

ANTIMICROBIAL RESISTANCE IN LIVESTOCK PATHOGENS WITH MOLECULAR MECHANISMS HOST IMMUNE RESPONSES AND PATHOLOGICAL CONSEQUENCES

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ABSTRACT

Antimicrobial resistance (AMR) has become a major challenge to the effective treatment of bacterial infections in livestock, with consequences for animal health, productivity, welfare, and the sustainability of veterinary antimicrobial therapy. Although AMR is commonly discussed in relation to antimicrobial use, prevalence, transmission, and surveillance, comparatively less attention has been given to its biological consequences within the infected host. This review integrates molecular mechanisms of resistance with bacterial fitness, virulence-associated traits, host immune responses, pathological injury, and therapeutic outcome in livestock infections. Resistance may arise through chromosomal mutations, horizontal acquisition of resistance determinants, antimicrobial-inactivating enzymes, target modification, altered permeability, active efflux, metabolic bypass, and biofilm-associated tolerance or persistence. These mechanisms can interact with bacterial physiology and adaptation, although antimicrobial resistance should not be interpreted as synonymous with increased virulence or greater pathological severity. Resistant pathogens may persist during ineffective therapy, prolonging host-pathogen interactions and sustaining inflammatory responses that contribute indirectly to chronic infection, tissue damage, recurrence, and treatment failure. Innate and adaptive immune responses remain central determinants of infection outcome, while biofilm formation, intracellular survival, and immune-evasion strategies may further complicate bacterial clearance. Advances in whole-genome sequencing, metagenomics, transcriptomics, and integrated pathology offer new opportunities to connect resistance genotype with expressed phenotype and disease progression.

Future veterinary AMR research should therefore integrate antimicrobial susceptibility, pathogen genomics, virulence, host immunity, lesion characterization, and clinical outcome to better understand resistant infections as complex biological disease processes.

Keywords: Antimicrobial resistance, Livestock pathogens, Molecular mechanisms, Host immune response, Pathological consequences

INTRODUCTION

Antimicrobial resistance (AMR) has become an increasingly important constraint on the effective treatment of bacterial diseases in food-producing animals, with consequences for animal health, welfare, productivity, and the sustainability of veterinary antimicrobial therapy. Antimicrobial exposure creates selective pressure that favors the survival and expansion of resistant bacterial populations, while intensive production systems can provide opportunities for their persistence and dissemination. Global analyses have documented substantial increases in resistance among bacteria recovered from food-producing animals, particularly in rapidly expanding livestock systems in low- and middle-income countries (Van Boeckel et al., 2020). Clinical investigations further demonstrate that multidrug-resistant organisms are frequently recovered from diseased livestock, including *Escherichia coli* and *Staphylococcus* spp., emphasizing that AMR represents an immediate veterinary therapeutic problem rather than solely a potential public-health threat (Haulisah et al., 2021).

The biological basis of AMR is highly diverse and includes antimicrobial inactivation, modification or protection of drug targets, decreased membrane permeability, active efflux, metabolic adaptation, and acquisition of resistance determinants through mobile genetic elements. Long-term surveillance of pathogenic *E. coli* from cattle, swine, and poultry has demonstrated that resistance patterns can change markedly over time and differ among livestock populations and antimicrobial classes (Boireau et al., 2018). Advances in molecular diagnostics and whole-genome sequencing have further revealed that resistant livestock pathogens may simultaneously harbor multiple antimicrobial resistance genes, integrative genetic elements, and determinants associated with pathogenicity. In respiratory

bacteria isolated from dairy heifers, for example, genomic analysis identified extensive co-occurrence of resistance genes affecting several antimicrobial classes (Depenbrock et al., 2024).

Importantly, antimicrobial resistance does not operate independently of other components of bacterial biology. Resistance determinants may coexist with genes involved in adhesion, invasion, toxin production, secretion systems, biofilm formation, and other virulence-associated processes. A molecular investigation of resistant *Salmonella* from cattle, pigs, and chickens demonstrated extensive concurrent carriage of resistance and virulence determinants, illustrating the complex genomic background within which resistance evolves (Gu et al., 2023). Nevertheless, increased antimicrobial resistance should not automatically be interpreted as increased virulence. The relationship between resistance, bacterial fitness, pathogenicity, and host adaptation is context dependent and may involve fitness costs, compensatory evolution, or changes in persistence and colonization.

Despite extensive research on the prevalence and transmission of AMR in livestock, comparatively less attention has been directed toward integrating resistance mechanisms with host-pathogen interactions, immune responses, tissue injury, and clinical disease progression. Pathological outcome ultimately reflects the interaction between microbial traits and host defense rather than resistance phenotype alone. Understanding these relationships is therefore important for interpreting therapeutic failure and persistent infection and for designing more biologically informed control strategies. This review critically examines antimicrobial resistance in livestock pathogens by integrating molecular resistance mechanisms with bacterial virulence, host immune responses, pathological consequences, diagnostic advances, and the major research priorities required to understand

resistant infections as complex biological disease processes. The proposed biological continuum linking antimicrobial resistance with bacterial

adaptation, host pathogen interactions, pathological injury, and clinical outcome is summarized in Figure 1.

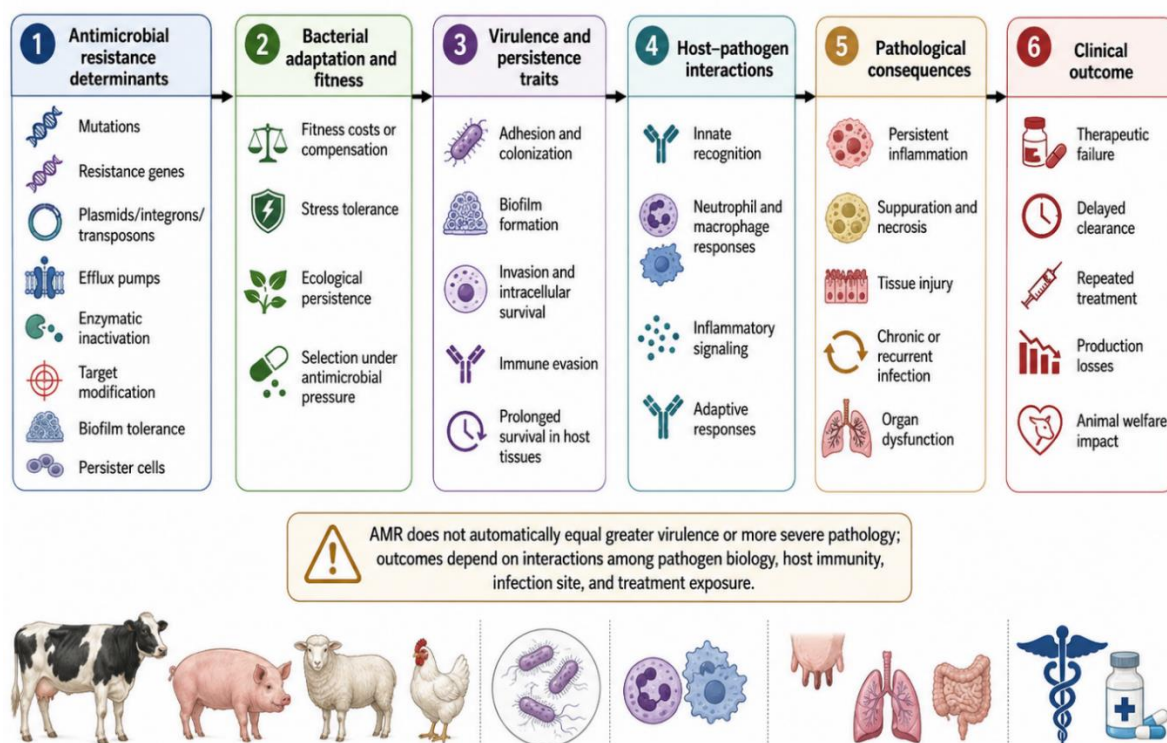


Figure 1. Biological pathway linking antimicrobial resistance to disease outcomes in livestock infections

2. Antimicrobial Resistance in Livestock Pathogens

2.1 Major Resistant Bacterial Pathogens in Livestock

Bacterial infections remain an important cause of morbidity, mortality, reduced productivity, reproductive impairment, and economic loss in livestock production. Antimicrobial therapy therefore continues to play an essential role in the management of respiratory, enteric, mammary, reproductive, and systemic bacterial diseases. However, the increasing occurrence of antimicrobial resistance has reduced the therapeutic reliability of several commonly used antimicrobial classes. Resistance has been reported among both Gram-negative and Gram-positive livestock pathogens, including *Escherichia coli*, *Salmonella enterica*, *Campylobacter* spp., *Pasteurella multocida*, *Mannheimia haemolytica*, *Staphylococcus aureus*, *Streptococcus* spp., and

Enterococcus spp. The distribution of resistance is highly variable and is influenced by bacterial species, antimicrobial exposure, production practices, geographic region, infection type, and previous treatment history. Importantly, resistant organisms are frequently recovered from clinically diseased animals, demonstrating that AMR is not only a concern related to environmental dissemination or food safety but also a direct challenge to veterinary disease management. Investigations of bacterial isolates from diseased cattle, sheep, goats, pigs, and poultry have documented substantial multidrug resistance among clinically important organisms, particularly *E. coli* and staphylococci (Haulisah et al., 2021).

2.2 Multidrug Resistance and Clinically Important Resistance Phenotypes

The accumulation of resistance to multiple antimicrobial classes is particularly concerning

because it can substantially restrict therapeutic options. Enteric bacteria are among the most extensively investigated livestock-associated organisms in this context. Pathogenic *E. coli* may cause neonatal diarrhea, septicemia, post-weaning disease, mastitis, and other extraintestinal infections, while *Salmonella* can produce enteric or systemic disease and may persist in both symptomatic and carrier animals. Molecular studies increasingly demonstrate that resistant isolates frequently harbor several antimicrobial resistance determinants together with virulence-associated genes and mobile genetic elements. Antimicrobial-resistant *Salmonella* isolated from cattle, pigs, and chickens and identified a broad array of resistance genes together with multiple virulence determinants, illustrating the complex genetic background in which resistance and pathogenicity may coexist (Gu et al., 2023).

Resistance is also of major concern in respiratory disease complexes. *M. haemolytica*, *P. multocida*, and *Histophilus somni* are important bacterial contributors to bovine respiratory disease, where antimicrobials may be used therapeutically or metaphylactically. Genomic and phenotypic investigations of respiratory isolates have demonstrated resistance to several clinically important antimicrobial groups and the co-occurrence of multiple resistance determinants within individual bacterial populations. Recent work on *P. multocida* and *M. haemolytica* from calves with respiratory disease has similarly shown substantial resistance to commonly used veterinary antimicrobials together with the presence of resistance and virulence-associated genes (Lachowicz-Wolak et al., 2025). These findings highlight the need to interpret antimicrobial susceptibility within the broader biological characteristics of the infecting organism.

2.3 Clinical Significance of Resistance in Livestock Disease

The clinical importance of AMR extends beyond the laboratory classification of an isolate as susceptible or resistant. Disease outcome is determined by interactions among antimicrobial susceptibility, bacterial burden, virulence,

infection site, host immunity, tissue accessibility of the drug, treatment timing, and pharmacological exposure. A resistant organism may survive otherwise appropriate antimicrobial therapy, allowing continued multiplication or persistence within infected tissues. This may contribute to prolonged disease, recurrent infection, repeated treatment, increased production losses, and greater risk of poor clinical outcome. However, resistance itself should not automatically be interpreted as evidence of increased virulence or greater pathological severity.

3. Molecular Mechanisms Underlying Antimicrobial Resistance

Antimicrobial resistance in livestock pathogens develops through a combination of inherited genetic variation, acquisition of exogenous resistance determinants, and physiological adaptations that reduce antimicrobial activity. These mechanisms rarely operate in isolation. A single bacterial isolate may simultaneously carry genes encoding antimicrobial-inactivating enzymes, altered targets, active efflux systems, and additional determinants located on mobile genetic elements. The resulting phenotype is therefore shaped by interactions among chromosomal mutations, horizontally acquired genes, regulatory networks, bacterial physiology, and antimicrobial exposure. Understanding these processes is particularly important in livestock infections because resistant organisms often circulate within bacterial populations exposed repeatedly to therapeutic, metaphylactic, or prophylactic antimicrobial pressure.

3.1 Genetic Acquisition and Dissemination of Resistance

Resistance may arise through spontaneous chromosomal mutations or through horizontal acquisition of antimicrobial resistance genes. Mutational resistance is particularly relevant when antimicrobial activity depends on highly defined cellular targets. Alterations in genes encoding DNA gyrase and topoisomerase IV, for example, can reduce susceptibility to fluoroquinolones, whereas modification of

ribosomal targets can impair the activity of several protein-synthesis inhibitors. Mutations may also influence regulatory pathways controlling membrane permeability or multidrug efflux. Under antimicrobial selection, bacterial populations carrying advantageous mutations can expand rapidly, although the biological cost of these changes varies according to the organism, resistance mechanism, and surrounding genetic background.

Horizontal gene transfer has an even broader role in the development of multidrug resistance because resistance determinants can move within and between bacterial populations. Conjugation enables direct transfer of plasmids and other conjugative elements, while transformation permits uptake of extracellular DNA and transduction allows bacteriophages to mediate genetic exchange. Plasmids are particularly important because they may carry multiple antimicrobial resistance genes together with integrons, insertion sequences, transposons, and other mobile elements capable of reorganizing or mobilizing resistance regions. A recent genomic analysis of poultry-associated *E. coli*, for example, identified 37 antimicrobial resistance genes and 27 plasmid replicon types among 100 isolates; all isolates contained at least one resistance gene and one plasmid replicon, illustrating the extensive genetic mobility that can occur within livestock-associated bacterial populations (Cho et al., 2025).

3.2 Cellular and Biochemical Mechanisms of Resistance

At the cellular level, bacteria resist antimicrobial activity through several complementary strategies. Enzymatic inactivation is one of the most important mechanisms and is particularly prominent in resistance to β -lactam antimicrobials. β -Lactamases hydrolyze the β -lactam ring before the antimicrobial can effectively interact with its target. The diversity of these enzymes includes narrow-spectrum β -lactamases, extended-spectrum β -lactamases and AmpC-type enzymes, among others. In poultry-derived *E. coli*, *bla*CMY-2 as the most frequent β -lactamase determinant, together with *bla*TEM

and *bla*CARB-2. Similar resistance architectures have been documented among Enterobacterales from livestock, demonstrating that enzymatic degradation remains a major route by which the effectiveness of β -lactam therapy can be compromised (Cho et al., 2025).

Reduced intracellular antimicrobial concentration represents a further major strategy. Gram-negative bacteria can decrease antimicrobial entry through changes in outer-membrane permeability and porin expression, while active efflux systems export structurally diverse compounds from the bacterial cell. These mechanisms become especially important when overexpressed or combined with target modification and enzymatic resistance. In a study of multidrug-resistant bacteria isolated from bovine mastitis, several efflux-associated genes, including *acrB*, *acrE*, *acrF*, *emrB*, and *norB*, together with β -lactam, tetracycline, aminoglycoside, and other resistance determinants. Their coexistence illustrates how multidrug resistance can emerge through the cumulative activity of different cellular mechanisms rather than through a single genetic event (Janus et al., 2024).

3.3 Biofilm Associated Tolerance and Bacterial Persistence

Classical antimicrobial resistance should be distinguished from antimicrobial tolerance and persistence. Resistance generally refers to the ability of a bacterial population to grow at antimicrobial concentrations that inhibit susceptible organisms and is commonly reflected by an increased minimum inhibitory concentration. Tolerance, in contrast, describes the capacity to survive prolonged antimicrobial exposure without necessarily showing a corresponding increase in the MIC. Persister cells represent a small phenotypic subpopulation that survives otherwise lethal antimicrobial concentrations and can re-establish a susceptible population after treatment is withdrawn. These distinctions are clinically relevant because treatment failure can occur even when conventional susceptibility testing does not identify high-level genetic resistance.

The relevance of these processes to livestock disease is particularly evident in bovine mastitis. Biofilm formation among multidrug-resistant staphylococci, *E. coli*, and *Klebsiella* isolates obtained from clinical and subclinical mastitis, alongside antimicrobial resistance genes and efflux systems (Janus et al., 2024). Experimental work with *S. aureus* isolated from recurrent bovine mastitis has further demonstrated that biofilm populations can contain antibiotic-tolerant cells that remain difficult to eradicate even at high antimicrobial concentrations (Lin et al., 2023). Earlier studies of mastitis-associated *S. aureus* also showed that intracellular localization and biofilm formation can reduce antimicrobial effectiveness, illustrating how bacterial persistence may arise from the combined influence of antimicrobial susceptibility and protected ecological niches within the infected host (Kamaruzzaman et al., 2017).

3.4 Fitness Costs Compensatory Adaptation and Maintenance of Resistance

Acquisition of antimicrobial resistance is not biologically neutral. Resistance mutations or newly acquired genes may impose metabolic costs by altering essential cellular functions, slowing bacterial growth, or reducing competitive fitness in the absence of antimicrobial exposure. However, such costs are highly variable and can be reduced through compensatory mutations, regulatory adjustment, or adaptation of the surrounding genome. Consequently, resistant populations may remain stable even after antimicrobial selection decreases.

4. Interplay among Antimicrobial Resistance Virulence and Host Pathogen Interactions

Antimicrobial resistance and bacterial virulence are often investigated as separate biological traits, although both contribute to the ability of a pathogen to survive antimicrobial exposure, persist within the host, and cause clinically important diseases. Their relationship is complex and cannot be reduced to the assumption that resistant bacteria are inherently more pathogenic than susceptible organisms. Acquisition of a resistance determinant may impose a fitness

burden, have little detectable effect on bacterial physiology, or, in some genetic backgrounds, become associated with adaptations that improve persistence or colonization. Conversely, resistance and virulence determinants may coexist because they are genetically linked on the same mobile element or have been selected within successful bacterial lineages without one trait directly causing the other. Interpretation of this relationship therefore requires consideration of bacterial fitness, genomic context, virulence phenotype, and host environment.

4.1 Antimicrobial Resistance and Bacterial Fitness

Evidence from livestock-associated *Salmonella* illustrates this dynamic relationship. *Salmonella* Typhimurium collected from animals over approximately 25 years and found that antimicrobial resistance plasmids were strongly associated with particular livestock clades (Mellor et al., 2022). Several plasmids, including hybrid resistance–virulence structures, persisted within defined lineages over long periods rather than undergoing constant horizontal exchange. Their persistence suggests that successful integration of plasmids into particular genomic backgrounds can allow resistant populations to remain stable despite the potential biological costs associated with carrying additional genetic material.

Adaptation within the animal host may further modify the fitness of resistant organisms. Multidrug-resistant *Salmonella* during colonization of chickens and identified adaptive evolution associated with a mutation in the small protein-encoding gene *STM14_1829* (He et al., 2024). The evolved bacterial population showed increased fitness in asymptomatic hosts and enhanced survival under several environmental stresses. Experimental characterization indicated that the adaptation influenced flagellar expression and was accompanied by improved invasion and persistence in phagocytic cells. Importantly, this observation does not indicate that multidrug resistance itself directly increased virulence. Rather, it demonstrates that resistant bacterial populations can undergo subsequent

host-driven evolution that improves biological fitness and pathogenic potential.

Fitness is also shaped by interactions with competing members of the microbiota. Antimicrobial-resistant *Salmonella* Heidelberg strain originating from poultry production, demonstrated that co-culture with a commensal *E. coli* strain altered the expression of numerous genes involved in bacterial growth, metabolism, chemotaxis, biofilm formation, virulence, and antimicrobial resistance (Khan et al., 2025). Several drug-efflux and resistance-associated pathways were downregulated in the presence of the commensal organism. These findings emphasize that the fitness of resistant pathogens is not determined solely by their resistance genotype but also by ecological interactions occurring within complex microbial communities.

4.2 Coexistence of Resistance and Virulence Determinants

A major concern in livestock-associated bacterial populations is the simultaneous carriage of genes that promote antimicrobial resistance and pathogenicity. Such coexistence has been reported in *E. coli*, *Salmonella*, staphylococci, *Campylobacter*, and several respiratory pathogens. However, statistical or genomic association between resistance and virulence determinants should not be interpreted automatically as evidence of functional synergy.

In a large investigation of 500 *E. coli* isolates from diarrheic sheep, 75.4% of isolates were multidrug resistant and 94.8% carried at least one virulence gene. The isolates displayed substantial diversity in phylogenetic background, resistance phenotype, virulence profile, and biofilm-forming ability (Zhao et al., 2021). Associations between individual resistance phenotypes and virulence determinants were identified, indicating that pathogenic livestock populations can simultaneously accumulate traits relevant to antimicrobial survival and disease production. Nevertheless, because the study was observational, these correlations do not establish that resistance caused enhanced virulence or vice versa.

A more direct genetic connection can occur when resistance and virulence determinants are physically located on the same plasmid. Hybrid Z/I1 plasmids in *S. Typhimurium* originating from Australian swine production and identified complex structures containing genes associated with both antimicrobial resistance and virulence. These plasmids carried determinants conferring resistance to several antimicrobial classes as well as virulence-associated genetic material, illustrating how mobile elements can potentially preserve and disseminate multiple adaptive traits simultaneously (Wyrsh et al., 2019). Hybrid AMR–virulence plasmids within animal-associated *S. Typhimurium* populations and showed that these elements could remain associated with particular clades for extended periods (Mellor et al., 2022).

4.3 Effects on Colonization Persistence and Pathogenicity

Virulence ultimately depends on the ability of a pathogen to overcome host barriers, colonize appropriate tissues, acquire nutrients, evade or withstand immune defenses, and produce tissue damage. Resistance may influence these processes indirectly when the genetic or physiological changes responsible for antimicrobial survival alter bacterial behavior. Relevant traits include adhesion, motility, invasion, intracellular survival, toxin production, secretion systems, biofilm formation, and resistance to host-generated stresses.

Experimental evidence demonstrates that antimicrobial-resistant livestock-associated isolates can retain substantial pathogenic potential. *Salmonella enterica* isolates from UK livestock and imported meat that differed in antimicrobial resistance and virulence-gene content. Selected isolates were evaluated using an infection model, where invasive *S. Enteritidis*, *S. Gallinarum*, multidrug-resistant *S. Kentucky*, and *S. Typhimurium* DT104 produced substantial mortality and tissue injury. Notably, the presence of a recognized plasmid virulence in *S. Typhimurium* did not consistently correspond to greater mortality. This illustrates an important principle: neither antimicrobial resistance nor

possession of an individual virulence determinant reliably predicts the overall pathogenic phenotype (Card et al., 2016).

Persistent colonization may nevertheless provide resistant organisms with an important biological advantage by extending the period during which they survive within animal populations. The chicken colonization adaptive evolution of an MDR *Salmonella* population enhanced invasion and persistence in phagocytic cells while simultaneously improving resistance to environmental stresses (He et al., 2024). These adaptations could increase opportunities for continued shedding, environmental contamination, and transmission. Similarly, biofilm-forming resistant organisms may persist at infection sites because the biofilm state protects bacterial populations from both antimicrobial exposure and components of host defense.

Persistence can also contribute indirectly to the evolution and dissemination of resistance. In an experimental *Salmonella* model, tissue-associated persister populations can function as long-lived reservoirs that subsequently reseed the intestinal lumen and facilitate horizontal transfer of resistance plasmids to other Enterobacteriaceae (Bakkeren et al., 2019). Although this work was not restricted to a naturally occurring livestock disease outbreak, the underlying mechanism is highly relevant to enteric pathogens capable of establishing persistent infection in food-producing animals. It provides a mechanistic link among host persistence, antimicrobial tolerance, and subsequent genetic dissemination of resistance.

5. Host Immune Responses during Infections with Antimicrobial Resistant Livestock Pathogens

The outcome of infection with an antimicrobial-resistant pathogen is determined not only by antimicrobial susceptibility but also by the effectiveness and regulation of the host immune response. Innate immune mechanisms provide the first line of defense against invading bacteria, whereas adaptive responses contribute to pathogen-specific control and immunological memory. Importantly, antimicrobial resistance

itself does not necessarily create a distinct immunological phenotype. Host responses are primarily shaped by bacterial species, virulence factors, inoculum, tissue tropism, infection stage, and host physiological status. Nevertheless, resistant pathogens may indirectly alter immune-pathogen dynamics when therapeutic failure permits prolonged bacterial survival, when resistance is genetically or functionally associated with virulence traits, or when biofilm formation and intracellular persistence reduce immune-mediated clearance. The immunological significance of AMR therefore lies largely in the interaction between resistant bacterial survival strategies and host defense mechanisms rather than in resistance phenotype alone.

5.1 Innate Recognition and Phagocytic Responses

Recognition of bacterial pathogens begins when host pattern-recognition receptors detect conserved microbial structures and initiate inflammatory signaling. Toll-like receptors and other innate sensors activate pathways that promote the production of cytokines, chemokines, antimicrobial molecules, and mediators responsible for recruiting phagocytic cells to infected tissues. The magnitude and composition of this response can differ markedly according to the infecting organism. In bovine whole blood, distinct cytokine profiles following exposure to *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus uberis*, with IFN- γ , IL-1 β , and TNF- α contributing strongly to pathogen-specific differences. Such findings emphasize that the immune environment encountered by a resistant bacterium is determined substantially by its species-specific molecular characteristics rather than by antimicrobial resistance alone (Lesueur et al., 2022).

Neutrophils are particularly important in bacterial infections of livestock because of their rapid recruitment and ability to phagocytose organisms, generate reactive oxygen species, release antimicrobial granule components, and form extracellular traps. In respiratory infection, high-dimensional immune profiling used to demonstrate extensive recruitment and

differentiation of neutrophil populations following *Actinobacillus pleuropneumoniae* infection (Li et al., 2023). Distinct neutrophil subsets were associated with different stages of pulmonary infection, and inflammatory monocyte/macrophage populations also expanded. These observations illustrate that bacterial pneumonia induces a highly dynamic innate response in which efficient microbial clearance must be balanced against the potential for inflammatory tissue injury.

The importance of host innate responsiveness has also been demonstrated directly during challenges with an antimicrobial-resistant organism. Genetically distinct broiler lines with vancomycin-resistant *Enterococcus gallinarum* and found lower frequencies of bacterial recovery from internal organs in birds characterized by stronger innate immune responsiveness. Although the experiment did not compare resistant and susceptible *Enterococcus* strains, it provides evidence that host innate immune competence can materially influence systemic invasion by an antimicrobial-resistant livestock-associated bacterium (Swaggerty et al., 2005). Thus, resistance to antimicrobial therapy does not imply resistance to innate immune clearance, and variation in host defense can remain a major determinant of disease outcome.

Pathogens may nevertheless interfere with phagocyte function. Recent work with bovine neutrophils showed that *Staphylococcus chromogenes* could reduce the efficiency of phagocytosis and bacterial killing despite substantial intracellular reactive oxygen species production, suggesting that livestock-associated staphylococci can persist through mechanisms involving altered pathogen recognition and impaired neutrophil function (Srithanasuwan et al., 2024). Such immune-evasion strategies may become particularly problematic when they coexist with antimicrobial resistance because both pharmacological and cellular mechanisms of bacterial elimination can then be compromised.

5.2 Inflammatory and Adaptive Immune Responses

Inflammation is required for effective bacterial control, but its timing and magnitude strongly influence pathological outcome. Bacterial infections can induce local and systemic production of TNF- α , IL-1 β , IL-6, chemokines, interferons, and other inflammatory mediators that promote leukocyte recruitment and activation. Excessive or sustained signaling, however, can contribute to vascular injury, tissue necrosis, and impaired organ function. Conversely, an insufficient or delayed response may permit bacterial persistence and the development of chronic infection.

Bovine mastitis provides a well-characterized example of pathogen-specific differences in inflammatory behavior. Experimental intramammary infections have shown that *E. coli* commonly induces a rapid and intense inflammatory response, whereas *S. aureus* can generate a weaker or delayed response that favors chronic persistence. Substantially lower concentrations of inflammatory cytokines and complement-derived chemoattractant activity during *S. aureus* infection than during *E. coli* mastitis, providing a mechanistic explanation for differences in neutrophil recruitment and persistence (Riollet et al., 2000). More recent cytokine profiling similarly confirms that bovine immune responses differ substantially according to bacterial species (Lesueur et al., 2022). These observations are relevant to AMR because resistant *S. aureus* strains that also possess persistence-associated characteristics may remain within a host environment in which inflammatory clearance is already relatively inefficient.

Single-cell approaches are now providing greater resolution of these host responses. Blood and milk immune-cell populations during experimentally induced chronic *S. aureus* mastitis in Holstein cattle and identified granulocytes as the predominant immune population, with mammary gland-associated granulocyte subsets showing transcriptional programs linked to cell migration, myeloid differentiation, and inflammatory responses (Wiarda et al., 2025).

Such studies demonstrate that persistent bacterial infection involves substantial local immune remodeling even when sterilizing clearance is not achieved. This is particularly important when considering resistant infections, in which continued survival during antimicrobial treatment may prolong the interaction between bacterial populations and inflammatory leukocytes.

5.3 Immune Evasion Persistence and the AMR Knowledge Gap

The most biologically relevant connection between AMR and immunity may arise through bacterial persistence. Biofilm formation, intracellular survival, altered surface structures, suppression of immune recognition, and resistance to phagocyte-mediated killing can extend the duration of infection. When these properties coexist with antimicrobial resistance, conventional therapy may fail to rapidly reduce bacterial burden, allowing repeated or prolonged stimulation of host inflammatory pathways. Persistent bacterial exposure can therefore maintain leukocyte recruitment and cytokine production and, depending on the affected tissue, contribute to chronic inflammation and progressive structural damage.

6. Pathological Consequences and Therapeutic Failure

The pathological consequences of bacterial infection arise from a combination of microbial proliferation, virulence-associated injury, host inflammatory responses, tissue tropism, and the duration of infection. Antimicrobial resistance can modify this process principally by reducing the probability of effective bacterial clearance during treatment. When an infecting organism survives antimicrobial exposure, continued replication or persistence may prolong inflammatory stimulation and increase the opportunity for local tissue injury, systemic dissemination, or recurrence. However, AMR should not be regarded as an independent pathological determinant. A resistant isolate does not necessarily produce more severe lesions than a susceptible isolate, because disease severity is

also strongly influenced by virulence, bacterial load, immune competence, infection site, treatment timing, and pharmacokinetic exposure. The pathological relevance of resistance is therefore most evident when reduced antimicrobial efficacy allows an otherwise pathogenic infection to persist.

6.1 Persistent Infection and Tissue Injury

Failure to rapidly eliminate bacterial pathogens can transform an acute infectious process into persistent or recurrent disease. Continued bacterial presence sustains recruitment of neutrophils and mononuclear inflammatory cells, maintains cytokine and chemokine production, and exposes tissues to microbial toxins, degradative enzymes, reactive oxygen species, proteases, and other inflammatory mediators. Depending on the organism and affected organ, the resulting lesions may include suppuration, necrosis, fibrin deposition, abscess formation, epithelial disruption, vascular injury, fibrosis, or chronic inflammatory remodeling. These lesions are produced by the combined effects of bacterial pathogenicity and host-mediated injury rather than by antimicrobial resistance alone.

Respiratory disease illustrates this interaction particularly well. Bacterial components of bovine respiratory disease, including *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*, can produce extensive pulmonary injury when infection is not adequately controlled. Lesions associated with bacterial bronchopneumonia may include fibrinous or suppurative inflammation, alveolar exudation, consolidation, necrosis, and pleural involvement. Antimicrobial susceptibility is therefore clinically relevant because persistence of viable organisms after therapy may allow an active inflammatory lesion to continue. In cattle diagnosed with bovine respiratory disease, antimicrobial susceptibility of bacterial isolates while also monitoring clinical outcomes and demonstrated substantial variation in nonsusceptibility according to bacterial species and previous metaphylactic exposure (Jobman et al., 2023). Earlier diagnostic laboratory data similarly showed that resistance among respiratory isolates

increased with the number of previous antimicrobial treatments, particularly in *M. haemolytica*, *P. multocida*, and *H. somni* recovered from clinical cases (Magstadt et al., 2018). These findings support an association between repeated treatment history and recovery of increasingly resistant bacterial populations, although they do not establish that resistance itself produces more severe pulmonary pathology.

The importance of linking microbiological detection with actual tissue lesions is equally evident in swine disease. Gross and histological examinations of nursery pigs and identified frequent lesions in the respiratory tract, gastrointestinal tract, skin, and joints. Detection of *E. coli*, *Streptococcus suis*, and *S. aureus* in tissues was supplemented by immunohistochemical confirmation to establish whether bacterial presence corresponded to genuine tissue infection (Barington et al., 2023). The study demonstrated that microbiological isolation alone cannot always establish causation and that histopathology and in situ pathogen detection substantially strengthen interpretation. This principle is particularly important in AMR research, where demonstration of a resistance gene or resistant isolate should not automatically be equated with the pathological cause of a lesion.

6.2 Chronicity Recurrence and Organ Specific Consequences

Persistent bacterial infections are especially important in tissues where organisms can occupy protected extracellular or intracellular niches. Bovine mastitis represents a major example. *S. aureus* can establish chronic intramammary infection through adhesion, biofilm formation, intracellular survival, small-colony-variant formation, and modulation of the mammary immune response. When antimicrobial resistance accompanies these persistence traits, the probability of successful bacterial eradication may be further reduced. Repeated inflammatory episodes can contribute to epithelial injury, disruption of secretory tissue, increased somatic cell counts, altered milk composition, and progressive reduction in mammary function.

Multidrug-resistant isolates from bovine mastitis frequently combined resistance genes with biofilm formation and efflux-associated mechanisms. Among the MDR isolates examined, biofilm production was highly prevalent, suggesting that several mechanisms capable of impairing antimicrobial efficacy may coexist within the same pathogen population. Importantly, this finding does not prove that MDR isolates necessarily produce more severe mammary lesions, but it provides a plausible biological basis for persistence and incomplete therapeutic clearance. Chronic infection may consequently maintain local inflammation even when overt clinical signs fluctuate (Janus et al., 2024).

This distinction between resistance and pathogenicity is central to interpreting pathological data. Resistant isolates may recover from animals with severe lesions simply because previous therapy failed to eliminate them, producing a form of selection bias toward difficult clinical cases. Conversely, susceptible bacteria can cause rapidly fatal disease if virulence is high and treatment is delayed. Therefore, pathology-focused AMR studies should ideally compare bacterial genotype and phenotype with lesion severity, bacterial burden, treatment history, and clinical progression in the same animals.

6.3 Therapeutic Failure and Clinical Outcome

Therapeutic failure occurs when treatment does not achieve the expected microbiological or clinical response. Antimicrobial resistance is one important cause, but inadequate drug concentration at the infection site, inappropriate antimicrobial selection, delayed treatment, biofilm formation, intracellular localization, poor host immunity, extensive pre-existing tissue damage, and incorrect diagnosis can produce similar outcomes. Thus, in vitro resistance should be interpreted within the pharmacological and pathological context of infection.

Evidence from bovine respiratory disease demonstrates the complexity of this relationship. Respiratory bacterial isolates obtained from previously treated cattle were more likely to show

resistance than isolates from untreated animals, and resistance increased with additional antimicrobial treatments. This may reflect selection of resistant subpopulations during unsuccessful treatment (Magstadt et al., 2018). Similarly reported greater nonsusceptibility among isolates from cattle exposed to metaphylaxis. These observations indicate that repeated antimicrobial exposure and clinical treatment failure can become epidemiologically linked, potentially generating a cycle in which persistent disease results in further antimicrobial use and additional selection for resistance (Jobman et al., 2023).

Mastitis presents comparable challenges because bacteriological cure and clinical improvement do not always occur simultaneously. A reduction in inflammation may occur while bacteria persist within mammary tissue, allowing subsequent recurrence. Conversely, extensive inflammatory damage may continue after bacterial numbers have fallen. Culture and susceptibility testing are therefore most informative when interpreted together with clinical findings, somatic cell responses, treatment history, and pathogen characteristics. In an investigation of clinical bovine mastitis, substantial pathogen-specific differences in antimicrobial susceptibility and emphasized the importance of identifying the causative organism when guiding therapy (Duse et al., 2021).

The most informative future investigations will therefore integrate antimicrobial susceptibility, resistance genotype, bacterial load, virulence profile, pharmacological exposure, clinical response, and quantitative pathology within the same experimental or naturally infected animals. Such an approach would allow resistant infection to be evaluated as a biological continuum from microbial survival to persistent inflammation, lesion progression, treatment failure, and impaired organ function. This integrated perspective also provides the rationale for diagnostic strategies capable of linking resistance phenotype and genotype with clinically meaningful pathological outcomes.

7. Diagnostic and Genomic Advances with Future Research Priorities

Accurate interpretation of antimicrobial-resistant infections requires more than identification of the bacterial species or detection of an individual resistance gene. The clinically relevant phenotype emerges from interactions among antimicrobial susceptibility, resistance mechanisms, virulence determinants, bacterial burden, host responses, tissue localization, and pathological progression. Conventional culture and antimicrobial susceptibility testing remain essential because they demonstrate viable organisms and directly measure phenotypic response to antimicrobial exposure. Molecular and genomic technologies, however, increasingly provide information that cannot be obtained from susceptibility testing alone, including the genetic architecture of resistance, mobile genetic elements, virulence-associated determinants, strain relatedness, and within-population diversity. The major diagnostic challenge is therefore not simply to replace conventional methods with sequencing, but to integrate microbiology, molecular genetics, host-response measurements, and pathology into a biologically meaningful assessment of resistant livestock disease.

7.1 Phenotypic and Molecular Diagnosis of Antimicrobial Resistance

Bacterial culture followed by antimicrobial susceptibility testing remains the reference framework for determining whether a viable isolate expresses a clinically relevant resistance phenotype. Disk diffusion and broth microdilution are widely used, while determination of the minimum inhibitory concentration provides a quantitative estimate of antimicrobial susceptibility. Interpretation requires appropriate veterinary clinical breakpoints or epidemiological cut-off values, where available. This is particularly important because resistance classifications developed for human isolates or unrelated host-pathogen combinations may not accurately predict therapeutic response in livestock.

Conventional PCR, multiplex PCR, and quantitative PCR provide rapid and sensitive

detection of defined resistance genes and can be particularly useful when culture is slow, difficult, or affected by previous treatment. Their principal limitation is that the investigator must largely know what to search for in advance. Detection of a gene also does not necessarily demonstrate its expression or confirm phenotypic resistance. Conversely, resistance caused by novel mutations or untested determinants may be missed. Gene-targeted molecular assays are also increasingly used in veterinary infectious-disease diagnostics; for example, the *VirB5* encoding gene has been investigated as a molecular diagnostic target for caprine and ovine brucellosis (Ullah et al., 2026). For these reasons, molecular detection and phenotypic susceptibility testing should be regarded as complementary rather than interchangeable diagnostic approaches.

7.2 Whole Genome Sequencing Metagenomics and Integrated Omics

Whole genome sequencing has substantially expanded the resolution at which resistant livestock pathogens can be characterized. A single genome can provide information on resistance genes, resistance-associated mutations, plasmid replicons, integrons, virulence determinants, sequence type, phylogenetic relationships, and potential transmission patterns. This is particularly valuable for multidrug-resistant organisms in which several mechanisms may coexist within the same isolate.

The ability to predict antimicrobial phenotypes directly from genomic data is developing rapidly. Combined whole-genome sequencing and antimicrobial susceptibility data from 980 disease-associated *E. coli* isolates obtained from farm and companion animals. Their modelling approach achieved high overall predictive performance across multiple antimicrobial agents, demonstrating the potential of genotype-based prediction in veterinary pathogens. Importantly, prediction differed for some antimicrobial-host combinations, indicating that the relationship between genotype and expressed phenotype is not universally constant. This heterogeneity reinforces the continuing importance of phenotypic validation rather than assuming that

detection of a known resistance determinant will invariably correspond to clinical resistance (Chung et al., 2023).

Metagenomic sequencing extends genomic investigation beyond isolated colonies by examining microbial DNA directly within complex samples. This may be particularly valuable for polymicrobial respiratory or enteric disease, where routine culture can incompletely represent microbial communities. Application of bacterial enrichment followed by long read metagenomic sequencing to deep nasopharyngeal samples from feedlot cattle. Enrichment increased detection of bovine respiratory disease-associated bacteria and permitted identification of antimicrobial resistance genes with species-level resolution. Agreement between genomic resistance detection and conventional susceptibility testing varied by organism and antimicrobial class, demonstrating both the potential and the current limitations of culture-independent AMR diagnosis (Herman et al., 2024).

Transcriptomics adds a functional dimension by identifying genes actively expressed during infection or antimicrobial exposure. RNA sequencing can reveal changes in bacterial stress responses, efflux regulation, metabolism, virulence expression, and host inflammatory pathways that cannot be inferred from genomic sequence alone. Parallel host transcriptomics may be especially informative for determining whether a resistant infection is associated with altered immune activation, bacterial persistence, or tissue-specific responses. Single-cell RNA sequencing provides even greater resolution by identifying immune-cell populations and transcriptional states within infected tissues. Single-cell transcriptomics to characterize circulating and mammary immune-cell populations during chronic *S. aureus* mastitis, revealing granulocyte-associated programs linked to migration and local inflammatory responses (Wiarda et al., 2025).

7.3 Linking Resistance Genotype with Pathological Phenotype

A major weakness of current AMR research is the limited integration between microbiological resistance data and lesion-level pathology. Many studies describe resistance genes without characterizing tissue damage, whereas pathological investigations frequently identify bacterial agents without detailed genomic analysis. This separation makes it difficult to determine whether particular resistant lineages, mobile elements, or resistance-associated adaptations influence persistence, inflammatory response, or disease progression.

Pathology should therefore become an explicit component of advanced AMR investigation. Gross lesions and histopathology can identify the anatomical distribution, type, severity, and

chronicity of tissue injury, while immunohistochemistry, in situ hybridization, or other localization methods can demonstrate whether the detected organism is actually present within the lesion. The value of combining gross examination, histopathology, bacterial culture, immunohistochemistry, and qPCR when investigating disease in nursery pigs. Importantly, microbiological detection did not always establish lesion causality, illustrating why genomic or culture results should be interpreted within their pathological context (Barington et al., 2023). An optimal future diagnostic framework would therefore connect phenotypic susceptibility, resistance genotype, virulence profile, bacterial localization and burden, host response, lesion phenotype, and therapeutic outcome within an integrated analytical framework (Figure 2).

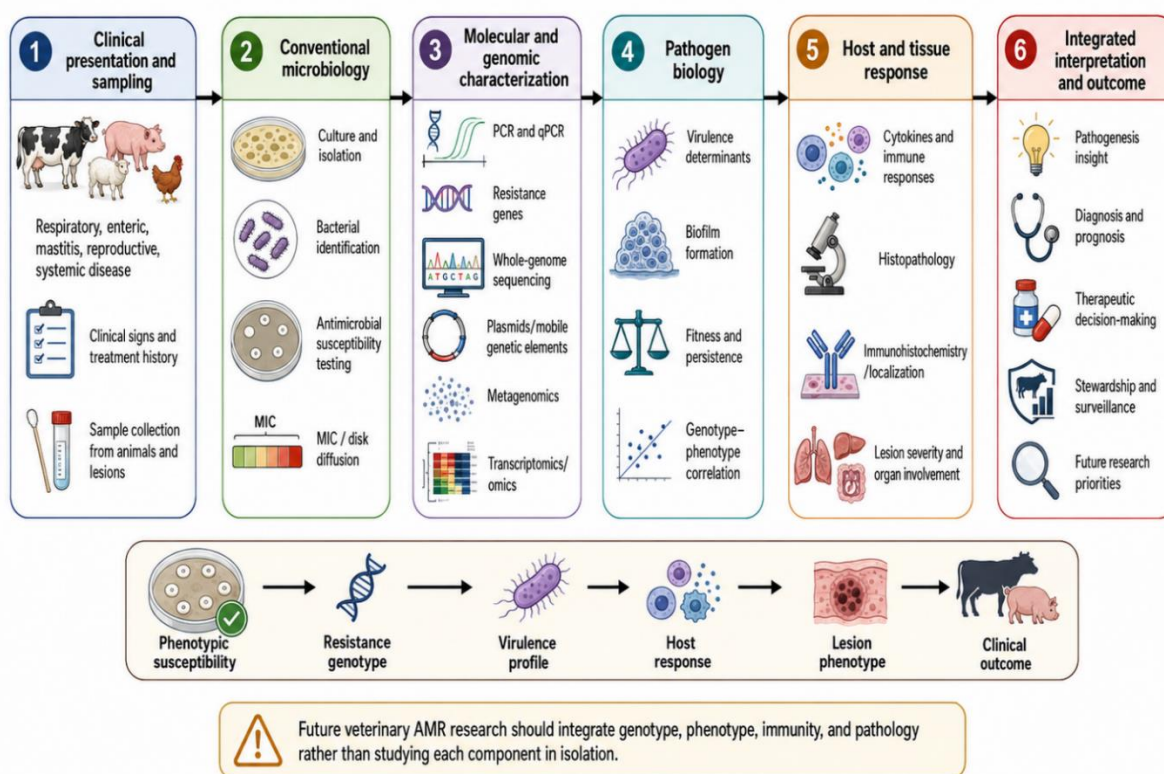


Figure 2. Integrated diagnostic and research framework for antimicrobial-resistant livestock infections.

7.4 Research Gaps and Future Priorities

Several major knowledge gaps currently prevent this level of interpretation. First, direct experimental comparisons between closely related antimicrobial-resistant and susceptible isolates

remain uncommon in livestock. Genetically matched strains differing in a defined resistance determinant would provide much stronger evidence of causal effects on fitness, immune

interactions, or pathology than comparisons among epidemiologically unrelated isolates.

Second, genotype-phenotype relationships require broader validation for veterinary pathogens. The performance of genomic resistance prediction depends on the completeness of resistance databases, sequencing quality, bacterial species, host origin, and antimicrobial being assessed. Large collections containing paired MIC and high-quality genomic data are therefore needed across diverse livestock pathogens and production systems.

Third, studies should integrate resistance with virulence and host biology rather than examining these components separately. Experimental designs combining WGS with bacterial transcriptomics, host transcriptomics, cytokine profiling, quantitative bacterial load, histopathological scoring, and treatment response could clarify whether resistant pathogens behave differently within infected tissues. Emerging artificial intelligence-assisted approaches may further support the integration and interpretation of complex diagnostic, imaging, molecular, and surveillance datasets in veterinary medicine and zoonotic disease monitoring (Maqbool et al., 2025).

Finally, longitudinal studies are needed to follow resistant infections from initial exposure through treatment, persistence, lesion development, recovery, recurrence, or transmission. Such studies would provide temporal information that cross-sectional surveillance cannot capture. The future of veterinary AMR diagnostics therefore lies not in sequencing alone, but in integrated genotype-phenotype-pathology analysis capable of explaining how resistance affects the biological course and clinical outcome of livestock infection.

8. CONCLUSION

Antimicrobial resistance in livestock pathogens is more than a laboratory susceptibility problem because its biological consequences may extend into pathogen survival, host interaction, disease persistence, and therapeutic outcome. Resistance develops through diverse genetic and cellular mechanisms that can interact with bacterial

fitness, virulence-associated traits, biofilm formation, and persistence. However, antimicrobial resistance should not be interpreted as synonymous with increased virulence or greater pathological severity. Disease outcome ultimately reflects the combined effects of microbial genotype, host immunity, infection site, antimicrobial exposure, and tissue response. When resistant pathogens evade effective antimicrobial clearance, prolonged bacterial survival may sustain inflammation, promote chronic or recurrent infection, and contribute indirectly to tissue injury and clinical treatment failure. Future research should therefore integrate phenotypic susceptibility testing with genomics, host-response profiling, and quantitative pathology. Such approaches will clarify the mechanistic sequence linking microbial adaptation, resistance, host pathogen interactions, immune responses, pathological injury, and clinical outcome in livestock disease.

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