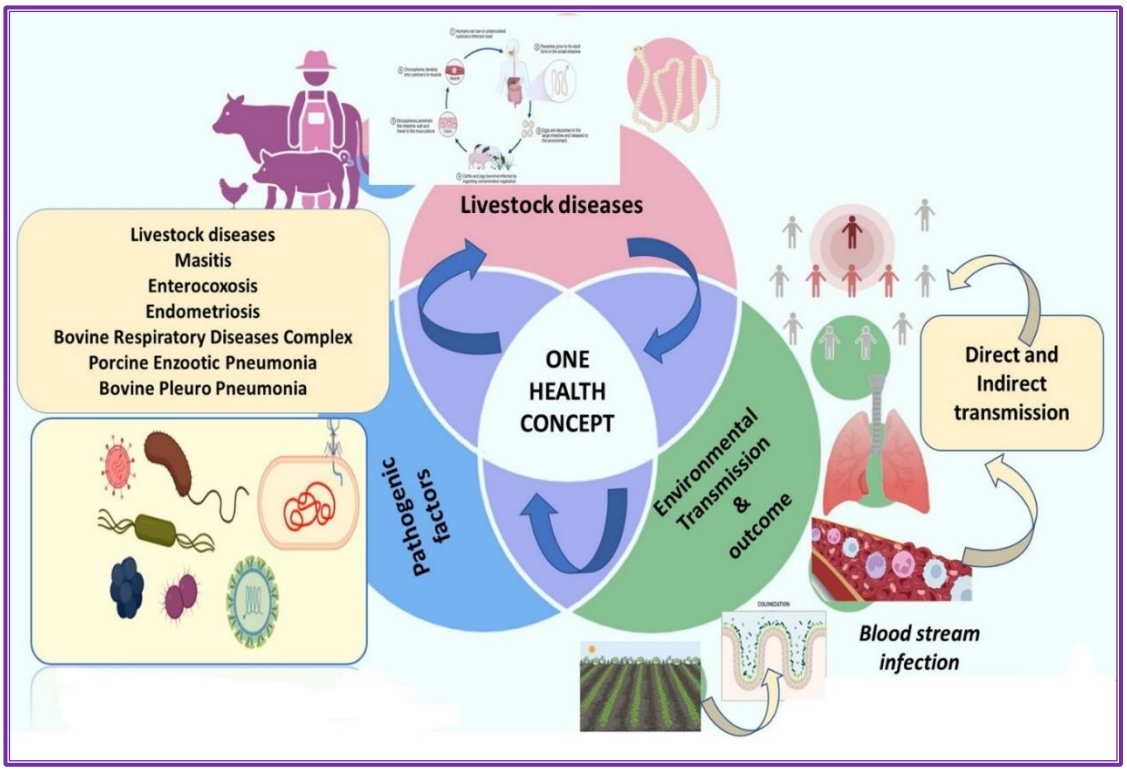


Figure 1: *Schematic Representation of One Health Concept in Correlation with Livestock Diseases [Reproduced by Self with Assistance of Biorender.Com]*

Methodology

A structured literature search process was performed to identify relevant reports on bacterial biofilms in livestock diseases, focusing on host-pathogen interactions, antimicrobial resistance, and therapeutic approaches in pathogenic conditions. Searches were conducted using major biomedical and multidisciplinary databases, including PubMed, Web of Science, Google Scholar, and Semantic Scholar, to ensure comprehensive reporting of peer-reviewed veterinary, microbiology, and translational literature. The search strategy combined specific keywords related to the topic, such as “bacterial biofilm,” “livestock,” “cattle,” “cow,” “pig,” “goat,” “mastitis,” “endometritis,” “respiratory disease,” “endometritis,” “*Escherichia coli*,” “*Staphylococcus aureus*,” “*Streptococcus*,” “*Mycoplasma*,” “biofilm formation,” “antimicrobial resistance,” and “quorum sensing.” Boolean operators such as AND and OR were applied to refine the combined search results where appropriate. Reference lists of selected articles were also scrutinized by forward and backward tracking to identify additional studies not captured in the database search.

Inclusion and Exclusion Criteria

Inclusive studies incorporated relevant original research articles, review articles, and book chapters published in English that addressed bacterial biofilm formation in livestock species or its role in disease pathogenesis, persistence, diagnosis, and treatment. Studies were included if they provided experimental, observational, or mechanistic evidence relevant to biofilm-associated infections in food-producing animals. Articles focused solely on human biofilms, non-bacterial biofilms, unrelated or distantly related microbial systems, or non-livestock species were excluded. Outdated or duplicated records, inaccessible full texts, conference abstracts without sufficient data, and articles lacking proper indexing and relevance to the central theme of the review were also excluded.

The search was performed to acquire and summarize current literature available up to the time of manuscript preparation, as possible. Retrieved and selected articles were screened in a stepwise manner by summarizing title and abstract, followed by evaluation. Priority was given to studies with clear methodological descriptions, explanations of specific pathogenicity and sufficient experimental

data supporting their conclusions. This approach ensured that the narrative review synthesizes updated, reliable, and scientifically robust evidence on bacterial biofilms in livestock diseases.

Bacterial Biofilm Biology

Bacterial biofilms are populations of bacterial cell that are surface-associated and consist of microcolonies of bacteria surrounded by exopolysaccharides (Danese *et al*., 2000; Sauer *et al*., 2022). It is predicted that greater than 99% of bacteria on Earth reside in organized biofilm communities, and it is observed that 65% of nosocomial infections and 61% of zoonotic human infections are associated with biofilm formation (Alotaibi, 2021). The extracellular polymeric matrix, which is mostly created by the microbes themselves, gives the biofilm its structure and allows for survival under harsh conditions, including resistance to different generations of antibiotics (Bright *et al*., 2023; Limoli *et al*., 2015). The various steps involved in biofilm attachment, colonization and maturation have been illustrated in Figure 2.

Biofilm Structure

The formation and establishment of bacterial biofilms are dynamic, intricate processes controlled by intrinsic biological characteristics and physical factors like physicochemical characteristics. Since biofilms absorb a lot of water through hydrogen bonding, they are complex, highly hydrated structures with water channels (Alotaibi, 2021).

DNA, extracellular polysaccharides constitute the framework of the EPS found in biofilms. Exopolysaccharides are high-molecular-weight sugar polymers that are either intra- or extracellularly produced and released into the external environment (Chakraborty *et al*., 2018). *Streptococcus mutans* possesses glucan-binding proteins (Gbps), which bind bacteria and exopolysaccharides and are essential for preserving biofilm formation (Lynch *et al*., 2007). The structure of biofilms is further enhanced by amyloids, which are insoluble fibrous proteins. Biofilm production is enhanced in *Pseudomonas* species when Fap amyloids are overexpressed (Chakraborty *et al*., 2018). Along with polysaccharides and proteins, extracellular DNA (eDNA) also plays an important role in the architecture of bacterial biofilms, as it helps bacteria attach to the substratum (Singh *et al*., 2010). The AMR characteristics of biofilms is another crucial factor, and negatively charged eDNA can chelate positively charged antibiotics, thereby conferring antibiotic resistance (Lewenza, 2013). Microbial cells make up around 2–5% of the total amount of the biofilm matrix, whereas extracellular polymeric compounds comprise approximately 95–98% (Chakraborty *et al*., 2018).

Biofilm Maturation

The interactions of biological, chemical and physical processes induce in the development of biofilms formation (Perni *et al*., 2014). The initial adhesion and successive aggregation into multicellular structures are the crucial initial stages in the biofilm growth. Adhesive forces are necessary for biofilm growth during surface colonization, as well as for the interaction between cells. The development of fluid-filled channels, which are essential for the transport of nutritional substances, metabolites, and oxygen to every biofilm cell and give the biofilm its characteristic three-dimensional structure, is mostly attributed to disruptive forces. Bacterial microcolonies subsequently grow into mature biofilms, which are eventually followed by the cells in the biofilm dispersing into the surrounding environment (Figure 2).

Initial Reversible Attachment

Individual cells adhere to surfaces in contact with an aqueous solution to initiate the formation of a biofilm, which is then trailed by the formation of a conditioning layer. Once the bacteria adhere to the surface, there is a greater chance that additional free-floating microorganisms may be transported, leading to coaggregation and the formation of multiple layers (Perni *et al*., 2014). Cellular or microbial adherence is greatly influenced by the conditioning film, which can govern by the number of organic moieties. It has been observed that increased surface roughness effectively promotes early biofilm

growth and microbial colonization. The attachment of the bacteria to the conditioning layer depends on the motility of the bacterium.

Irreversible Attachment to the Substratum

In the step of irreversible attachment, bacteria irreversibly attach to the substratum and start to synthesize extracellular polymeric substances (EPS). Planktonic microorganisms can adhere to various surface-bound organisms or to one another during this stage, forming aggregates on the substratum. If no physical or chemical intervention is applied, the adhesion becomes irreversible, resulting in the bacterial cells becoming firmly attached to the surface substratum (Alotaibi, 2021).

Development of Biofilm Architecture

After attachment to the substratum, an exponential phase of growth can be observed, leading to the aggregation of microcolonies. The irreversibly adhered cells originate to proliferate and divide while utilizing the available nutrients in the conditioning film. Bacteria start to produce polysaccharide intercellular adhesion (PIA) polymers for firm attachment (Sharma *et al*., 2023).

Maturation of Biofilm

In this phase, attached small colonies multiply and take up new planktonic bacteria and debris from the surroundings, developing into a mature biofilm with a distinctive 3-dimensional structure that depends on EPS. The biofilm uses quorum sensing, a kind of cell signalling, when cell density is high. After maturation, the structure of a biofilm is composed of three consecutive layers: a connecting film that attributes the biofilm to the surface substratum; a base matrix with bacterial cells accumulation; and a surface film allowing free-floating bacteria dispersion and transmission.

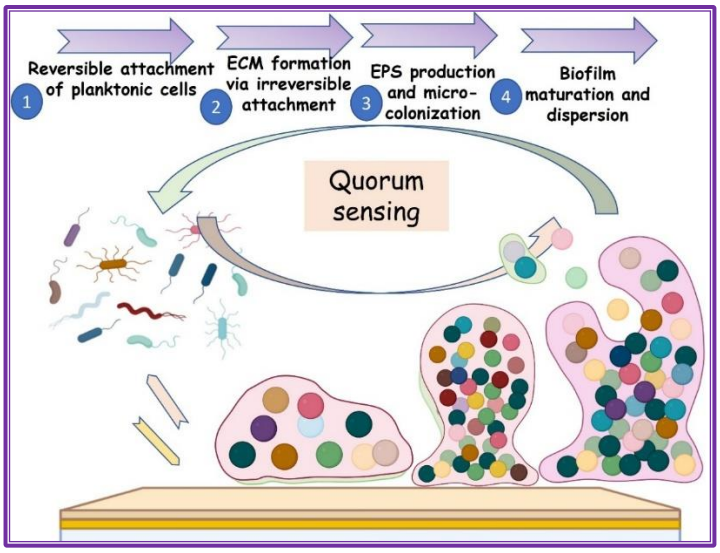


Figure 2: *Sequential Steps of Biofilm Maturation, 1. Reversible Attachment of Planktonic Cells, 2. ECM (Extracellular Matrix) Formation Via Irreversible Attachment, 3. EPS (Extracellular Polymeric Substance) Production and Micro-Colonization, 4. Biofilm Maturation and Dispersion [Reproduced by Self with Assistance of Biorender.com]*

The Final Stage of Dispersal

The biofilm process ends with dispersal, in which attached cells separate and spread to occupy a new niche. The dispersal of a mature biofilm can be achieved either by shedding daughter cells or by the influence of other factors, such as fluid dynamics, nutrient limitation etc. In general, a total of three substages comprise the dispersal stage, namely sloughing, erosion, and abrasion. Sloughing is the process by which large biofilm biomass particles are released, and erosion is the continual elimination of individual cells or small biofilm segments. When the loss of biofilm is due to suspended particles, it is termed abrasion (Kaplan, 2010). Hypoxic or less nutrient condition at the base of the biofilm, as well

as an abrupt rise in nutrient concentration, result in sloughing. Any cells that are liberated may be transported to new locations, where they may recommence the biofilm formation process, and this is believed to be the main reason for the propagation of infections (Maklakova *et al*., 2024).

Basic Molecular Insight to Biofilm Formation

Several molecular strategies are involved in biofilm growth and proliferation, which can lead to microbial infection followed by antimicrobial resistance. This affects the treatment outcome and produces antimicrobial resistance mechanisms, thereby affecting therapeutic outcome. The following section deals with various mechanistic insights and their correlation with biofilm maturation.

Quorum Sensing

The different phases of biofilm formation depend on the class of bacteria and surface colonization pattern. Bacteria have quorum sensing (QS) mechanisms that are involved in host colonization to the production of various virulence determinants (Juszczuk-Kubiak, 2024). According to genetic studies, based on various RNA synthesis studies, the genes responsible may be broadly classified into 4 classes according to their functions (Sánchez-Jiménez *et al*., 2023). Genes related to cell life and growth are included in the first group; genes regulating cell behaviour in the environment are included in the second group; and genes related to horizontal gene transfer are included in the third group. The genes belonging to the fourth group are intricate in the formation of virulence determinants (Sun *et al*., 2022).

Various virulence factors including coagulase, elastase, lectins, and proteases, are denoted by gene groups that are showing upregulation by the QS system. The gene like the las operons, Rhl operons, Pqs and Lgs operons are predominantly overexpressed. In Gram negative bacteria specifically acyl homoserine lactones (AHL) act as autoinducers and modulate the signalling pathways of the QS system. In this regard, the widely investigated AHL mediated QS mechanism in Gram negative bacteria is LuXI/LuxR system, which was first isolated in *Vibrio fischeri* (Juszczuk-Kubiak, 2024).

Serratamolide and other lipopeptide biosurfactants from the serrawettin family of lipopeptides are produced by *Serratia marcescens*. These compounds improve surface motility, which is linked to the first stages of biofilm progress and may trigger hemolytic activity. The matrix contains extracellular DNA, and DNase activity alters adhesion and dispersion, suggesting that eDNA is functional in Serratia biofilms. Transcriptomic studies of clinical isolates with mutations in the GumB gene, an inner-membrane Rcs regulatory component, show that envelope-stress signalling via the Rcs phosphorelay system closely controls biofilm-associated gene expression in *Serratia marcescens* (Boldeanu *et al*., 2025). Several methods have been observed where Gram positive bacteria produce autoinducers (AIP) that directly affect the QS system. Additionally, AIP induces a membrane signalling cascade via sequential phosphorylation events. It interacts with transcription factors that ultimately govern target gene expression via ATP binding cassette transporters within the cell (Neiditch *et al*., 2017; Juszczuk-Kubiak, 2024).

In livestock-associated biofilm development in case of *Staphylococcus aureus* is controlled by a coordinated network of adhesion factors, matrix-producing genes, and global regulatory systems. First, a capsular antigen polysaccharide/adhesin (PS/A) causes bacterial cells to attach to a surface. After that, biofilm development forms a multilayered biofilm that stimulates the development of polysaccharide intercellular adhesin (PIA). PIA and PS/A production in staphylococcal species is triggered by the intercellular adhesion (ica) operon, which is comprised of the regulatory gene icaR and the structural genes icaA, icaB, icaC, and icaD, which encode the proteins IcaA, IcaB, IcaC, and IcaD (Ibrahim *et al*., 2022; Song *et al.,* 2024).

Cyclic-di-GMP Signalling

Cyclic di-GMP (c-di-GMP), an internal signalling molecule, governs several important bacterial processes and mechanisms. High quantities of c-di-GMP have been shown to be required, mainly for bacterial biofilm formation and establishment. Bacterial cellular modifications have been demonstrated to be driven by variations in c-di-GMP levels. An increase in c-di-GMP levels is linked to a shift from an

aggressive to a chronic infection strategy or from a motile, fast-spreading cell to a slow-growing biofilm (Valentini & Filloux, 2019).

For Gram-negative bacterial cells, the second messenger molecule c-di-GMP controls motility and biofilm development. A flagellum-dependent biofilm regulatory (FDBR) response is triggered when the flagellum is not present due to the loss of the gene responsible for producing flagellin. This reaction boosts biofilm formation, increases cellular c-di-GMP levels, and increases the expression of biofilm related genes (Wu *et al*., 2020).

Biofilm-driven Persistence and Dynamics in Livestock Diseases

Persister cells (PCs) are dormant, non-growing, yet drug-susceptible bacterial cells that survive antibiotic exposure through a transient phenotypic tolerance and can regrow and re-establish infection. The persisters found in biofilms were later identified as the cause of chronic persistent infections, recurrence following therapy, and the difficulty associated with treating biofilm infections (Niu *et al*., 2024). According to a study, the stringent response (RelA, SpoT) and toxin-antitoxin (TA) modules are necessary for persister development, and antibiotic treatment increases the expression of genes involved in these mechanisms. Additionally, *P. aeruginosa* biofilm persister cells are much more resistant to antibiotics than non-persister cells due to the heterogeneity of biofilm formation and different growth phases (Patel *et al*., 2022). Two antibiotics, ciprofloxacin and colistin, enhance PC formation in *P. aeruginosa*, as they promote the type II TA system (RelBE, VapBC) during the log and stationary phases of growth (Zadeh *et al*., 2022). A feed-forward loop, where persistence and biofilm production mutually promote one another, is suggested by the fact that mutations enhancing persister formation also result in much higher biofilm biomass in livestock pathogens such as *M. bovis* and *M. hyopneumoniae* (Greve *et al*., 2025; Hoelzle *et al*., 2020).

Horizontal gene transfer (HGT) is more commonly involved in biofilm formation, as extracellular polymeric substances, including DNA and mobile genetic material, promote the likelihood of gene exchange (Michaelis & Grohmann, 2023). Through transformation inside biofilms, ampicillin-tolerant *E. coli* persisters carry out HGT, obtaining plasmid DNA from dead cells at a frequency of 10^{-7}–10^{-6} per cell (Nasu & Maeda, 2024). Sessile cells leave the extracellular matrix to colonize new surfaces by an active, controlled process called biofilm dispersal, which is controlled by chemical signalling pathways. Quorum sensing and cyclic-di-GMP signalling control dispersal. Native dispersal in *P. aeruginosa* depends on elements essential to environmentally mediated dispersal, such as BdlA, DipA, RbdA, and AmrZ, and occurs concurrently with decreased c-di-GMP levels (Kalia *et al*., 2023). The cidABC operon controls biofilm dispersal in *S. aureus* by regulating cell lysis inside the biofilm, while proteases help sessile cells spread to colonize new surfaces (Aboelnaga *et al*., 2024).

Significance of Biofilms in Major Livestock Diseases

Microbes are typically recognized as potent host immunological responses and participate in a wide spectrum of defence mechanisms to adapt to environmental stress. Microorganisms derived from biofilms have altered metabolic pathways and are genetically adapted to induce host-pathogen interactions in livestock organisms. Biofilm infection affects several organs and tissues in livestock. The related disease consequences have been discussed effectively in the following section.

Endometritis Disease

In the dairy industry, postpartum reproductive abnormalities result in significant financial losses. Mucometra, endometritis, pyometra and metritis are examples of uterine infections. Endometritis is one of the main gynaecological issues influencing the economy of milk production and reproductive efficacy in dairy cows among all these uterine inflammatory illnesses. In dairy cows, postpartum endometritis is defined as endometrial inflammation that appears 21 days or more after parturition and is not associated by systemic symptoms. Purulent flakes in the uterine discharge are a clinical characteristic of this condition (Mandhwani *et al*., 2017).

It is brought on by bacteria growing in the uterine endometrium. *Escherichia coli*, an opportunistic and pathogenic bacterium, primarily induces a local cellular immune response that is detectable in a cytological test as leukocyte infiltration. Long-lasting inflammation can be caused by opportunistic microorganisms in the uterus secreting chemicals that damage the local immune responses of the endometrium. *Bacillus licheniformis*, *Fusobacterium necrophorum*, *Trueperella pyogenes*, *E. coli*, *Streptococcus* spp., and *Prevotella* spp. are the most frequently isolated bacterial agents from the uterus of postpartum cows. Research assessing the appearance and smell of vaginal mucus has revealed that *E. coli*, *T. pyogenes*, and non-hemolytic *Streptococcus* spp. are linked to foul-smelling exudates, while *Proteus* spp., *F. necrophorum*, and *T. pyogenes* are linked to mucopurulent or purulent discharge that is visible in the vaginal mucus (Maklakova *et al*., 2024).

The inflammation of the endometrium without any visible symptoms and frequently without any indication of infection is known as subclinical endometritis. This disorder may have its roots in changes to the postpartum inflammatory response. Numerous effects of bacterial endotoxins on reproduction are known to exist: (a) they may interfere with the synthesis of LH, disrupt the secretion of progesterone and estradiol, change follicular growth and the corpus luteum's normal development, and induce ovulation failure.; (b) enhance PGE_2 production and extend the corpus luteum's lifespan; and (c) induce embryo mortality (Arias *et al*., 2018). In cows with metritis and clinical endometritis, known pathogens such *E. coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum*, or *Prevotella* spp. are often isolated from the uterus. Studies have demonstrated that, in comparison to healthy cows, cows with clinical and subclinical endometritis have higher serum concentrations of pro-inflammatory mediators and greater expression of endometrial mRNA. Conditions in which inflammation persists even after bacterial contamination has been eliminated might arise when low levels of anti-inflammatory mediators are produced in conjunction with increased eicosanoids, delaying the return of the affected tissues to equilibrium (Söllner *et al*., 2021).

Mastitis Disease

Mastitis in cows is an inflammatory response of the udder tissue of the mammary gland caused by injury or infection (Nesse *et al*., 2023), (Cheng & Han, 2020). During lactation, bovine mastitis (BM) mainly takes place. The primary organism of BM is *Staphylococcus aureus*. Other organisms associated with bovine mastitis are *Streptococcus agalactiae* (causing contagious mastitis), coagulase-negative staphylococci (CoNS), *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *S. dysgalactiae*, *S. uberis*, *Enterococcus* spp., and *Pseudomonas* spp (Nesse *et al*., 2023).

It is considered that bacterial intramammary infections (IMIs) are the primary cause of mastitis in cows. These diseases can be classified as either infectious or environmental based on the source of the pathogens. "Contagious mastitis" is defined as mastitis that can be transmitted from cow. In addition to less common organisms like *Mycoplasma bovis* and *Corynebacterium* spp., which colonize and grow inside the teat canal, infectious pathogens like *Staph. aureus* and *Strep. agalactiae* are found in the udder and teat skin of cows. Two strategies to reduce environmental infection include lowering the teat ends' exposure to environmental pathogens and boosting the cows' resistance to IMIs by vaccination and antibiotic intervention (Cheng & Han, 2020).

Table 1: *List of Pathogens causing Different Types of Mastitis*

Pathogen	Mastitis Type	Biofilm Formation	References
Staphylococcus Aureus	Subclinical Mastitis	+	Darwish & Asfour, 2013; Fabres-Klein *et al*., 2015
Coagulase-Negative Staphylococci	Subclinical Mastitis	+	Darwish & Asfour, 2013
Escherichia coli	Clinical mastitis	+	Fabres-Klein *et al*., 2015; Milanov *et al*., 2015
Enterococcus faecalis	Clinical mastitis	+	Elhadidy & Zahran, 2014
Streptococcus uberis	Clinical mastitis	+	Crowley *et al*., 2011; Krömker *et al*., 2014
Streptococcus agalactiae	Mastitis	+	Rosini & Margarit, 2015
Serratia marcescens	Mastitis	+	Boldeanu *et al*., 2025

Research has shown that, in mastitis pathogenesis, the Bap protein contributes to biofilm formation in staphylococci. It was detected that, in subclinical mastitis, bap-positive *S. aureus* colonizes the mammary gland in vivo (Cheng & Han, 2020) (Table 1).

Bovine Respiratory Disease Complex (BRDC)

The respiratory illness that affects cattle, known as bovine respiratory disease (BRD), shipping fever pneumonia, or undifferentiated fever. The main bacterial agents that cause it are typically *Mannheimia haemolytica* and, less commonly, *Pasteurella multocida*, *Histophilus somni*, or *Mycoplasma bovis*. Major factors involved in pathogenesis ultimately leads to the lung inflammation followed by cell death. Bacterial byproducts including capsular polysaccharides, several enzymes, iron binding proteins, lipoologosaccharides have the ability to weaken the immune system, cause immunological damage, and induce a serious inflammatory reaction (Campbell, 2023).

Porcine Enzootic Pneumonia

The principal cause of enzootic pneumonia, *Mycoplasma hyopneumoniae*, is found all over the world and costs the pig industry a significant amount of money. The bacterium adheres to the respiratory tract's ciliated epithelium and causes damage to it. Pigs with the disease exhibit persistent coughing, decreased performance, and increased susceptibility to other respiratory illnesses. Furthermore, it is thought that *M. hyopneumoniae* is one of the main culprits causing porcine respiratory disease complex (PRDC) (Maes *et al*., 2008).

M. hyopneumoniae interferes with the mucosal clearance process through damaging the cilia on the epithelial surface. Furthermore, the bacterium alters the respiratory tract's immune system (Thacker *et al*., 2000). As a result, *M. hyopneumoniae* puts animals at risk of co-infections with viruses, bacteria, and parasites that cause respiratory diseases (Maes *et al*., 2008).

Porcine Pleuropneumonia

Although animals of all ages are susceptible, pigs between the ages of 12 and 15 weeks are the most common victims of porcine pleuropneumonia. It is a highly infectious caused by Gram-negative *Actinobacillus pleuropneumoniae*. A fibrinous to fibrinonecrotic bronchopneumonia is present in the more acute forms, whereas diminished weight gain and the formation of pulmonary sequestra are characteristics of the chronic form. Clinical manifestations can range from subacute to chronic. The high mortality rate and high expense of chemotherapy associated with swine pleuropneumonia outbreaks result in significant economic losses. Herd performance may be compromised by the disease's preclinical and chronic manifestations (Nahar *et al*., 2021).

Numerous virulence factors expressed by *A. pleuropneumoniae* are required for tissue injury, immunological clearance, and colonisation. Vaccines are used to prevent *A. pleuropneumoniae* infection in swine herds, although they are not completely effective in doing so; in many cases, they only provide protection against the serovar or serovars that were used to make the vaccine (Nusrat Nahar *et al*., 2021). Porcine pleuropneumonia's causal agent, *A. pleuropneumoniae*, is incredibly adaptable when it comes to producing RTX toxins. In this species, four distinct RTX toxins have been found. ApxI, ApxII, and ApxIII are three of them that have undergone substantial research. Strongly hemolytic and strongly cytotoxic for phagocytic cells, ApxI (110 kDa) shares 41% of its amino acid sequence with *M. haemolytica* LktA and 56% of its amino acid sequence with *E. coli* HlyA. Compared to ApxI (46% identity) and *E. coli* HlyA (47% identity), ApxII (102.5 kDa) demonstrates mild hemolytic and moderate cytotoxic activity. As a result, ApxII contains only eight consecutive nonapeptide repeats, similar to *M. haemolytica* LktA (67% identity). On the other hand, ApxI contains 13 repeats. ApxIII (113 kDa, 13 glycine-rich repetitions in the repeat domain) exhibits little hemolytic activity but exhibits significant cytotoxic action against neutrophils and porcine alveolar macrophages (Söllner *et al*., 2021).

Animals suffering from acute outbreaks must receive parenteral antibiotic therapy immediately, as well as those in nearby pens. This can lower mortality in addition to adding antibiotics to the diet or water for a few weeks. Common medications used for prophylaxis and/or therapy include tulathromycin,

tiamulin, ceftiofur, chlortetracycline, tilmicosin, florfenicol, enrofloxacin, and procaine penicillin G. Tetracycline resistance is common in these strains. Treatment usually has no effect on chronic patients.

Porcine Bronchopneumonia

Bordetella bronchiseptica is a Gram-negative bacterium that naturally infects a variety of domestic, wild, and pet animals. It is closely related to *Bordetella pertussis* and *Bordetella parapertussis*. *B. bronchiseptica* is prevalent in swine and has a significant role in respiratory disorders. Bronchopneumoniae is predominantly expressed in young pigs and develops secondary pneumonia in older pigs. Also, *B. bronchiseptica* is the prime causative agent of bronchopneumonia among young pigs and plays a pivotal role in developing secondary pneumonia in cats (Nicholson *et al*., 2017).

According to recent investigations, *B. bronchiseptica* can form a biofilm on abiotic substratum and also in the respiratory tract of mice due to the production of exopolysaccharide Bps (Bordetella polysaccharide) (Nicholson *et al*., 2017; Sloan *et al*., 2007; Cattelan *et al*., 2016). The Bordetella virulence gene BvgAS signal transduction system directs the expression of genes encoding secreted, surface, membrane, regulatory, and secretory proteins, along with those involved in the metabolism and physiology of the bacterium. BvgA and BvgS are the regulatory signal-transducing proteins. These studies demonstrate that *Bordetella bronchiseptica* biofilm production is regulated and controlled by BvgAS (Cattelan *et al*., 2016).

According to the report by Cattelan *et al.,* (2016) during the initial phase of *B. bronchiseptica* biofilm formation, bvg-activated genes were in the repressed mode, and there was an induction of bvg-repressed genes. Bvg-activated proteins include filamentous hemagglutinin (FHA), pertactin, fimbriae, and toxins such as adenylate cyclase toxin (ACT), whereas bvg-repressed proteins include flagellar proteins. This suggests that motility and flagellar movement are important for initial attachment to the substrate during biofilm formation. The bpsA-D locus was expressed more frequently in biofilms of *B. bronchiseptica*, but the expression of bpsA-D is not under the regulation of BvgAS genes (Sloan *et al.,* 2007).

Pathogen-associated Livestock Diseases

Staphylococcus Species

Several clinical and subclinical forms of mastitis are associated with the Gram-positive bacterium *Staphylococcus aureus*. The mammary gland is persistently infected with this bacterium. It causes a weaker immunological response than *E. coli*, which results in chronic mastitis that persists for several months (Cheng & Han, 2020). Because methicillin-resistant *S. aureus* (MRSA) strains are often resistant to multiple classes of antimicrobials, antimicrobials have become less effective in treating *S. aureus* infections due to the antimicrobial resistance that frequently occurs in these organisms.

According to some research, spillover from adjacent pig farms was the cause of livestock-associated MRSA clonal complex (CC) 398 infections linked to healthcare. Faecal–oral transmission is another way that *S. aureus* strains might reach humans via the food chain. MSCRAMMs, or microbial surface components recognizing adhesive matrix molecules, are the primary protein family implicated in *S. aureus* adhesion. Various proteins, including fibronectin-binding proteins A and B, laminin-binding protein, collagen-binding protein, fibrinogen-binding protein and elastin-binding protein, are members of the MSCRAMMs family (Figueroa-Valenzuela *et al.,* 2022; Silva *et al.,* 2022).

Coagulase-Negative Staphylococci (CNS)

CNS is thought to be the most frequent cause of cow mastitis. In dairy cows, the CNS is the primary source of subclinical mastitis, which can lead to herd problems. The most common CNS species in the study herds were *S. haemolyticus*, *S. epidermidis*, *S. simulans*, *S. chromogenes*, and *S. xylosus*. Among cows with subclinical mastitis, intramammary infections (IMIs) caused by *S. epidermidis* were mainly seen in multiparous cows, whereas *S. chromogenes* IMIs were found in primiparous cows (Thorberg *et al.,* 2009). CNS are generally found on the teats, nasal mucosa, and the hands of milking personnel. Low levels of CNS represent the normal flora, whereas high levels represent poor udder

sanitation or contamination of equipment. In dairy herds, CNS can cause chronic infections that lower milk quality and increase the milk somatic cell count (SCC). One detrimental effect of CNS infection in dairy herds is that it can damage udder tissue, which causes dairy cows to produce less milk (Cheng & Han, 2020).

Escherichia Coli

Escherichia coli is considered to be one of the major causative agents of BM. The fimH gene, encoding type 1 fimbriae, is responsible for biofilm formation and is found in all isolates (Gomes *et al.,* 2016; Thorberg *et al.,* 2009). Gram-negative *E. coli* bacteria enter bovine udders through the teat, then multiply and proliferate, and trigger an inflammatory reaction in dairy cows. *E. coli* induced severe clinical mastitis can cause complete loss of milk production, irreversible damage to the mammary gland tissue, and in rare cases, even the death of dairy cows. Because of its capacity to form biofilms to varying degrees, *E. coli* can survive in the mammary gland and result in difficult-to-treat mastitis infections (Goulart & Mellata, 2022). Adhesins, invasins, and toxins are some of the virulence factors that *E. coli* contains. These factors allow the bacteria to colonize and grow in the udder while resisting the action of serum complement proteins and the host's selection pressure (Gomes *et al.,* 2016).

Enterococcus Species

The Gram-positive bacterium *Enterococcus faecalis* is responsible for bovine mastitis by invading the mammary glands. Experimental data have demonstrated a connection between *E. faecalis* biofilm development and adherence or invasion of the mammary gland epithelium. Studies have shown that there may be a possibility of the involvement of Esp, specific cell surface protein responsible for biofilm formation. The genetic sequence of the Esp protein is similar to that of the Bap protein, which is also involved in biofilm formation. *E. faecalis* has an innate resistance to several antimicrobial agents, thereby exhibiting increased resistance (Elhadidy & Zahran, 2014; Elhadidy & Elsayyad, 2013).

Streptococcus Species

Streptococcus uberis is an environmental bacterium that develops mastitis and is related to both clinical and subclinical infections. The components of milk, α- and β-casein, have been shown to promote the development of biofilms that allow *Strep. uberis* may withstand environmental stress and resist antimicrobial therapy. The rectum, rumen, respiratory tract, lips, skin, oral cavity, tonsils, sick udders, faeces, and wounds are among the animal body sites where it has been discovered (Elhadidy & Zahran, 2014). The Gram-positive bacterium *Streptococcus agalactiae* causes infectious mastitis in cattle. It is found in the gastrointestinal system of cows and in the surroundings of dairy cows. It can spread by the oral–faecal route and through milking machines, especially through contaminated drinking water (Cheng & Han, 2020). By maturing a biofilm enables it to adhere to the mammary gland and resulting enhances AMR. *Streptococcus agalactiae* can survive in the mammary gland of cows indefinitely ultimately affects in decreased milk output (Cheng & Han, 2020).

Pseudomonas Aeruginosa

Animals and humans can become ill due to the common opportunistic bacterium *Pseudomonas aeruginosa*. This bacterium can be transmitted through contaminated drinking water in livestock. Diseases that decrease fertility and reproduction have an impact on animal productivity. The two most common reproductive diseases in cows, cattle, camels, and mares that lower overall milk supply are endometritis and metritis (Nesse *et al.,* 2023; Mahmoud *et al.,* 2022).

Several antibiotics, including aminoglycosides, quinolones, and β-lactams, are ineffective against *P. aeruginosa*. Three main categories of mechanisms are thought to be responsible for *P. aeruginosa* antimicrobial resistance: intrinsic, acquired, and adaptive resistance. *P. aeruginosa* exhibits intrinsic antimicrobial resistance because of the development of efflux pumps, the limited permeability of its outer membrane, and the generation of enzymes that inactivate antibiotics (Mahmoud *et al.,* 2022; Ferris *et al.,* 2017). The quorum-sensing (QS) system, a cell-to-cell signalling mechanism that regulates gene expression in accordance to variations in cell population density, is primarily responsible for

governing *P. aeruginosa* associated biofilm formation. Three QS systems have been found in *P. aeruginosa*: LasI-LasR, RhlI-RhlR, and PQS-MvfR. These systems all produce autoinducers, which diffuse into host and bacterial cells and cause transcriptional regulation that lowers the immune response to infection and increases bacterial survival (Mahmoud *et al.,* 2022; Tuon *et al.,* 2022).

Therapeutic Insights in Livestock Disease Management

Significant therapeutic insights for livestock disease management are emerging from various aspects, specifically early screening and targeted intervention. This includes next-generation vaccines, monoclonal antibody design and CRISPR-based strategies.

Insight into AMR

AMR is a concerning public health issue because of its substantial clinical repercussions, which include increased morbidity and mortality, the development of comorbidities, and the financial burden on the healthcare industry. Antimicrobial-resistant bacteria are highly prevalent among farmers and veterinarians. From several investigations it was found that farm animals are the major source of AMR bacteria, including *E. coli*, *Salmonella* spp., *S. aureus*, and *K. pneumoniae*, and that associated genes are transmitted from livestock to humans (Meijs *et al.,* 2021).

According to the study by Meijs *et al* . (2021), there is evidence of the prevalence and occurrence of extended-spectrum β-lactamase (ESBL)-producing *E. coli* and AmpC β-lactamase-producing *K. pneumoniae* among Dutch farm workers and family members. The ESBL gene shared by agricultural labourers was present in 13% of household members, and 17.4% of gene is derived from *K. pneumoniae* and *E. coli*. The table depicts the antibiotic resistance genes (ARG) associated genes in livestock pathogens (Meijs *et al.,* 2021) (Table 2).

Table 2: *ARGs and Bacteria Often Found in Livestock (Bava et al., 2024)*

Name of the Bacteria	Name of the ARGs
Clostridium sp., *Acinetobacter* sp., *E. coli, K. pneumoniae, P. aeruginosa, Salmonella* sp.	β-Lactams: ^{bla}CTX−M−1, ^{bla}CTX−M−8, ^{bla}CTX−M−14, mecA, ampC. Aminoglycosides: aac, aad

Current Therapeutics

It is essential to manage antibiotic resistance to prevent bacterial infections in livestock. The protective matrix that biofilms produce for bacteria may make antibiotic monotherapy ineffective against bacterial biofilms. It is suggested by many research investigations that combinations of antibiotics that target different bacterial populations aid in overcoming biofilm heterogeneity. Mala *et al* . (2022) examined the combination therapy of antibiotics, gentamicin and zinc pyrithione, to assess the elimination of biofilms of *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Streptococcus dysgalactiae* for livestock skin diseases (Mala *et al.,* 2022). A flavonoid called baicalin was extracted from the roots of *Scutellaria baicalensis* and used as a quorum-sensing inhibitor with gentamicin, tobramycin, and kanamycin in different combinations (Guchhait *et al.,* 2026).

Adhesin proteins are responsible of bacterial biofilms. The purpose of adhesin targeting is to interfere with these adhesion processes through surface alterations in order to prevent the formation of biofilms. Researchers successfully targeted fimH and the associated genes luxS and bolA using single-guide RNAs (sgRNAs) in a recent study using the contemporary CRISPR/Cas9-HDR technique (Alshammari *et al.,* 2023). Antimicrobial peptides are another new strategy to disrupt microbial membranes and prevent biofilm formation (Guchhait *et al.,* 2026). Tables 3 and 4 highlight the key parameters, including antibiofilm management and its various features towards livestock disease management and therapeutic applications.

Table 3: *Comparative Table Highlighting Major Livestock Pathogens, Resistance Mechanisms, and Therapeutic Outcomes*

Livestock Pathogen	Host	Biofilm Genes	Name of the Disease Caused	Resistance Mechanism	Therapeutic Applications	Reference
Staphylococcus aureus	Cattle, pigs, sheep, chickens	ica operon, bap, agr,sar	Bovine mastitis, skin/soft tissue infections, septic arthritis	Biofilm-mediated tolerance; multidrug resistance; MRSA	Antibiotics, combination therapy, biofilm-targeted approaches	Cheng & Han, 2020
Coagulase-negative staphylococci *S. epidermidis*, *S. simulans*, *S. chromogenes*, *S. haemolyticus*, *S. xylosus*)	Cattle	Biofilm-associated staphylococcal adhesion systems; ica-independent in some goats	Subclinical bovine mastitis	Persistent infection; biofilm-associated tolerance	Antibiotics	Cheng & Han, 2020
Escherichia coli	Cattle	fimH; fimbrial adhesins	Clinical mastitis, endometritis	Biofilm formation, serum-complement evasion, chronic persistence	Antibiotics; combination approaches; adhesin targeting	Goulart & Mellata, 2022
Streptococcus uberis	Cattle	Biofilm promoted by milk proteins; adhesion-associated factors	Clinical and subclinical mastitis	Biofilm-mediated resistance to antibiotics and environmental stress	Antibiotics; vaccination	Elhadidy & Zahran, 2014
Streptococcus agalactiae	Cattle	Biofilm-associated adhesion, persistence factors	Infectious mastitis	Biofilm-driven persistence in mammary gland	Antibiotics; milking hygiene	Nesse *et al.,* 2023
Pseudomonas aeruginosa	Sheep, cattle	lasI-lasR, rhlI-rhlR, PQS/mvfR;	Fleece rot disease, reproductive infections	Intrinsic, acquired, and adaptive resistance; biofilm formation	Antibiotics, QS inhibition, anti-biofilm strategies	Lee & Zhang, 2015
Mannheimia haemolytica	Cattle	Adhesins, capsular polysaccharides, OMPs, LOS, toxins	Bovine respiratory disease	Inflammation-driven pathogenesis; tissue damage; immune evasion	Antibiotics; vaccination	Campbell, 2023
Histophilus somni	Cattle	Biofilm	Myocarditis, vasculitis	Persistence in respiratory/systemic sites	Antibiotics	Andrés-Lasheras *et al.,* 2022
Mycoplasma hyopneumoniae	Pigs	Adhesins such as P97, P159,	Porcine enzootic pneumonia	Chronic colonization,	Vaccination, antibiotics,	Nesse *et al.,* 2023;

		110 kDa and 146 kDa proteins		immune modulation		Stakenborg *et al.,* 2006
Actinobacillus pleuropneumoniae	Pigs	Apx toxins (apxI, apxII, apxIII)	Porcine pleuropneumonia	Toxin-mediated virulence; chronic disease persistence	Parenteral antibiotics, prophylaxis, vaccines	Nahar *et al.,* 2021
Bordetella bronchiseptica	Pigs	bvgAS, bpsA-D locus; FHA, pertactin, fimbriae	Porcine bronchopneumonia, secondary pneumonia	Biofilm formation; virulence switching	Antibiotics	Nicholson *et al.,* 2017
Trueperella pyogenes	Cattle	Biofilm-associated persistence	Endometritis, metritis, pyometra	Chronic uterine persistence	Antibiotics	Maklakova *et al.,* 2024

Table 4: *Summarising Antibiofilm Strategies and Their Advantages/Disadvantages in Livestock Disease Management*

Anti-Biofilm Strategies	**Mechanisms**	**Advantages**	**Limitations**	**Veterinary Applications**	**Reference**
Combination antibiotics	Targets distinct bacterial subpopulations inside the biofilm by using two or more antimicrobial agents	In comparison to monotherapy, greater efficacy against killing the biofilm	Can still be unsuccessful against deeply established biofilms; resistance may persist	Mastitis and skin infections, especially against *Staphylococcus aureus, Streptococcus agalactiae*, and *Streptococcus dysgalactiae*	Bright *et al.,* 2023
QS inhibitors	Disrupt cellular crosstalk that regulates virulence and biofilm development	May reduce virulence, adhesion, and maturation without direct selection as antibiotics.do	Often experimental; efficacy may vary by species and biofilm stage.	Potential application against diseases associated with biofilms	Juszczuk-Kubiak, 2024
Adhesin targeting	Prevents the adhesion proteins required for surface colonization and initial attachment.	Prevents biofilm initiation; useful in early stage infection control	Once a mature biofilm is formed, it becomes less effective	*E. coli* associated infections through targeting fimH and related adhesion pathways	Ibrahim *et al.,* 2022
Antimicrobial peptides	Disrupt microbial membranes and prevent the formation of biofilms	Broad anti biofilm potential; can act on membrane integrity	Stability, cost, and in vivo delivery remain challenges	Promising adjuncts for veterinary biofilm control	Guchhait *et al.,* 2026
Zinc pyrithione combinations	Used in addition to antibiotics to improve biofilm clearance	Improves biofilm clearance when combined with gentamicin	Not a standalone solution; safety and formulation need consideration	Tested for livestock skin diseases caused by staphylococci and streptococci	Mala *et al.,* 2022
CRISPR-Cas9-HDR targeting	Genome editing to disrupt biofilm-related genes such as fimH, luxS, and bolA	Highly specific; can directly silence key biofilm genes	Delivery and regulatory challenges still exist; most of the research is still ongoing.	Future precision control of biofilm-associated livestock pathogens	Wang *et al.,* 2020

Emerging Technologies and Future Perspectives

Bacterial biofilms profoundly instigate the pathogenesis of livestock diseases like bovine respiratory disease (BRD), mastitis, and pneumonia, ultimately indicating a resistance mechanism to antimicrobials and host defence processes (Zhan *et al.,* 2021). Conventional antimicrobial therapeutics merely trigger biofilm-associated infections, as biofilms are structurally dense matrices comprising multicellular entities in an extracellular polymeric substance (EPS), thereby altering drug functionality and promoting bacterial growth. This manifests as a recurrent infection rate followed by severe colonization and persistent AMR (Mishra *et al.,* 2023). Various interconnected mechanisms explain the inadequacies of conventional therapy, wherein polysaccharides, proteins, nucleic acids, and lipid molecules impede the diffusion process, thereby preventing antimicrobial contact with affected cells. Simultaneously, biofilm growth can be induced by subpopulation growth within deep layers (Mishra *et al.,* 2023). Also, quorum sensing and horizontal gene transfer are governing factors for regulating virulence factors and AMR. Looking ahead, these features suggest that standard antibiotics are often inefficient at eradicating biofilms, leading to relapsing resistant variants (Table 5).

Table 5: *Investigation of Viral Resistance/Resilience Studies in Livestock*

Name of the Host	Name of the Virus	Target Gene / Protein	Method Used	References
Pig	Influenza viruses	Mouse Mx1 gene	DNA construct	Müller *et al.,* 1992
	FMDV	Non-structural protein 2B and polymerase 3D	Gene engineering approach	Hu *et al.,* 2021
	FMDV	Viral protein 1	RNA interference	Hu *et al.,* 2015
	PRRSV	CD163 receptor gene	CRISPR/Cas9	Whitworth *et al.,* 2016
	PRRSV	CD163 SRCR5 domain	CRISPR/Cas9	Burkard *et al.,* 2017
	PRRSV	CD163 homolog	CRISPR/Cas9	Wells *et al.,* 2017
	CSFV	NS4B viral protein	CRISPR/Cas9 and RNA interference	Xie *et al.,* 2018
	PRRSV, TGE3V, PDCoV	CD163 SRCR5 and ANPEP genes	CRISPR/Cas9	Xu *et al.,* 2020
	TGEV, PEDV	ANPEP receptor gene	CRISPR/Cas9	Whitworth *et al.,* 2019
	TGEV, PEDV	ANPEP gene (alternative modification)	CRISPR/Cas9	Luo *et al.,* 2019
	PEDV	CMAH gene	CRISPR/Cas9	Tu *et al.,* 2019
	ASFV	RELA gene	Zinc-finger nucleases	Lillico *et al.,* 2013
	ASFV	CD163 gene	CRISPR/Cas9	Popescu *et al.,* 2017
	PERVs	gag and pol genes	RNA interference	Ramsoondar *et al.,* 2009
	PERVs	pol2 gene	RNA interference	Dieckhoff *et al.,* 2008
	PERVs	pol gene	CRISPR/Cas9	Niu *et al.,* 2017
Chicken	MDV	gB glycoprotein B gene and ICP4	RNA interference	Chen. *et al.,* 2009
	MDV	ICP4 gene	CRISPR/Cas9	Challagulla *et al.,* 2021
	AIV	Viral polymerase gene	RNA interference	Lyall *et al.,* 2011

Recent trends in diagnostics, AI analytics, anti-biofilm agents, and CRISPR editing tools predominantly offer notable solutions to overcome theranostic challenges (Figure 3). Therefore, future research necessitates integrated CRISPR platforms for biofilm disruption in livestock with polymicrobial infections. This directly provides synergism and minimizes the lacuna at the viral-bacterial interface. CRISPR application strategies directly aim at biofilm-related livestock viruses and inhibit livestock infections (Söllner *et al.,* 2021). Various genome editing tools like CRISPR/Cas, TALENs (transcription activator-like nucleases), and zinc-finger nucleases facilitate DNA denaturation within an organism (Rouet *et al.,* 1994; Subhadra *et al.,* 2023).

Besides that, homology-directed repair (HDR) is not common mechanistic strategy as it derives from DNA replication phase. It instigates the knock-in (KI) strategies ultimately developing diagnostic tools to modulate and screen the viral outbreaks. Recently HERLOCK (Gootenberg *et al.,* 2017), HOLMES (Li *et al.,* 2018), and DETECTR are now being prominently explored as the major CRISPR-mediated viral detection methods governed by Cas12a and Cas13a proteins (Chen *et al.,* 2018). By tuning the specificity and sensitivity, a few on-site detection tools for African swine fever virus (ASFV) have been

miniaturized utilizing Cas12a cleavage (Bai *et al.,* 2019; Lu *et al.,* 2020; Yuan *et al.,* 2020) and Cas9 (Wang *et al.,* 2020). Regarding ethical concerns in CRISPR livestock editing, primary issues encompass off-target mutations, which risk animal health defects, and welfare impacts from unintended phenotypes. Regulatory frameworks for CRISPR-edited livestock (e.g., PRRS-resistant pigs) must progress for anti-biofilm material development, AMR inhibition, genetic susceptibility, and AI modulation (Ishii, 2017). Advanced nanotechnological tools are also versatile in inhibiting biofilm formation against bacterial pathogens. Various nanoparticles, including silver (Ag) produced through biosynthetic routes, could be highly efficient in inhibiting biofilm maturation (Ali *et al.,* 2023; Hosnedlova *et al.,* 2022) (Figure 3).

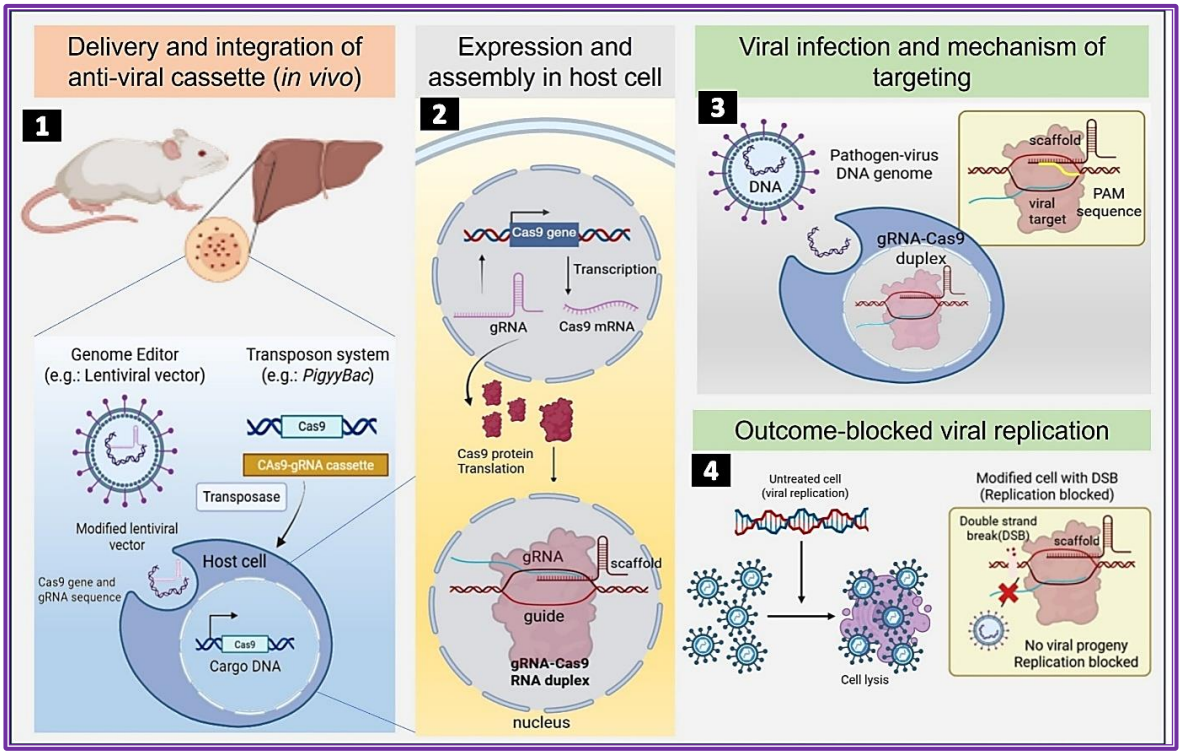


Figure 3*: Mechanistic Insight into In Vivo Pathogen Genome-Targeting in Disease Management. 1. In Vivo Delivery of Cas9-Grna Cassette Using Viral/Trasnsposon Vectors, 2. Cas9-Grna Expression and Complex Formation, 3. Specific Recognition of Viral DNA Target Adjacent to The Protospacer Adjacent Motif (PAM) Sequence, 4. Cas9 Mediated Double Starnd Break (DSB) In the Viral Genome and Disruption of The Viral Replication, Inhibition of Viral Progeny Productio[Reproduced by Self with Assistance of Biorender.Com]*

In summary, amalgamating 3D tissue engineering network with multi-omics approaches would engender deeper insight into the bacterial genomic factors in accordance with biofilm studies followed by antimicrobial resistance and therapeutic interventions (Bano *et al.,* 2023).

Conclusion

In accordance with recent research trends, bacterial biofilms are explored as fundamental drivers of persistent and recurrent livestock infections, reshaping disease heterogeneity, treatment failure, and AMR. By shielding embedded bacteria from host immunity and antibiotic penetration, biofilms sustain chronic conditions such as bovine mastitis and respiratory complexes while acting as reservoirs for horizontal gene transfer, thereby amplifying risks across animal, human, and environmental domains. Although mechanistic insights into biofilm architecture, extracellular matrix composition, quorum-sensing (QS) circuits, and host–microbe crosstalk have recently advanced, major gaps remain. Conventional antimicrobials inadequately remove mature biofilms and select for tolerant or resistant subpopulations, while host-specific biofilm determinants in major livestock pathogens are rarely

characterized. In this juncture One Health concepts that integrate antimicrobial resistivity, early biofilm diagnostics and therapeutics approaches provide a prominent route for mitigating disease prevalence thereby broadening the global public health impact.

Data Availability

No datasets were generated or analysed during the current study.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

The authors acknowledge Gargi Roy Chowdhury, Dept. of Biotechnology, Brainware University, West Bengal, IndiaDr. Gargi Saha Kesh, Associate Professor, Department of Microbiology, Vijaygarh Jyotish Ray College, Kolkata, West Bengal, India; Nityananda Paul, Brainware University and Falak Neaz, Department of Microbiology, Vijaygarh Jyotish Ray College, Kolkata, West Bengal, India for supporting this study.

References

Aboelnaga, N., Elsayed, S. W., Abdelsalam, N. A., Salem, S., Saif, N. A., Elsayed, M., ... & Elhadidy, M. (2024). Deciphering the dynamics of methicillin-resistant Staphylococcus aureus biofilm formation: from molecular signaling to nanotherapeutic advances. *Cell Communication and Signaling*, *22*(1), 188. https://doi.org/10.1186/s12964-024-01511-2

Ali, A., Zahra, A., Kamthan, M., Husain, F. M., Albalawi, T., Zubair, M., ... & Noorani, M. S. (2023). Microbial biofilms: applications, clinical consequences, and alternative therapies. *Microorganisms*, *11*(8), 1934. https://doi.org/10.3390/microorganisms11081934

Alotaibi, G. F. (2021). Factors influencing bacterial biofilm formation and development. *American Journal of Biomedical Science & Research*, *12*(6), 617-626. https://doi.org/10.34297/AJBSR.2021.12.001820

Alshammari, M., Ahmad, A., AlKhulaifi, M., Al Farraj, D., Alsudir, S., Alarawi, M., ... & Alyamani, E. (2023). Reduction of biofilm formation of Escherichia coli by targeting quorum sensing and adhesion genes using the CRISPR/Cas9-HDR approach, and its clinical application on urinary catheter. *Journal of Infection and Public Health*, *16*(8), 1174-1183. https://doi.org/10.1016/j.jiph.2023.05.026

Andrés-Lasheras, S., Ha, R., Zaheer, R., Lee, C., Booker, C. W., Dorin, C., ... & McAllister, T. A. (2021). Prevalence and risk factors associated with antimicrobial resistance in bacteria related to bovine respiratory disease—a broad cross-sectional study of beef cattle at entry into Canadian feedlots. *Frontiers in Veterinary Science*, *8*, 692646. https://doi.org/10.3389/fvets.2021.692646

Andrés-Lasheras, S., Jelinski, M., Zaheer, R., & McAllister, T. A. (2022). Bovine respiratory disease: conventional to culture-independent approaches to studying antimicrobial resistance in North America. *Antibiotics*, *11*(4), 487. https://doi.org/10.3390/antibiotics11040487

Arias, L. A. Q., Fernández, M. V., González, J. J. B., López, M. B., Herradón, P. J. G., & Martínez, A. I. P. (2018). Subclinical Endometritis in Dairy Cattle. In New Insights into Theriogenology. *IntechOpen.* https://doi.org/10.5772/intechopen.80229

Bai, J., Lin, H., Li, H., Zhou, Y., Liu, J., Zhong, G., ... & Huang, L. (2019). Cas12a-based on-site and rapid nucleic acid detection of African swine fever. *Frontiers in Microbiology*, *10*, 2830. https://doi.org/10.3389/fmicb.2019.02830

Bano, S., Hassan, N., Rafiq, M., Hassan, F., Rehman, M., Iqbal, N., ... & Kang, Y. Q. (2023). Biofilms as battlefield armor for bacteria against antibiotics: challenges and combating strategies. *Microorganisms*, *11*(10), 2595. https://doi.org/10.3390/microorganisms11102595

Bava, R., Castagna, F., Lupia, C., Poerio, G., Liguori, G., Lombardi, R., ... & Palma, E. (2024). Antimicrobial resistance in livestock: A serious threat to public health. *Antibiotics*, *13*(6), 551. https://doi.org/10.3390/antibiotics13060551

Bjarnsholt, T., Alhede, M., Alhede, M., Eickhardt-Sørensen, S. R., Moser, C., Kühl, M., ... & Høiby, N. (2013). The in vivo biofilm. *Trends in Microbiology*, *21*(9), 466-474. https://doi.org/10.1016/j.tim.2013.06.002

Boldeanu, L., Boldeanu, M. V., Novac, M. B., Assani, M. Z., & Radu, L. (2025). Serratia marcescens: A versatile opportunistic pathogen with emerging clinical and biotechnological significance. *International Journal of Molecular Sciences*, *26*(23), 11479. https://doi.org/10.3390/ijms262311479

Bouari, C. M., Nadăş, G. C., Crăciun, S., & Fiț, N. I. (2026). Staphylococcus aureus in Bovine Mastitis: Pathogenesis, Antimicrobial Resistance, and Emerging Control Strategies. *Microorganisms*, *14*(5), 1125. https://doi.org/10.3390/microorganisms14051125

Bright, L. M. E., Garren, M. R. S., Douglass, M., & Handa, H. (2023). Synthesis and characterization of nitric oxide-releasing Ampicillin as a potential strategy for combatting bacterial biofilm formation. *ACS Applied Materials & Interfaces*, *15*(12), 15185-15194. https://doi.org/10.1021/acsami.3c00140

Burkard, C., Lillico, S. G., Reid, E., Jackson, B., Mileham, A. J., Ait-Ali, T., ... & Archibald, A. L. (2017). Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. *PLoS pathogens*, *13*(2), e1006206. https://doi.org/10.1371/journal.ppat.1006206

Campbell, J. (2023). Unhappily Ever After: Hardy, Tess, and the Fairy Tale Manqué. *The Comparatist*, *47*(1), 289-308. https://doi.org/10.1353/com.2023.a911944

Cattelan, N., Dubey, P., Arnal, L., Yantorno, O. M., & Deora, R. (2016). Bordetella biofilms: a lifestyle leading to persistent infections. *FEMS Pathogens and Disease*, *74*(1), ftv108. https://doi.org/10.1093/femspd/ftv108

Centers for Disease Control and Prevention. (2022). One Health Basics. U.S. Department of Health and Human Services. https://www.cdc.gov/onehealth/basics/index.html

Chakraborty, S., Dutta, T. K., De, A., Das, M., & Ghosh, S. (2018). Impact of bacterial biofilm in veterinary medicine: An overview. *nternational Journal of Current Microbiology and Applied Sciences, 7*(4), 3228-3239. https://doi.org/10.20546/ijcmas.2018.704.366

Challagulla, A., Jenkins, K. A., O'Neil, T. E., Shi, S., Morris, K. R., Wise, T. G., ... & Schat, K. A. (2021). In vivo inhibition of Marek's disease virus in transgenic chickens expressing Cas9 and gRNA against ICP4. *Microorganisms*, *9*(1), 164. https://doi.org/10.3390/microorganisms9010164

Chatterjee, B., Banerjee, A., Chatterjee, D., Chakraborty, O., Dey, S., Mitra, A.K. (2025). Biofilm Threats in Livestock: A Growing Concern. In: Lahiri, D., Nag, M., Bhattacharya, D., Ray, R.R. (eds) Biofilm Associated Livestock Diseases and their Management. Livestock Diseases and Management. Springer, Singapore. https://doi.org/10.1007/978-981-96-1885-9_17

Chen, J. S., Ma, E., Harrington, L. B., Da Costa, M., Tian, X., Palefsky, J. M., & Doudna, J. A. (2018). CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*, *360*(6387), 436-439. https://doi.org/10.1126/science.aar6245

Chen, M., Payne, W. S., Dunn, J. R., Chang, S., Zhang, H. M., Hunt, H. D., & Dodgson, J. B. (2009). Retroviral delivery of RNA interference against Marek's disease virus in vivo. *Poultry Science*, *88*(7), 1373-1380. https://doi.org/10.3382/ps.2009-00070

Cheng, W. N., & Han, S. G. (2020). Bovine mastitis: Risk factors, therapeutic strategies, and alternative treatments—A review. *Asian-Australasian Journal of Animal Sciences*, *33*(11), 1699. https://doi.org/10.5713/ajas.20.0156

Crowley, R. C., Leigh, J. A., Ward, P. N., Lappin-Scott, H. M., & Bowler, L. D. (2011). Differential protein expression in Streptococcus uberis under planktonic and biofilm growth conditions. *Applied and Environmental Microbiology*, *77*(1), 382-384. https://doi.org/10.1128/AEM.01099-10

Danese, P. N., Pratt, L. A., & Kolter, R. (2000). Exopolysaccharide production is required for development of Escherichia coli K-12 biofilm architecture. *Journal of Bacteriology*, *182*(12), 3593-3596. https://doi.org/10.1128/JB.182.12.3593-3596.2000

Darwish, S. F., & Asfour, H. A. (2013). Investigation of biofilm forming ability in Staphylococci causing bovine mastitis using phenotypic and genotypic assays. *The Scientific World Journal*, *2013*(1), 378492. https://doi.org/10.1155/2013/378492

Dieckhoff, B., Petersen, B., Kues, W. A., Kurth, R., Niemann, H., & Denner, J. (2008). Knockdown of porcine endogenous retrovirus (PERV) expression by PERV-specific shRNA in transgenic pigs. *Xenotransplantation*, *15*(1), 36-45. https://doi.org/10.1111/j.1399-3089.2008.00442.x

Elhadidy, M., & Elsayyad, A. (2013). Uncommitted role of enterococcal surface protein, Esp, and origin of isolates on biofilm production by Enterococcus faecalis isolated from bovine mastitis. *Journal of Microbiology, Immunology and Infection*, *46*(2), 80-84. https://doi.org/10.1016/j.jmii.2012.02.002

Elhadidy, M., & Zahran, E. (2014). Biofilm mediates Enterococcus faecalis adhesion, invasion and survival into bovine mammary epithelial cells. *Letters in Applied Microbiology*, *58*(3), 248-254. https://doi.org/10.1111/lam.12184

Fabres-Klein, M. H., Caizer Santos, M. J., Contelli Klein, R., Nunes de Souza, G., & de Oliveira Barros Ribon, A. (2015). An association between milk and slime increases biofilm production by bovine Staphylococcus aureus. *BMC Veterinary Research*, *11*(1), 3. https://doi.org/10.1186/s12917-015-0319-7

Ferris, R. A., McCue, P. M., Borlee, G. I., Glapa, K. E., Martin, K. H., Mangalea, M. R., ... & Borlee, B. R. (2017). Model of chronic equine endometritis involving a Pseudomonas aeruginosa biofilm. *Infection and Immunity*, *85*(12), 10-1128. https://doi.org/10.1128/IAI.00332-17

Figueroa-Valenzuela, C., Montes-García, J. F., Vazquez-Cruz, C., Zenteno, E., Pereyra, M. A., & Negrete-Abascal, E. (2022). Mannheimia haemolytica OmpH binds fibrinogen and fibronectin and participates in biofilm formation. *Microbial pathogenesis*, *172*, 105788. https://doi.org/10.1016/j.micpath.2022.105788

Gomes, F., Saavedra, M. J., & Henriques, M. (2016). Bovine mastitis disease/pathogenicity: evidence of the potential role of microbial biofilms. *FEMS Pathogens and Disease*, *74*(3), ftw006. https://doi.org/10.1093/femspd/ftw006

Gootenberg, J. S., Abudayyeh, O. O., Lee, J. W., Essletzbichler, P., Dy, A. J., Joung, J., ... & Zhang, F. (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science*, *356*(6336), 438-442. https://doi.org/10.1126/science.aam9321

Goulart, D. B., & Mellata, M. (2022). Escherichia coli mastitis in dairy cattle: etiology, diagnosis, and treatment challenges. *Frontiers in Microbiology*, *13*, 928346. https://doi.org/10.3389/fmicb.2022.928346

Greve, N. B., Fei, X., Håkansson, A. P., Olsen, J. E., & Thomsen, L. E. (2025). Identification of a novel gene associated with group B Streptococcus persister cell formation and biofilm production. *Scientific Reports*, *16*(1), 4217. https://doi.org/10.1038/s41598-025-34333-4

Guchhait, K. C., Dey, S., Majumder, S., Raul, P., Ballav, S., Jana, D., ... & Ghosh, C. (2026). Modern therapeutic strategies to mitigate biofilm-associated infection in livestock organisms. *Antonie van Leeuwenhoek*, *119*(1), 5. https://doi.org/10.1007/s10482-025-02220-7

Hoelzle, K., Ade, J., & Hoelzle, L. E. (2020). Persistence in livestock mycoplasmas—a key role in infection and pathogenesis. *Current Clinical Microbiology Reports*, *7*(3), 81-89. https://doi.org/10.1007/s40588-020-00149-1

Høiby, N., Bjarnsholt, T., Givskov, M., Molin, S., & Ciofu, O. (2010). Antibiotic resistance of bacterial biofilms. *International Journal of Antimicrobial Agents*, *35*(4), 322-332. https://doi.org/10.1016/j.ijantimicag.2009.12.011

Hosnedlova, B., Kabanov, D., Kepinska, M., B Narayanan, V. H., Parikesit, A. A., Fernandez, C., ... & Kizek, R. (2022). Effect of biosynthesized silver nanoparticles on bacterial biofilm changes in S. aureus and E. coli. *Nanomaterials*, *12*(13), 2183. https://doi.org/10.3390/nano12132183

Hu, S., Qiao, J., Fu, Q., Chen, C., Ni, W., Wujiafu, S., ... & Ouyang, H. (2015). Transgenic shRNA pigs reduce susceptibility to foot and mouth disease virus infection. *Elife*, *4*, e06951. https://doi.org/10.7554/eLife.06951

Hu, W., Zheng, H., Li, Q., Wang, Y., Liu, X., Hu, X., ... & Li, N. (2021). shRNA transgenic swine display resistance to infection with the foot-and-mouth disease virus. *Scientific Reports*, *11*(1), 16377. https://doi.org/10.1038/s41598-021-95853-3

Ibrahim, E. S., Arafa, A. A., Dorgam, S. M., Eid, R. H., Atta, N. S., El-Dabae, W. H., & Sadek, E. G. (2022). Molecular characterization of genes responsible for biofilm formation in Staphylococcus aureus isolated from mastitic cows. *Veterinary World*, *15*(1), 205-212. https://doi.org/10.14202/vetworld.2022.205-212

Ishii, T. (2017). Genome-edited livestock: Ethics and social acceptance. *Animal Frontiers*, *7*(2), 24-32. https://doi.org/10.2527/af.2017.0115

Jacques, M., & Malouin, F. (2022). One health—one biofilm. *Veterinary Research*, *53*(1), 51. https://doi.org/10.1186/s13567-022-01067-4

Juszczuk-Kubiak, E. (2024). Molecular aspects of the functioning of pathogenic bacteria biofilm based on quorum sensing (QS) signal-response system and innovative non-antibiotic strategies for their elimination. *International Journal of Molecular Sciences*, *25*(5), 2655. https://doi.org/10.3390/ijms25052655

Kalia, M., Amari, D., Davies, D. G., & Sauer, K. (2023). cis-DA-dependent dispersion by Pseudomonas aeruginosa biofilm and identification of cis-DA-sensory protein DspS. *Mbio*, *14*(6), e02570-23. https://doi.org/10.1128/mbio.02570-23

Kaplan, J. Á. (2010). Biofilm dispersal: mechanisms, clinical implications, and potential therapeutic uses. *Journal of Dental Research*, *89*(3), 205-218. https://doi.org/10.1177/0022034509359403

Krömker, V., Reinecke, F., Paduch, J. H., & Grabowski, N. (2014). Bovine Streptococcus uberis intramammary infections and mastitis. *Clinical Microbiology: Open Access*, *3*. https://doi.org/10.25968/opus-873

Lee, J., & Zhang, L. (2015). The hierarchy quorum sensing network in Pseudomonas aeruginosa. Protein & Cell, 6(1), 26–41. https://doi.org/10.1007/s13238-014-0100-x

Lewenza, S. (2013). Extracellular DNA-induced antimicrobial peptide resistance mechanisms in Pseudomonas aeruginosa. *Frontiers in Microbiology*, *4*, 21. https://doi.org/10.3389/fmicb.2013.00021

Li, S. Y., Cheng, Q. X., Wang, J. M., Li, X. Y., Zhang, Z. L., Gao, S., ... & Wang, J. (2018). CRISPR-Cas12a-assisted nucleic acid detection. *Cell Discovery*, *4*(1), 20. https://doi.org/10.1038/s41421-018-0028-z

Lillico, S. G., Proudfoot, C., Carlson, D. F., Stverakova, D., Neil, C., Blain, C., ... & Whitelaw, C. B. A. (2013). Live pigs produced from genome edited zygotes. *Scientific Reports*, *3*(1), 2847. https://doi.org/10.1038/srep02847

Limoli, D. H., Jones, C. J., & Wozniak, D. J. (2015). Bacterial extracellular polysaccharides in biofilm formation and function. *Microbiology spectrum*, *3*(3), 10-1128. https://doi.org/10.1128/microbiolspec.MB-0011-2014

Liu, F., Zhao, Q., Jia, Z., Zhang, S., Wang, J., Song, S., & Jia, Y. (2022). N-3-oxo-octanoyl homoserine lactone primes plant resistance against necrotrophic pathogen Pectobacterium carotovorum by coordinating jasmonic acid and auxin-signaling pathways. *Frontiers in Plant Science*, *13*, 886268. https://doi.org/10.3389/fpls.2022.886268

Lu, S., Li, F., Chen, Q., Wu, J., Duan, J., Lei, X., ... & Yin, H. (2020). Rapid detection of African swine fever virus using Cas12a-based portable paper diagnostics. *Cell Discovery*, *6*(1), 18. https://doi.org/10.1038/s41421-020-0151-5

Luo, L., Wang, S., Zhu, L., Fan, B., Liu, T., Wang, L., ... & Zhang, K. (2019). Aminopeptidase N-null neonatal piglets are protected from transmissible gastroenteritis virus but not porcine epidemic diarrhea virus. *Scientific Reports*, *9*(1), 13186. https://doi.org/10.1038/s41598-019-49838-y

Lyall, J., Irvine, R. M., Sherman, A., McKinley, T. J., Núñez, A., Purdie, A., ... & Tiley, L. (2011). Suppression of avian influenza transmission in genetically modified chickens. *Science*, *331*(6014), 223-226. https://doi.org/10.1126/science.1198020

Lynch, D. J., Fountain, T. L., Mazurkiewicz, J. E., & Banas, J. A. (2007). Glucan-binding proteins are essential for shaping Streptococcus mutans biofilm architecture. *FEMS Microbiology Letters*, *268*(2), 158-165. https://doi.org/10.1111/j.1574-6968.2006.00576.x

Maes, D., Segales, J., Meyns, T., Sibila, M., Pieters, M., & Haesebrouck, F. (2008). Control of Mycoplasma hyopneumoniae infections in pigs. *Veterinary Microbiology*, *126*(4), 297-309. https://doi.org/10.1016/j.vetmic.2007.09.008

Mahmoud, S. F., Fayez, M., Swelum, A. A., Alswat, A. S., Alkafafy, M., Alzahrani, O. M., ... & Yusuf, S. (2022). Genetic diversity, biofilm formation, and antibiotic resistance of Pseudomonas aeruginosa isolated from cow, camel, and mare with clinical endometritis. *Veterinary Sciences*, *9*(5), 239. https://doi.org/10.3390/vetsci9050239

Maklakova, M., Villarreal-Gomez, L. J., Nefedova, E., Shkil, N., Pestryakov, A., & Bogdanchikova, N. (2024). Role of biofilm formation in the drop of bacterial resistance to antibiotics after animal therapy with silver nanoparticles. *ACS Applied Nano Materials*, *7*(14), 16553-16563. https://doi.org/10.1021/acsanm.4c02566

Mala, L., Lalouckova, K., Skrivanova, E., Houdkova, M., Strakova, M., & Kokoska, L. (2022). In vitro growth-inhibitory synergistic effect of zinc pyrithione in combination with gentamicin against bacterial skin pathogens of livestock. *Antibiotics*, *11*(7), 960. https://doi.org/10.3390/antibiotics11070960

Mandhwani, R., Bhardwaz, A., Kumar, S., Shivhare, M., & Aich, R. (2017). Insights into bovine endometritis with special reference to phytotherapy. *Veterinary World*, *10*(12), 1529-1532. https://doi.org/10.14202/vetworld.2017.1529-1532

Meijs, A. P., Gijsbers, E. F., Hengeveld, P. D., Dierikx, C. M., de Greeff, S. C., & van Duijkeren, E. (2021). ESBL/pAmpC-producing Escherichia coli and Klebsiella pneumoniae carriage among veterinary healthcare workers in the Netherlands. *Antimicrobial Resistance & Infection Control*, *10*, 147. https://doi.org/10.1186/s13756-021-01012-8

Michaelis, C., & Grohmann, E. (2023). Horizontal gene transfer of antibiotic resistance genes in biofilms. *Antibiotics*, *12*(2), 328. https://doi.org/10.3390/antibiotics12020328

Milanov, D., Prunić, B., Velhner, M., Todorović, D., & Polaček, V. (2015). Investigation of biofilm formation and phylogenetic typing of Escherichia coli strains isolated from milk of cows with mastitis/Ispitivanje Formiranja Biofilma I Filogenetska Tipizacija Sojeva Escherichia Coli Izolovanih Iz Mleka Krava Sa Mastitisom. *Acta Veterinaria*, *65*(2), 202-216. https://doi.org/10.1515/acve-2015-0017

Mishra, S., Gupta, A., Upadhye, V., Singh, S. C., Sinha, R. P., & Häder, D. P. (2023). Therapeutic strategies against biofilm infections. *Life*, *13*(1), 172. https://doi.org/10.3390/life13010172

Müller, M., Brenig, B., Winnacker, E. L., & Brem, G. (1992). Transgenic pigs carrying cDNA copies encoding the murine Mx1 protein which confers resistance to influenza virus infection. *Gene*, *121*(2), 263-270. https://doi.org/10.1016/0378-1119(92)90130-H

Nahar, N., Turni, C., Tram, G., Blackall, P. J., & Atack, J. M. (2021). Actinobacillus pleuropneumoniae: The molecular determinants of virulence and pathogenesis. *Advances in Microbial Physiology*, *78*, 179-216. https://doi.org/10.1016/bs.ampbs.2020.12.001

Nasu, T., & Maeda, S. (2024). Escherichia coli persisters in biofilm can perform horizontal gene transfer by transformation. *Biochemical and Biophysical Research Communications*, *738*, 150549. https://doi.org/10.1016/j.bbrc.2024.150549

Neiditch, M. B., Capodagli, G. C., Prehna, G., & Federle, M. J. (2017). Genetic and structural analyses of RRNPP intercellular peptide signaling of Gram-positive bacteria. *Annual Review of Genetics*, *51*, 311-333. https://doi.org/10.1146/annurev-genet-120116-023507

Nesse, L. L., Osland, A. M., & Vestby, L. K. (2023). The role of biofilms in the pathogenesis of animal bacterial infections. *Microorganisms*, *11*(3), 608. https://doi.org/10.3390/microorganisms11030608

Nicholson, T. L., Brockmeier, S. L., Sukumar, N., Paharik, A. E., Lister, J. L., Horswill, A. R., ... & Deora, R. (2017). The Bordetella Bps polysaccharide is required for biofilm formation and enhances survival in the lower respiratory tract of swine. *Infection and Immunity*, *85*(8), 10-1128. https://doi.org/10.1128/iai.00261-17

Niu, D., Wei, H. J., Lin, L., George, H., Wang, T., Lee, I. H., ... & Yang, L. (2017). Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. *Science*, *357*(6357), 1303-1307. https://doi.org/10.1126/science.aan4187

Niu, H., Gu, J., & Zhang, Y. (2024). Bacterial persisters: molecular mechanisms and therapeutic development. *Signal Transduction and Targeted Therapy*, *9*(1), 174. https://doi.org/10.1038/s41392-024-01866-5

Patel, H., Buchad, H., & Gajjar, D. (2022). Pseudomonas aeruginosa persister cell formation upon antibiotic exposure in planktonic and biofilm state. *Scientific Reports*, *12*, 16151. https://doi.org/10.1038/s41598-022-20323-3

Perni, S., Preedy, E. C., & Prokopovich, P. (2014). Success and failure of colloidal approaches in adhesion of microorganisms to surfaces. *Advances in Colloid and Interface Science*, *206*, 265-274. https://doi.org/10.1016/j.cis.2013.11.008

Popescu, L., Gaudreault, N. N., Whitworth, K. M., Murgia, M. V., Nietfeld, J. C., Mileham, A., ... & Rowland, R. R. (2017). Genetically edited pigs lacking CD163 show no resistance following infection with the African swine fever virus isolate, Georgia 2007/1. *Virology*, *501*, 102-106. https://doi.org/10.1016/j.virol.2016.11.012

Ramsoondar, J., Vaught, T., Ball, S., Mendicino, M., Monahan, J., Jobst, P., ... & Ayares, D. (2009). Production of transgenic pigs that express porcine endogenous retrovirus small interfering RNAs. *Xenotransplantation*, *16*(3), 164-180. https://doi.org/10.1111/j.1399-3089.2009.00525.x

Rosini, R., & Margarit, I. (2015). Biofilm formation by Streptococcus agalactiae: influence of environmental conditions and implicated virulence factors. *Frontiers in Cellular and Infection Microbiology*, *5*, 6. https://doi.org/10.3389/fcimb.2015.00006

Rouet, P., Smih, F., & Jasin, M. (1994). Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. *Molecular and Cellular Biology*, *14*(12), 8096-8106. https://doi.org/10.1128/mcb.14.12.8096-8106.1994

Rybtke, M., Hultqvist, L. D., Givskov, M., & Tolker-Nielsen, T. (2015). Pseudomonas aeruginosa biofilm infections: community structure, antimicrobial tolerance and immune response. *Journal of Molecular Biology*, *427*(23), 3628-3645. https://doi.org/10.1016/j.jmb.2015.08.016

Sánchez-Jiménez, A., Llamas, M. A., & Marcos-Torres, F. J. (2023). Transcriptional regulators controlling virulence in Pseudomonas aeruginosa. *International Journal of Molecular Sciences*, *24*(15), 11895. https://doi.org/10.3390/ijms241511895

Sauer, K., Stoodley, P., Goeres, D. M., Hall-Stoodley, L., Burmølle, M., Stewart, P. S., & Bjarnsholt, T. (2022). The biofilm life cycle: expanding the conceptual model of biofilm formation. *Nature Reviews Microbiology*, *20*(10), 608-620. https://doi.org/10.1038/s41579-022-00767-0

Schwarz, D., Diesterbeck, U. S., Failing, K., König, S., Bruegemann, K., Zschöck, M., ... & Czerny, C. P. (2010). Somatic cell counts and bacteriological status in quarter foremilk samples of cows in Hesse, Germany—A longitudinal study. *Journal of Dairy Science*, *93*(12), 5716-5728. https://doi.org/10.3168/jds.2010-3223

Sharma, S., Mohler, J., Mahajan, S. D., Schwartz, S. A., Bruggemann, L., & Aalinkeel, R. (2023). Microbial biofilm: A review on formation, infection, antibiotic resistance, control measures, and innovative treatment. *Microorganisms*, *11*(6), 1614. https://doi.org/10.3390/microorganisms11061614

Silva, V., Correia, E., Pereira, J. E., González-Machado, C., Capita, R., Alonso-Calleja, C., ... & Poeta, P. (2022). Biofilm formation of Staphylococcus aureus from pets, livestock, and wild animals: relationship with clonal lineages and antimicrobial resistance. *Antibiotics*, *11*(6), 772. https://doi.org/10.3390/antibiotics11060772

Singh, R., Ray, P., Das, A., & Sharma, M. (2010). Penetration of antibiotics through Staphylococcus aureus and Staphylococcus epidermidis biofilms. *Journal of Antimicrobial Chemotherapy*, *65*(9), 1955-1958. https://doi.org/10.1093/jac/dkq257

Sloan, G. P., Love, C. F., Sukumar, N., Mishra, M., & Deora, R. (2007). The Bordetella Bps polysaccharide is critical for biofilm development in the mouse respiratory tract. *Journal of Bacteriology, 189*(22), 8270–8276. https://doi.org/10.1128/JB.00785-07

Söllner, J. H., Mettenleiter, T. C., & Petersen, B. (2021). Genome editing strategies to protect livestock from viral infections. *Viruses*, *13*(10), 1996. https://doi.org/10.3390/v13101996

Song, M., Tang, Q., Ding, Y., Tan, P., Zhang, Y., Wang, T., ... & Ma, X. (2024). Staphylococcus aureus and biofilms: transmission, threats, and promising strategies in animal husbandry. *Journal of Animal Science and Biotechnology*, *15*, 44. https://doi.org/10.1186/s40104-024-01007-6

Stakenborg, T., Vicca, J., Maes, D., Peeters, J., de Kruif, A., Haesebrouck, F., & Butaye, P. (2006). Comparison of molecular techniques for the typing of Mycoplasma hyopneumoniae isolates. *Journal of Microbiological Methods*, *66*(2), 263-275. https://doi.org/10.1016/j.mimet.2005.12.002

Stewart, P. S. (2002). Mechanisms of antibiotic resistance in bacterial biofilms. *International Journal of Medical Microbiology*, *292*(2), 107-113. https://doi.org/10.1078/1438-4221-00196

Subhadra, B., Cao, D., Jensen, R., Caswell, C., & Inzana, T. J. (2023). Identification and initial characterization of Hfq-associated sRNAs in Histophilus somni strain 2336. *PLoS One*, *18*(5), e0286158. https://doi.org/10.1371/journal.pone.0286158

Sun, Z., Xi, J., Yang, C., & Cong, W. (2022). Quorum sensing regulation methods and their effects on biofilm in biological waste treatment systems: A review. *Frontiers of Environmental Science & Engineering*, *16*, 87. https://doi.org/10.1007/s11783-021-1495-2

Thacker, E. L., Thacker, B. J., Young, T. F., & Halbur, P. G. (2000). Effect of vaccination on the potentiation of porcine reproductive and respiratory syndrome virus (PRRSV)-induced pneumonia by Mycoplasma hyopneumoniae. *Vaccine*, *18*(13), 1244-1252. https://doi.org/10.1016/S0264-410X(99)00395-3

Thorberg, B. M., Danielsson-Tham, M. L., Emanuelson, U., & Waller, K. P. (2009). Bovine subclinical mastitis caused by different types of coagulase-negative staphylococci. *Journal of Dairy Science*, *92*(10), 4962-4970. https://doi.org/10.3168/jds.2009-2184

Tu, C. F., Chuang, C. K., Hsiao, K. H., Chen, C. H., Chen, C. M., Peng, S. H., ... & Chen, C. M. (2019). Lessening of porcine epidemic diarrhoea virus susceptibility in piglets after editing of the CMP-N-glycolylneuraminic acid hydroxylase gene with CRISPR/Cas9 to nullify N-glycolylneuraminic acid expression. *PLoS One*, *14*(5), e0217236. https://doi.org/10.1371/journal.pone.0217236

Tuon, F. F., Dantas, L. R., Suss, P. H., & Tasca Ribeiro, V. S. (2022). Pathogenesis of the Pseudomonas aeruginosa biofilm: A review. *Pathogens*, *11*(3), 300. https://doi.org/10.3390/pathogens11030300

Uruén, C., Chopo-Escuin, G., Tommassen, J., Mainar-Jaime, R. C., & Arenas, J. (2020). Biofilms as promoters of bacterial antibiotic resistance and tolerance. *Antibiotics*, *10*(1), 3. https://doi.org/10.3390/antibiotics10010003

Valentini, M., & Filloux, A. (2019). Multiple roles of c-di-GMP signaling in bacterial pathogenesis. *Annual Review of Microbiology*, *73*(1), 387-406. https://doi.org/10.1146/annurev-micro-020518-115555

Wang, X., Xiong, E., Tian, T., Cheng, M., Lin, W., Wang, H., ... & Zhou, X. (2020). Clustered regularly interspaced short palindromic repeats/Cas9-mediated lateral flow nucleic acid assay. *ACS Nano*, *14*(2), 2497-2508. https://doi.org/10.1021/acsnano.0c00022

Wells, K. D., Bardot, R., Whitworth, K. M., Trible, B. R., Fang, Y., Mileham, A., ... & Rowland, R. R. (2017). Replacement of porcine CD163 scavenger receptor cysteine-rich domain 5 with a CD163-like homolog confers resistance of pigs to genotype 1 but not genotype 2 porcine reproductive and respiratory syndrome virus. *Journal of Virology*, *91*(2), 10-1128. https://doi.org/10.1128/JVI.01521-16

Whitworth, K. M., Rowland, R. R., Ewen, C. L., Trible, B. R., Kerrigan, M. A., Cino-Ozuna, A. G., ... & Prather, R. S. (2016). Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. *Nature Biotechnology*, *34*(1), 20-22. https://doi.org/10.1038/nbt.3434

Whitworth, K. M., Rowland, R. R., Petrovan, V., Sheahan, M., Cino-Ozuna, A. G., Fang, Y., ... & Prather, R. S. (2019). Resistance to coronavirus infection in amino peptidase N-deficient pigs. *Transgenic Research*, *28*, 21-32. https://doi.org/10.1007/s11248-018-0100-3

World Health Organization, F. and A. O. of the U. N. W. O. for A. H. (2019). Taking a multisectoral, one health approach: A tripartite guide to addressing zoonotic diseases in countries. World Health Organization. https://www.fao.org/sustainable-development-goals-helpdesk/champion/article-detail/multisectoral-coordination-mechanisms-operational-tool.-an-operational-tool-of-the-tripartite-zoonoses-guide/en

Wu, D. C., Zamorano-Sánchez, D., Pagliai, F. A., Park, J. H., Floyd, K. A., Lee, C. K., ... & Yildiz, F. H. (2020). Reciprocal c-di-GMP signaling: Incomplete flagellum biogenesis triggers c-di-GMP signaling pathways that promote biofilm formation. *PLoS Genetics*, *16*(3), e1008703. https://doi.org/10.1371/journal.pgen.1008703

Xie, Z., Pang, D., Yuan, H., Jiao, H., Lu, C., Wang, K., ... & Ouyang, H. (2018). Genetically modified pigs are protected from classical swine fever virus. *PLoS Pathogens*, *14*(12), e1007193. https://doi.org/10.1371/journal.ppat.1007193

Xu, K., Zhou, Y., Mu, Y., Liu, Z., Hou, S., Xiong, Y., ... & Li, K. (2020). CD163 and pAPN double-knockout pigs are resistant to PRRSV and TGEV and exhibit decreased susceptibility to PDCoV while maintaining normal production performance. *Elife*, *9*, e57132. https://doi.org/10.7554/eLife.57132

Yuan, C., Tian, T., Sun, J., Hu, M., Wang, X., Xiong, E., ... & Zhou, X. (2020). Universal and naked-eye gene detection platform based on the clustered regularly interspaced short palindromic repeats/Cas12a/13a system. *Analytical Chemistry*, *92*(5), 4029-4037. https://doi.org/10.1021/acs.analchem.9b05597

Zadeh, R. G., Kalani, B. S., Ari, M. M., Talebi, M., Razavi, S., & Jazi, F. M. (2022). Isolation of persister cells within the biofilm and relative gene expression analysis of type II toxin/antitoxin system in Pseudomonas aeruginosa isolates in exponential and stationary phases. *Journal of Global Antimicrobial Resistance*, *28*, 30-37. https://doi.org/10.1016/j.jgar.2021.11.009

Zare, M., Vehreschild, M. J., & Wagenlehner, F. (2022). Management of uncomplicated recurrent urinary tract infections. *BJU International*, *129*(6), 668-678. https://doi.org/10.1111/bju.15630

Zhan, L., Zhang, J., Zhao, B., Li, X., Zhang, X., Hu, R., ... & Gao, Y. (2021). Genomic and transcriptomic analysis of bovine Pasteurella multocida serogroup a strain reveals insights into virulence attenuation. *Frontiers in Veterinary Science*, *8*, 765495. https://doi.org/10.3389/fvets.2021.765495