

THERAPEUTIC STRATEGIES FOR MANAGING BIOFILM-ASSOCIATED INFECTIONS IN LIVESTOCK: A COMPREHENSIVE REVIEW

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ABSTRACT

Biofilm-associated diseases in animals give rise to formidable challenges due to the biofilm forming bacteria shows resistance to the conventional antimicrobial therapy. These therapeutic strategies are explored in this chapter aimed at the influence of biofilm-associated pathogens in animal conditions will be lowered. Several techniques can be used for treating the biofilm-based disease in livestock, such as biofilm-penetrating antibiotics, probiotics, phage therapy, and various other methods which includes physical and mechanical intervention of ultrasound, high pressure cleaning and the ability to destroy and remove biofilms. Immunomodulation is turning up as a key, emphasizing the role of immunostimulants along with vaccines play in strengthening defence mechanism of the host against biofilm-forming pathogens. The disruptors of the biofilm, includes enzymes and specific chemicals which degrades the matrix formed by the bacterial biofilm. The integration of biosecurity measures, sanitation practices and quarantine protocols emphasize controlling the introduction and spread of biofilm-forming bacteria. The extensive research additionally emphasises the importance of routine procedures and diagnostic tools for timely diagnosis and effective implementation. highlights the relevance of multiple preventive measures in addition to therapies tailored with the distinctive features of the bacteria producing the entailed biofilm in consideration.

1. INTRODUCTION

The biofilm is an organized community of microbes living in an extracellular polymeric substance (EPS) matrix, it can exist either single or in communities known as biofilms, consisting of various or even identical species (P. Li et al., 2023). Microbes in biofilm adhere to one another on the surface of both living and nonliving, and their growth rate and gene expression vary. Microbiological infections become uncontrollable because of the formation of biofilms, which in turn aid in the evolution of antibiotic resistance and the establishment of persistent cells (X. Yang et al., 2020). Biofilms and bacteria are related through a few characteristics which include changes in phenotypic and gene expressions, resistance to specific antibiotics, reduced metabolic activity and growth rate, and synthesis of virulence-associated proteins (Makabenta et al., 2021). (H. Wu et al., 2015) data presents, that about 65% of all veterinary infections are attributed to microbes and the formation of biofilms. Bacteria have historically utilized biofilm development as a means of survival in unfavorable conditions. Biofilms protect cells against various hazards, including UV radiation, dehydration, pH stress, chemical exposure, phagocytosis, and antibiotics (Avershina et al., 2021). (Sun et al., 2013) mention,

biofilms have both direct and indirect influences on various infections and diseases, like prosthesis infection, chronic non-healing wounds, and mastitis. These issues have detrimental effects on the dairy industry and can cause a decline in milk yield due to mastitis. Biofilm increases the pressure on the veterinary industry to diagnose and treatment of infections and diseases faster and more effectively due to the intensity of the damage they cause (Sharma et al., 2016). Over a period, biofilms can develop elevated resistance to antimicrobial agents, complicating the treatment of the infection. Phage therapy and other alternative therapies are attracting renewed interest in the treatment of biofilm-associate microbes and due to the multidrug-resistant bacterial strains have emerged, constituting a main public health concern. Bacteriophage predators have shown promising results against bacterial biofilms (Koo et al., 2017). Biofilm-related infections can not only lead to more severe symptoms but also can increase the risk of death. With the elevation of the number of research investigating methods to eradicate microbial biofilms, it is important to have a comprehensive knowledge of the impact of biofilms on infections and to thoroughly evaluate the most recent effective antibiofilm tactics article. In this study, we examine the processes of

medication resistance in biofilms and discuss current improvements in alternative therapeutics and potential techniques to combat microbial biofilms. In addition, we provide a comprehensive analysis of the advantages and disadvantages of various tactics.

2. BIOFILM FORMATION IN LIVESTOCK

As mentioned by (Jacques et al., 2010) on any kind of given surface, biotic or abiotic, it serves as a platform for biofilm growth. Hydrophobic effects and weak Van der Waals's forces can be the reason for surface attachment. (Rudenko et al., 2021) their work says the initial mild attachment is not disturbed, and then colonies will remain permanently attached, with the cell adhesive structures such as flagellum, pili, and archaean pili. Gram-positive or gram-negative bacteria or whether they're motile or not, can readily combine to form biofilm. (Ammar et al., 2020) study mentions, that surface colonization (adhesion) allows quorum sensing characteristics, such as virulence factors, to be communicated by bacterial cells with the use of N-acetyl homoserine lactone. Cell recruitment and cell division are the two major mechanisms by which biofilm expands after colonization starts on the surface of the colony (Abdullahi et al., 2016a). Secondary metabolites of bacteria, including antibiotics, pigments, and siderophores, are among the signals that initiate biofilm formation (V. Silva et al., 2022), in addition to quorum-sensing molecules. (Komodromos et al., 2022) study reveals biofilm formation is induced by sub-inhibitory antibiotics, imipenem, and tobramycin. Bacteria creates a cocoon-like environment inside the biofilm (Horiuk et al., 2019), which is composed of polysaccharides matrix (d-glucose, d-mannose, and l-rhamnose). Protein, eDNA, and extracellular enzymes such as aminoglycoside modifying enzymes (AMEs) and β -lactamase are found in biofilm matrix with polysaccharides (Olson et al., 2002). Gram-positive and gram-negative bacteria form biofilms, certain bacteria are more prone to form biofilms while some are less (Guéneau et al., 2022). Biofilm formation consists of multiple stages, beginning with initial attachment. A total of five phases are involved in the development of biofilm. The dispersion phase follows initial reversal attachment, followed by irreversible attachment and maturation phase. Fungal, algal, protozoan, and bacterial organisms are all capable of producing biofilm.

2.2 SPECIFIC BIOFILM-FORMING PATHOGENS IN LIVESTOCK

(Milanov et al., 2015) studies investigate the majority of bacteria (99.9%) can produce biofilm on a wide range of surfaces, including both biological and non-reactive surfaces. Extracellular polymeric substances (EPS)

produced by microorganisms establish biofilm via surface adhesion (Ashraf & Imran, 2020). Due to antibiotic resistance and the illness, it causes in medical equipment, biofilm presents a substantial risk to public health. H. influenza has been shown to possess the ability to produce biofilm inside the human and animal body and escape the immune system. *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli* species, *Staphylococcus aureus*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* are the bacteria capable of producing biofilms. Several gram-positive bacteria, such as *Bacillus* spp., *Listeria monocytogenes*, *Staphylococcus* spp., *Lactobacillus plantarum*, and *Lactococcus lactis* as well as gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa*. Cyanobacteria also form biofilms in aquatic environments.

Gram-negative bacillus *E. coli* is responsible for a variety of nosocomial and community-acquired illnesses, such as urinary tract infections (UTIs) and prostatitis in humans, along with this it also poses a threat to the health of livestock as well. The microorganism can release toxins and polysaccharides, and it is also responsible for generating biofilm. *E. coli* capsules are macromolecules with a substantial molecular mass that adhere to the surface of cells. *E. coli* capsules indirectly contribute to the production of biofilms by inhibiting bacterial attachment to the surface. *E. coli* can produce biofilm influenced by several environmental variables. Because of the presence of exopolymers, biofilms formed by *E. coli* may reach a thickness of several hundred microns, which poses a challenge for antibiotic treatment and other therapeutic options. Along with *E. coli* another bacteria *Staphylococcus aureus* is resistant to several drugs and is responsible for causing various infections acquired in healthcare settings and the biggest threat in the dairy industry (Vlaeminck et al., 2020). *Staphylococcus aureus* utilizes intercellular protein recycling to generate the extracellular matrix. The capacity of cytoplasmic proteins to function as matrix proteins allows for increased flexibility and adaptability to *S. aureus* in the formation of biofilms in infectious conditions (Cheung et al., 2021). This promotes the development of biofilms consisting of many species in chronic wounds (Mourenza et al., 2020). Apart from these two bacteria, several other bacteria form biofilms and can be detrimental to livestock health, but *E. coli* and *S. aureus* are the deadliest bacterial infection that occurs in livestock and are majorly responsible for the huge economic losses in agricultural settings. Researchers have conducted various experiment on the pathogenic bacteria by collecting samples, the data is drawn in **Table 1**.

Table 1: Sample collected from pathogenic bacteria for treatment study.

Sl. no	Pathogenic bacteria	sample type	Focus of study	Reference
01.	<i>Enterococcus faecium</i>	Milk samples, subclinical bovine mastitis isolates	Study of phenotype, genotype, and virulence	(C. Wu et al., 2013)
02.	<i>S. uberis</i>	Clinical bovine mastitis isolates	Vaccination	(Khan, Pham, Oloketuyi, &

				Kim, 2020)
03.	<i>S. xyloso</i>	Milk samples	Antibacterial treatment	(Dias et al., 2018)
04.	<i>S. epidermidis</i>	Bovine mastitis isolates	Genomics and proteomics	(X. Liu et al., 2023)
05.	<i>E. coli</i>	Bovine mastitis isolates	Antibacterial treatment	(Nesse et al., 2023)
06.	<i>S. aureus</i>	Bovine mastitis isolates	vaccination	(L. A. D. S. De Silva & Heo, 2023)
07.	<i>S. aureus</i>	Bovine subclinical mastitis isolates	Molecular basis of biofilm	(Giaouris & Simões, 2018)

2.3 IMPACT OF BIOFILMS ON ANIMAL HEALTH AND PRODUCTION

Microorganisms get attached to surfaces and form biofilms, which have been associated with various infections of significant public health importance (Donlan, 2002). (Hotinger et al., 2021) Biofilms and several illnesses are correlated. Bacterial infections, which include persistent lung, wound, and ear infections in animals are often caused by biofilms. Biofilms can have control over medical devices such as catheters and implants (Elbourne et al., 2020). (González-Martín et al., 2022) mention biofilm accounts for about 80% of bacterial infections, which contribute to the majority of microbial diseases. These biofilms enhance the bacteria's ability to cause disease, while simultaneously protecting from external treatments that may eliminate them. Biofilm-related diseases exhibit resistance to both traditional biocides and the immune system of the host. The diagnosis and treatment of the illness pose challenges pulmonary ailment cystic fibrosis (CF) is specified for the production of viscous mucus that obstructs the airways, which results in respiratory difficulties for sufferers (Luong et al., 2020). *Pseudomonas aeruginosa* infects the majority, 80% of the population with cystic fibrosis over an extended time. *Pseudomonas aeruginosa* is present on medical gear, gadgets, and medical instruments. There is a scarcity of potent antibiotics for effectively treating chronic *P. aeruginosa* infections. Within the fields of animal and veterinary medicine, biofilm has profound effects on the health and livestock industries, resulting in substantial economic detriment. (Tremblay et al., 2014) study mentions, antibiotic resistance is one of the major obstacles posed by biofilm production; it can also pose a significant risk to human health through the food chain. Bacterial infections which are significant in the field of veterinary medicine exhibit resistance to antibiotic treatment. There is a lack of antibiotic treatment when specific disease conditions are addressed, these pathological conditions include mastitis caused by infections with *Streptococcus* and *Staphylococcus* species. Several bacterial infections, including *Salmonella* spp., generate biofilm in the poultry meat industry, which has the potential to cross-contaminate and negatively affect public health.

3. Biofilm-associated infection in livestock

Biofilm-associated infections are responsible for major challenges to livestock production and health. Certain bacteria pose biofilm formation, they utilize the surface within the host organism and create resilient and complex communities (Lieke et al., 2020). Livestock

face biofilm-associated infection in several anatomical sites, including the udder (leading to mastitis), respiratory tract infection, and gastrointestinal system. These infections impact the overall herd productivity and economic sustainability. In dairy cattle, bovine mastitis is a widespread disease. It affects the world's dairy industry (Zhang et al., 2022). The dairy industry suffers a massive loss in its business due to bovine mastitis. Mastitis is characterized as an inflammation of the mammary gland parenchyma that could be toxic, stressful, or infectious. Mastitis is identified by pathological abnormalities in the glandular udder tissue and physical, chemical, and typically bacteriological changes in the milk (Nie et al., 2020). Clinical symptoms, such as udder enlargement, tenderness to the touch, fever, and depression, are used to diagnose mastitis. A reduction in milk production is often observed. The diagnosis of mastitis may additionally hinge on ancillary tests, which in turn depend on factors such as the number of leukocytes in the milk, as a result of the high incidence of subclinical mastitis cases (B & A, 2021).

Livestock respiratory infections are a major problem, it is frequently associated with the formation of persistent biofilms. In the agricultural environment, it offers a prevalent serious problem. Especially bacteria can lead to several biofilm-associated respiratory diseases (Omoigberale, 2021). These biofilms, which are composed of an extracellular polymeric matrix, adhere to the respiratory system's mucosal surfaces and enable bacteria to persist and colonize them (Becker et al., 2020). These infections tend to be resistant to standard antimicrobial interventions due to the complexity of their structure, which also functions as a barrier against the host's immune response (Kabelitz et al., 2021). Thus, respiratory biofilm-associated diseases in livestock might have a long-term and recurring detrimental effect on health, adversely impacting the productivity of the herd as a whole and the overall well-being of the livestock (Howden et al., 2023).

Histophilus somni is an agent of respiratory and systemic diseases in bovines and readily forms biofilms in vitro. In a study it is found the capability of *H. somni* to form biofilms in cardiopulmonary tissue following experimental respiratory infection in the bovine, was examined by light microscopy, transmission electron microscopy, immunoelectron microscopy of ultrathin cryosections, scanning electron microscopy of freeze-fractured samples, and fluorescent in situ hybridization (Gao et al., 2020). Several other bacteria are also responsible for the bacterial respiratory disease in

livestock. The most widely recognized and deadly of these bacterial agents is *Mannheimia haemolytica*, which is also the most thoroughly studied. Other prominent bacterial pathogens associated with BRD include *Mycoplasma bovis*, *Histophilus somni*, and *Pasteurella multocida* (Guo *et al.*, 2020).

Livestock well-being and health in rural contexts are seriously jeopardized by biofilm-associated gastrointestinal illnesses. Several bacteria species exhibit a remarkable capacity to form biofilms on mucosal surfaces and in the gastrointestinal tract lumen within the complex environment of the digestive system. These microbial populations adhere to the extracellular polymeric components that make up the biofilm matrix, leading to colonization and the development of robust biofilm structures. Once livestock develop gastrointestinal diseases linked to biofilms, they frequently experience poor nutrient absorption, inflammatory reactions, and repeated health problems. *Escherichia coli* is a prevalent and typical resident of the gastrointestinal (GI) tract found in warm-blooded animals as well as in agricultural settings. *E. coli* infections in calves are a major contributor to infant mortality in dairy calves and can manifest as an enteric or septicemic illness. These biofilm-associated bacterial diseases in livestock can altogether affect economically and also impact the overall well-being of the livestock.

4. Consequences of biofilm infection in livestock

Biofilm formation in livestock has several negative impacts. Among them which is most evident is it is not easily treatable by conventional therapy i.e. antibiotic treatment. Biofilm formation by bacteria can occur in medical devices and several other tissues in the animal and it makes a protective layer that makes the biofilm resistant to the antibiotic treatment.

4.1 Reduced efficacy to the conventional treatments

The biofilms are considered as notorious as they evade the host's immune response as well as act as a barrier towards antibiotic treatment. A study was conducted in which four biofilm-forming bacteria were isolated from the wild animals they were *Acinetobacter* spp, *Klebsiella pneumonia*, *Shewanella putrefaciens*, and *Pseudomonas fluorescens*, and their antibiotic susceptibility was tested. They showed multi-drug resistance (MDR), and *K. pneumonia* was found to exhibit several beta-lactamase genes. This study also mentions that wild animals are the reservoirs and vectors for transferring the MDR bacteria. Broad spectrum and higher concentrations also failed to completely remove the biofilms.

4.2 Economical Impact of biofilm infection in livestock

Biofilm infection in livestock has a very serious economic impact on the agricultural sector and also a tremendous impact on the field of veterinary medicine, especially the livestock industry (Abdullahi *et al.*,

2016b). Among the most economically impacting diseases in livestock, mastitis holds a very strong position. The dairy industry has to undergo significant losses worldwide, caused by the decrease in milk production, increase in health care costs of the livestock, and increase in culling and death rates. Huge losses are reported in India which account for up to US\$526 million annually according to (Varshney & Ram Naresh, 2004). While another study indicated that there is a loss of Rs.1,607.20 crores due to bovine mastitis. The economics of milk production are affected by bovine mastitis, a main infectious disease. Mastitis is a systemic condition that affects more than just the mammary gland, it reduces milk supply, changes its quality, affects fertility, and causes diseases that raise healthcare and production expenditures. Economic losses occur when dairy sheep and goats have mastitis, this is due to the diseases reduces milk supply and quality, which in turn leads to undervaluation of milk with high somatic cell counts, treatment expenses, and death (Anter Saad, 2021).

4.3 Potential transmission risk

Transmission risk of livestock infection is a major problem, which affects both human as well as animal health. The livestock animals are a reservoir of several pathogens, which transmit the infection to the same species, cross-species even if it crosses the barrier and infects humans these are zoonotic diseases.

4.3.1 Zoonotic Diseases from the Livestock

As livestock are considered the harbor of the pathogen, it gets transmitted to humans and causes disease which is known as a zoonotic disease. It can be transferred through various routes like waterborne, foodborne, vectorborne, direct contact, etc. These diseases can be fatal in humans. When the infectious dose of a pathogen is less, then for the transmission of the pathogen a brief exposure is sufficient. It was revealed in Germany which focused on the carriage of LA-MRSA among the residents and farmers in an area with a high density of livestock farms. Farmers were found to be more prone to zoonotic disease when they were in contact with the pig, but it was also revealed that frequent visits to the market to buy eggs, meat, etc also put the non-farm residents at risk of zoonotic disease. In a study, it was found that a visit to an agricultural fair in the US resulted in the transmission of Swine influenza. Public access farm visitors in France were found to be infected with gastrointestinal infection with VTEC 0157 and Q fever. Zoonotic diseases caused by various microorganism along with their treatment in livestock is shown in **Table 2**.

Table 2: Zoonotic disease caused by the pathogenic microorganism.

Sl. No	Pathogenic microorganism	Disease caused	Treatment	Reference
1	Brucellosis abortus	Abortion in the last stage of pregnancy in female cattle, bursitis in male cattle, and orchitis	No practical treatment available	(Shin & Park, 2018)
2	Coxiella burnetii	Q fever	Animal vaccination	(Chlebicz & Śliżewska, 2018)
3	Mycobacterium bovis	Bovine tuberculosis	Specific antibiotics, with one year of treatment period	(Ali Gharieb et al., 2020)
4	Mycobacterium caprae	Caprine tuberculosis	Antibiotics for 9 to 10 months	(Nawaz, 2020)
5	Taenia saginata	Bovine cysticercosis	Anthelmintics can be provided for a long course, however no effective treatment for this disease	(MacKenzie et al., 2017)
6	Prions (Creutzfeldt-Jakob disease variants)	Bovine spongiform encephalopathy	No effective treatment	(Fan et al., 2022)
7	Campylobacter spp	Spotty liver disease	No treatment	(Percival et al., 2011)
8	Salmonella spp.	Fowl typhoid and pullorum disease	Ciprofloxacin and ceftriaxone	(García & Percival, 2011)
9	Balantidium coli	balantidiasis	Tetracycline, metronidazole, and iodoquinol.	(dos Santos et al., 2022)

4.3.2 Disease Transmission among Livestock Groups

The livestock is kept in close confinement, which favors the transmission of the rapid spread of infectious diseases among the animals. The pathogens are easily transferred as they are kept in close quarters. Airborne diseases are easily transmitted as airborne particles, such as respiratory droplets which have pathogens in it can be easily spread between animals in the same vicinity. In a study, it was revealed that *Mycobacterium bovis* is one of the major causes of respiratory infection in cattle. As it is transmitted through aerosols it can easily infect the healthy cattle which are near the diseased one. Apart from airborne, the pathogens can be transferred through the contaminated water source which is shared by the animals. In a study, It is mentioned that biofilm-based infection by *Salmonella* sp. in livestock can occur through infected gulls and passerines. As they are considered as the carrier. The wild birds are marked as a risk factor for transmission of the disease. It also revealed that *Vibrio cholerae* which is usually present in the aquatic environment, poses a significant health risk to livestock. It can lead to stomach upset, bloating, etc. It is usually transmitted by the consumption of shellfish and other fish.

4.3.3 Antimicrobial resistance in human

Antibiotics are used for several reasons in farm animals, among them the major is for treating a bacterial infection in the livestock, for treating metaphylactic, and sometimes it is also used in the growth enhancement of the farm animals. The consumption of such livestock treated with antimicrobials leads to the AMR. There are multiple ways through which resistant organisms can be transmitted to humans e.g. consumption of meat, or exposure to the environment. These antibiotics are transferred to humans through food chains and can have

a severe impact on humans while treating any bacterial disease (Osuoha et al., 2023). The main question arises what should be the proper dosage of the antibiotics being used for treating the bacterial biofilm-based infection? It has been studied that several resistant bacteria are emerging with AMR in farm animals like carbapenem, a potent antibiotic that is used when other antibiotics show resistance. When the product of this farm animal is being consumed it leads to developing carbapenem-resistant organism to develop. Antibiotic usage can be reduced by adopting several strategies like using symbiotic which is a mixture of prebiotic and probiotic which helps to prevent organisms from several diseases and does not lead to AMR in humans (Mousavi et al., 2021). For the long term, the genome of the livestock should have microbial-resistant genes which helps the livestock to prevent pathogens. Veterinary vaccines are also a very good choice. In a study, it was demonstrated that Methicillin-resistant *Staphylococcus aureus* infections, which at present do not have any effective vaccine, a multi-component vaccine was developed which targets *Staphylococcal* biofilm infection. When this vaccine was combined with vancomycin treatment in a rabbit model of chronic osteomyelitis, the vaccine was found to be effective by reducing the clinical and radiographic signs of infection by 67% and 82% respectively, compared to the vancomycin-only or untreated group (Ahmadipour et al., 2021). (Manaia, 2017) studies found that antibiotics are been intake by human through direct way by consuming antibiotics, or by indirect ways like, livestock product or environment, which is represented in **Figure 1**.

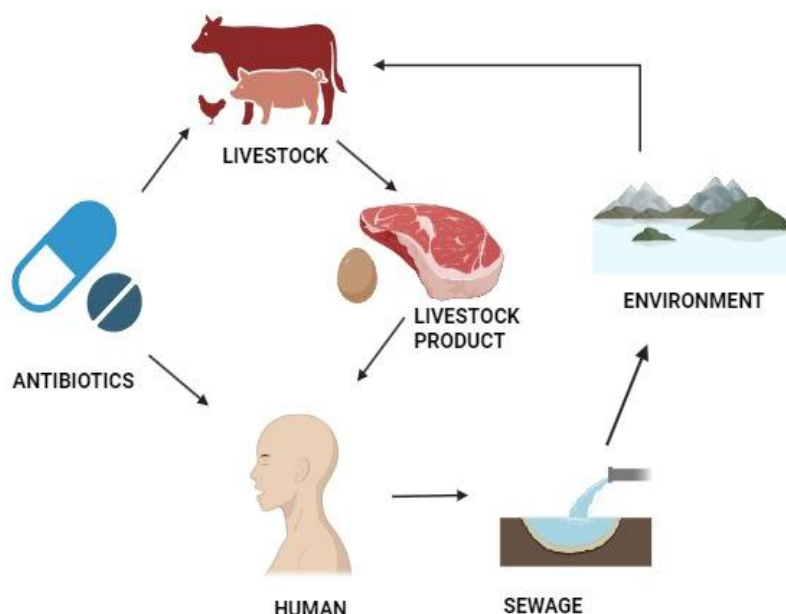


Figure 1: Routes of Transmission of Antimicrobial Resistance, live stocks are given antibiotics to treat infections, antibiotics end up in the livestock products, such as milk and meat. Humans consume these livestock products. The antibiotics in the livestock products can kill beneficial bacteria in the human gut which antibiotic-resistant bacteria to thrive in the human gut. Antibiotic-resistant bacteria is shed in human sewage. The sewage contaminates the environment. The environment can become a reservoir of antibiotic-resistant bacteria.

5. Therapeutic approaches

Novel therapies are needed for the disruption of the biofilm in livestock membrane, different methods like antimicrobial treatment, which include biofilm disruption by bacteriophage, antibiotics-based treatment.

5.1 Antimicrobial Treatment

Antibiotics and anti-microbial work by specifically targeting microorganisms that form biofilms. This breaks down the biofilms that defend these bacteria from current medical treatments like radiation therapy, which is often more efficient against planktonic cells than against those developed in a gel matrix. One of the most essential features for understanding potential pathways for innovative medication creation is efficacy against biofilms. By testing for bacterial resistance, patients can be diagnosed with potential biofilm-caused infections more rapidly and effectively, eliminating the need for empirical therapy-style testing. Medication only gives the germs that are already spreading inside implants like heart valves a short relief before they re-infect. All techniques, including bacteriophage therapy or the use of additional substances, interfere with and disintegrate biofilms that can fend off an antimicrobial treatment (Zia & Alkheraije, 2023).

5.1.1 Biofilm Disruption by Bacteriophage

The subtle process of phage-mediated biofilm disruption makes use of the complex interactions occurring between bacteria and bacteriophages during the development of biofilms (Ferriol-González & Domingo-Calap, 2021). Biofilms are intricate microbial communities made up of bacteria cultivated on an extracellular polymeric substance (EPS) matrix that they have generated on their

own. Viruses designated as phages exclusively infect bacteria. Certain phages have unique properties in the biofilm disruption landscape that make them suitable for this purpose (Elmberg et al., 2017). Several studies show that bacteriophage is commonly used in disrupting biofilm formed by multi-drug resistant bacteria like *P. aeruginosa*. In a study, it was found, that the bacteria that causes persistent mastitis in livestock, *Staphylococcus aureus*, can produce a biofilm that is very difficult to disrupt once it forms (MENZIES & NEILL, 2000). Disinfectants work well against *S. aureus*, although they are chemically hazardous to humans and livestock, destructive to equipment, and easily impacted by environmental conditions (Mingmongkolchai & Panbangred, 2018). The potential use of a bacteriophage as a narrow-spectrum disinfectant against *S. aureus* biofilms was examined in the study. It was concluded in the study that the bacteriophage vB_SauM_SDQ (short for SDQ) significantly reduced the biofilms formed in the polystyrene, milk, and mammary glands was significantly reduced and SDQ shows a constant bacteriostatic effect, further its efficacy can be improved by the addition of a nonionic detergent (Triton X-100) and It mimics the common environmental conditions. The investigation indicated that biofilm infections may be managed with SDQ, a particular lytic *S. aureus* phage (Ndlovu et al., 2023). Engineered bacteriophage binding to the surface of biofilm for biofilm disruption is shown in **Figure 2**.

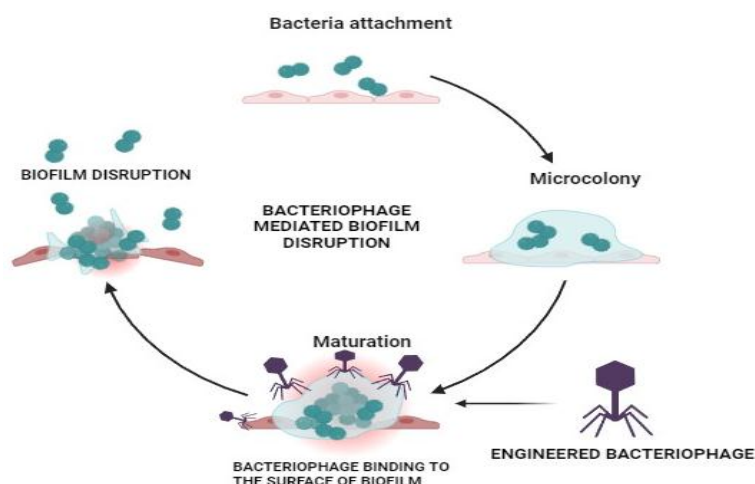


Figure 2: Biofilm Disruption by bacteriophage, firstly free-floating bacteria attaching themselves to a surface, once bacteria adhere to the surface, they begin to multiply and form microcolonies. As the microcolonies mature, they secrete extracellular polymeric substances (EPS). EPS creates a slimy substance that encases the bacteria and forms the biofilm structure. In the end the biofilm structures disrupt the bacteriophages.

Another study reveals that *E. coli* a common pathogen that causes respiratory infection in poultry can be treated with bacteriophage, an alternative to antibiotic treatment (Stecher et al., 2012). The bacteriophage SRP02, when injected directly into the air sac of the poultry shows a decline in the mortality rate, but inoculating it with water does not provide any significant result, (Nocera et al., 2021) The route of administration plays an important role in treating the biofilm-based infection. GIT diseases in poultry are another major problem, in a study revealed

that tail spike proteins (TSP) of P22 bacteriophage can reduce the colonization of *Salmonella* sp., (Brady et al., 2011) as it is one of the predominant pathogens in poultry forming biofilm leading to GIT diseases (Looft & Allen, 2012). P22 TSP shows complete resistance against GI trypsin and minute sensitivity against pepsin and chymotrypsin. Several other bacterial biofilm-mediated infections treated with bacteriophage have been outlined in the **Table 3**.

Table 3: Biofilm-forming bacteria along with their phage strain.

Sl. No	Bacteria forming bacteria	Phage strain	Disease	Reference
01.	Gardenerella	PM-477 (engineered lysin)	bacterial vaginosis	(Smith, 2015)
02.	<i>S. aureus</i>	PlySs2	mastitis in dairy-producing animals, bumblefoot" in chickens	(Smith, 2015)
03.	<i>K. pneumoniae</i>	Prophage/LysECD7	urinary tract infection, nasopharynx	(Effah et al., 2020)
04.	<i>S. aureus</i>	HY-133 (chimeric lysin)	Nasopharynx, pneumonia, or necrotizing fasciitis	(Kaspar et al., 2018; Schleimer et al., 2019)
05.	<i>S. aureus</i>	Bacterial specific phage/CF-301	osteomyelitis, endocarditis, and wound infection.	(H. Liu et al., 2023)
06.	<i>S. aureus</i>	187, bacterial specific phage/ClyF (chimeric lysin)	Sepsis, pulmonary disease	(H. Yang et al., 2017)
08.	<i>A. baumannii</i>	P307 and P307SQ-8C (engineered lysins)	bacteremia, skin infection	(Thandar et al., 2016)
09.	<i>A. baumannii</i>	Prophage/PlyF307	Bacteremia	(Ghose & Euler, 2020)
10.	<i>S. aureus</i>	ClyH (chimeric lysin)	pneumonia, endocarditis, osteomyelitis, meningitis, and bacteremia	(Hong et al., 2021)

5.1.2 Antibiotics-based treatment

Antibiotics are an essential aspect of the multimodal strategy for managing biofilm-mediated infections. High resistance to antimicrobials is a problem with biofilms in livestock, which are complex microbial communities ringed by an extracellular polymeric matrix (Vrancianu et al., 2020). With a focus on the bacterial species involved, several antibiotics are utilized to treat infections caused by biofilms. Antibiotic resistance is

another issue that poses a challenge; to save cattle lives from biofilm-mediated illnesses, cautious usage of antibiotics and combination therapies should be pursued in addition to investigating other therapeutic options like phage therapy (Murugaiyan et al., 2022). It is critical to comprehend how antibiotics function, the reasons behind biofilm's distinctive resistance to these medications, and the host-related variables that contribute to infection when developing workable, long-term treatment plans

for farm animals' wellbeing (Ling et al., 2022). Since chronic biofilm infections are renowned for being challenging to treat with antibiotics, biofilm formation may be the cause of mastitis instances that do not improve with conventional treatments. *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, and *Streptococcus uberis* are the most prevalent infecting agents associated with bovine mastitis (Kabelitz et al., 2021). Since *S. aureus* has a high retention rate and a low bacteriological cure rate in clinical mastitis cases, it is a prevalent and difficult mastitis pathogen (Nale & McEwan, 2023). Due to the high multi-drug resistance of *S. aureus*, it is often challenging to treat with antibiotics (X. Li et al., 2022).

The efficacy of a multicomponent vaccine in conjunction with antibiotic therapy for the eradication of *Staphylococcus aureus* biofilm infections—particularly those associated with methicillin-resistant strains (MRSA)—was investigated by in a study. After diligently choosing antigens that were expressed more prominently and persistently in biofilms. The researchers were able to include those antigens in a vaccination that was given in parallel. Antibiotic therapy was administered concurrently to non-attached planktonic cells (Osman et al., 2023). In comparison to animals treated with vancomycin alone or left untreated, results showed a significant decrease in clinical and radiographic indications of infection in a chronic osteomyelitis biofilm model in rabbits. Furthermore, in comparison to both antibiotic and vaccine monotherapies, the combination of vaccination plus antibiotic treatment showed a considerable clearance of MRSA biofilm infections in the majority of vaccinated animals (Mala et al., 2021).

There are several classes of antibiotics are being used for treating biofilm-based infections in livestock. The most frequently used antibiotics employed in targeting biofilm-based infection in livestock are the broad-spectrum antibiotics i.e., Tetracyclines, such as doxycycline are being used as they have a high efficacy rate against a wide range of bacteria which also includes the biofilm-forming bacteria (Sharun et al., 2021). Another antibiofilm-based antibiotic that is prevalently used is fluoroquinolones, exemplified by enrofloxacin, which are used for treating GIT infections in livestock. Administration of antibiotics should be based on the strain of the bacterial species involved and its sensitivity to the given antibiotic.

5.1.3 Challenges Due to Antibiotic Treatment

We need to consider the short- and long-term effects of administering antibiotics on livestock in farms. One major concern that worries is the development of microorganisms resistant to antibiotics. Livestock raised on farms frequently receive routine or prophylactic antibiotic treatments. This develops persistent bacterial strains. These pathogenic. This can occur from interacting with animals, consuming contaminated

strains are meat, or being in the environment. It's a significant matter for public health as it makes using antibiotics to treat infections more difficult. There may be increased mortality and longer-term illness among the affected population. The frequent use of antibiotics in livestock disturbs the resident microbiota. The animals may become less healthy, grow more slowly, and produce less as a result. Additionally, leftover antibiotics may be present in foods like milk and meat. This is a concern about food safety. Unknowingly consuming these leftovers could have negative health effects which lead to antibiotic resistance in humans. This makes the treatment complicated and traditional antibiotics are avoided due to resistance development. It also complicates the fight against antibiotic resistance worldwide (Royer et al., 2021).

Antibiotic treatment can lead to several other issues in livestock, like antibiotics may lead to an increased abundance of enteric *E. coli* in the gut of the livestock. This leads to severe diarrhea as well as mortality. In a study, a rapid genetic flow between Gram-negative Enterobacteriaceae has been identified using pangenome analysis. Still, little is known about the mechanisms promoting intraenterobacterial horizontal gene transfer, it has also been revealed *Salmonella* sp. induced enteropathy causes concurrent blooms of the pathogen and local commensal *Escherichia coli* using a mouse colitis model (Huemer et al., 2020).

A multifaceted approach is needed to deal with these problems. To eradicate antibiotic resistance certain guidelines need to be established for the use of antibiotics in farm animals, educating veterinarians on responsible prescription practices, and educating farmers on the negative effects of antibiotic overuse. Additionally, funding research into alternative treatments and preventative measures for illnesses in farm animals—such as probiotics and vaccinations—is advantageous (Kirtane et al., 2021). This has the potential to strengthen and extend the life of our farming system. Utilizing a comprehensive global network, we can address the challenging issues associated with the use of antibiotics in farm animals. This will guarantee the continued health of both humans and animals.

5.2 BIOFILM DISRUPTION

The composition of planktonic bacteria and bacteria living in biofilms are different, particularly when it comes to gene transcription and growth rate. Several veterinary diseases have been linked to biofilms, such as horse endometritis, liver abscesses, chronic skin sores, enteritis, and bovine mastitis. Biofilms exhibit intrinsic resistance to antibiotics and innate immune responses, leading to persistent and chronic infections that persist even after extended antibiotic therapy. Numerous nonantibiotic substances have been studied to disrupt biofilm formation, to enhance the effectiveness of concurrently delivered antibiotic therapies.

5.2.1 ENZYMATIC DISRUPTION

Distinct biofilms often need diverse categories of enzymes. Enzymatic compositions include several enzymes or additional antibacterial agents. The heterogeneity of the biofilm arises from the presence of many components contributing to its formation. To address the various compositions of biofilms, it is necessary to utilize a combination of enzymes, known as cocktails. The specific quantity and kind of enzymes must be carefully determined. The EPS matrix holds for about 90% of the overall biofilm. The primary constituents are polysaccharides of the EPS matrix, facilitating microbial adhesion to diverse surfaces. (Mohseni et al., 2023) The function of the enzymes is reduced by the structural integrity of the biofilm via the weakening of several components that make extracellular polymeric substances (EPS). (Versey et al., 2021) To achieve effective eradication of biofilm, it is important to first identify the structural components of EPS before applying the enzymes. EPS consists of several structural elements mostly produced from carbohydrates, lipids, nucleic acid, glycoproteins, glycolipids, and polysaccharides, among other substances. The enzymes are specifically applied to the EPS molecules to convert them into smaller units. The cell is to transfer these smaller components across its membranes and metabolize them. (Nandhini et al., 2022) For biofilm elimination, four categories of enzymes are used, proteolytic enzymes, polysaccharide-degrading enzymes, oxidative enzymes, and anti-quorum sensing (QS) enzymes (Sadeghzadeh et al., 2024). Proteases are enzymes that catalyze the hydrolysis of proteins. Several enzymes are coupled to enhance the efficiency in controlling biofilms. The extracellular polymeric substances (EPS) vary and are diverse from one biofilm to another. (França et al., 2021) Indicates, carbohydrates are the primary components of the EPS, while other studies emphasize the prevalence of proteins. Depending on the type of microbes that make up the biofilm, the quantity, shape, and various components of EPS might vary (Khan, Pham, Oloketuyi, Manivasagan, et al., 2020). It is necessary to apply several enzymes or treatments in conjunction with one another to eradicate a particular biofilm. Enzymes which is genetically engineered are being considered as the next big thing in enzyme-based biofilm management for optimum disruption of biofilm. Proteases, carbohydrases, oxidoreductases, anti-quorum sensing enzymes, and bacteriophage lysins are commonly seen in microbes during disruption (Thallinger et al., 2013).

5.2.2 DISPERSAL AGENTS

Systemic infections start with the biofilm-dispersal process, which is released when the bacteria have already entered the body. Several naturally occurring and synthesized proteins and peptides, including those derived from FK13, ApoB, and EntV (68), affect the suppression and dispersion of biofilms, with anti-inflammatory properties. (Cao et al., 2020)Reportedly, a marine strain of *Bacillus licheniformis* has an

extracellular protein that may dissolve biofilm. Bacteria that live in marine environments and are known as epibionts create chemicals that kill microbes in biofilms. Antibacterial activities of the isolated protein BL-DZL are evaluated from *Bacillus licheniformis* was conducted. Inhibitory concentration is the lowest and disruption of biofilms of several microbial taxa was used as a metric (Szczepanski & Lipski, 2014). Biofilms of common bacteria and fungal pathogens, such as *Candida albicans*, *Pseudomonas aeruginosa*, and *Bacillus pumilus*, were shown to be active against BL-DZ1. The production of the biofilm was reduced and preexisting biofilms were dispersed by BL-DZ1, in addition to inhibiting microbial growth. Efficacy exhibited against bacterial biofilms is shown by the antimicrobial peptides (AMPs), particularly lytic peptides. Lytic peptides attach to negative charge lipopolysaccharides and stimulate the openings in the outer membrane. (Parai et al., 2020) There is a loss of the integrity of the cell membrane, which ultimately disintegrates the cell. The antibacterial properties of cathelicidin lytic antimicrobial peptides (AMPs), obtained from cattle, and humans were compared to the efficacy of antibiotics often used to treat biofilms in lungs afflicted by cystic fibrosis, such as tobramycin. BMAP-27, BMAP-28, and SMAP-29 are the peptides that show the most efficacy against CF biofilms both in in-vivo and in vitro (Werkneh et al., 2023). This happens due to alpha-helical structure and positive charge, which lead to the rupture of the negatively charged membrane of *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa*, and *S. aureus* (X. Li et al., 2021) which leads to cell death and frees the resident live bacteria, dispersing bacteria can reinstate the process of biofilm formation when favorable environment is obtained (Kostakioti et al., 2013).

5.2.3 ANTI-BIOFILM COATING

Ag (silver) has a strong antibacterial character which led to its exploration as an antibiofilm coating material on implants in various investigations (de Barros et al., 2020). (Tong et al., 2020) Nanoscale layers of AgO were performed in antibacterial studies on *S. aureus* and *E. coli* utilizing titanium foil discs. (Fernández-Gómez et al., 2022) discovered that Ag⁺ ions have reduced in the inhibitory zone in the culture, suggesting that ions were the primary factor. (El-Kafrawy et al., 2023) Successfully attached silver nanoparticles to a self-assembled monolayer of polyethyleneimine, which results in an effective antibacterial effect against *E. coli* and *S. aureus*. A significant obstacle remains in the practical use of nanosilver for medical implants as a coating, namely the limited comprehension of the underlying physicochemical characteristics that contribute to its effectiveness in preventing biofilm formation (Mishra et al., 2020). The absolute dimension of nanosilver, coating thickness, and the major mechanism driving the antibiofilm activity of nanosilver coatings are still uncertain. (J. Liu, n.d.) The activity is mostly due to the release of Ag⁺ ions upon exposure to fluids or the direct interaction of bacteria with the

nanoparticles. It is necessary to perform a methodical examination of these parameters and their influence on the prevention of biofilm generation (Adegbeye *et al.*, 2021). The discharge of Ag⁺ ions occurs across the whole film, which impacts the antibiofilm action (Wollanke *et al.*, 2022). The wideness of the coating is precisely adjusted by regulating the interval of deposition when the nanoparticles are assembled on the surfaces via aerosol self-assembly. The activity of anti-biofilm is

influenced by the thickness of the material. (Pires *et al.*, 2020) It indicates that there is a certain small quantity of nanosilver and released Ag⁺ ions needed to effectively reduce biofilm formation. The coatings that had the largest release of Ag⁺ ions had the most powerful antibiofilm activity, which suggests that the activity against biofilms is primarily driven by the presence of Ag⁺ ions in nanosilver (Dutt *et al.*, 2022).

Silver nanoparticle disrupting the biofilm on livestock membrane

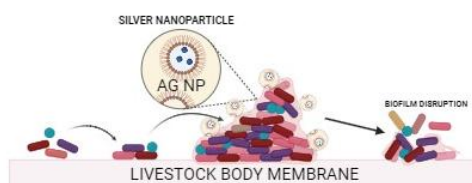


Figure 3: Nanoscale layer of Ag disrupting the biofilm on livestock membrane, Ag has antimicrobial properties, silver nanoparticles bind to the surface of the livestock body membrane and causing biofilm disruption.

5.3 IMMUNOTHERAPY

The therapy which blocks the body's early and late reactions to allergens is called immunotherapy. There is an increase in allergen-specific IgG, especially the IgG4 isotype, during immunotherapy. It happens because IgE is responsible for both the basophils' histamine release and the antigen presentation to T cells. Immunotherapy aims to alter T cell responses by promoting TH1 rather than TH2 reactions to allergens in the body's mucosa and peripheral tissues. Microbeads carrying immunological molecules are a novel immunotherapeutic strategy that has just been formed to augment traditional medical therapy.

5.3.1 VACCINES TARGETING BIOFILM-FORMING PATHOGENS

Vaccines are produced using bacteria cultivated as planktonic cells. These vaccinations may not provide complete protection against diseases that develop biofilms during infection. Biofilms play a major role in the development of many long-lasting diseases. They enhance antibiotic resistance and pathogenicity and also modify metabolic activity and protein profiles. The association of bacterial cells within the biofilm and the polysaccharide, extracellular protein, and DNA biofilm matrix are formed by the bacteria in a biofilm. The bacteria export and encapsulate antigens are identified in the extracellular matrix, and the antigens linked with biofilm cells remain within the bacterial cell. (Atshan *et al.*, 2023) The identification of antigens generated during biofilm formation by both bacterial cells and biofilm matrix has been an area of attention in the hunt to develop vaccines against important veterinarian infections. Metabolically active cells are needed for antibiotic treatment and biofilm active dispersion mechanisms, data suggests that persists, or dormant cells, inside biofilms, are crucial for drug tolerance. Treating method of biofilm that does not depend on the

presence of microbes is antimicrobial peptides (AMPs). A major component of the body's natural defense against pathogens is AMPs (Mohamed *et al.*, 2021). AMPs contain anti-biofilm properties and are referred to as "anti-biofilm peptides". (B & A, 2021) Human peptide LL-37 is the first documented anti-biofilm peptide. It may effectively hinder and reduce *P. aeruginosa* biofilms even at concentrations far less than those required for antimicrobial activity. An important benefit of AMPs is their extensive conservation, making them appealing as broad-spectrum antimicrobial treatments capable of targeting both bacterial and fungal biofilms. The combination of antibiotics with synthetic peptides can alter certain AMP sequences which shows inhibitory action and improved biofilm destruction of *Pseudomonas aeruginosa* in invertebrate infection animals.

5.3.2 IMMUNOMODULATORY APPROACHES

Bacteria produce bacteriocins which are peptides that can suppress the growth of other microbes, fungi, viruses, and parasites. Lactic acid bacteria (LAB) produce bacteriocins which may function as a substitute for antibiotics and are used to curb the development of bacteria resistant to them. Antimicrobial and probiotic applications of LAB and bacteriocins have been explored in the pharmaceutical and animal health fields. Bacteriocins' immunomodulatory impact is another important function (Stachelek *et al.*, 2021). Enzymatic degradation prevention and avoiding different dosages and high concentrations of bacteriocins may be attained by protecting them in capsules or nanocapsules. Bacteriocins in the gut are to use probiotics that produce LAB. Bacteriocins may be generated locally, and this tactic promotes bacterial colonization of the GI tract. The immune system can be stimulated, the intestinal barrier can be improved, digestion can be enhanced, and the microbiota may be modulated. The prevention of enteric pathogens can be done by colonizing the body, in a few

animals use of bacteriocin-producing LAB probiotics requires more research, also several animal studies have shown that certain lactic acid bacteria (LAB) found in the gastrointestinal tract may prevent villous atrophy during the post-weaning period, which facilitates the development of gastrointestinal lymphoid tissue, and exhibit immunomodulatory effects. (Uddin et al., 2021) that bacteriocins are responsible for *L. salivarius* B1's immunomodulatory activities. Bacteriocins' immunomodulatory impact is still not completely elucidated (Hancock et al., 2021). It is well-established that the concentration of bacteriocin employed decides the degree of immune system regulation. Their bacteriocin-induced immune response activation pathways contribute to the enhanced host defense, especially in the face of infections, due to their antibacterial activity (Algharib et al., 2020).

5.4 Probiotics and prebiotics in livestock

Prebiotics and probiotics are highly essential to a healthy digestive system as well as the entire body's well-being in the livestock. Probiotics refer to the live microorganisms including bacteria which are beneficial for health by maintaining a microflora in the gut (Barzegari et al., 2020). These microorganisms mostly found in fermented foods like yogurt, sauerkraut, and kimchi help in keeping a balanced population of gut flora, hence improving digestion and immunity (Tshibangu-Kabamba & Yamaoka, 2021). On the other hand, prebiotics are non-digestible fibers that provide nutrients for these types of bacteria. These non-digestible carbohydrates in some foods such as bananas, garlic, and onions promote the growth and activity of probiotic organisms forming a symbiotic relationship. Thus, they work together to create an optimal gut environment that improves nutrient uptake, and enhances immune response while possibly preventing some gastrointestinal disorders. Probiotics and prebiotics have a synergistic effect by improving the gut microflora in livestock (D. R. Silva et al., 2020).

Probiotics lead to dysbiosis which is the elimination of the pathogenic microorganism. Due to the ban on antibiotic promoters in the livestock sectors, as a feed supplement probiotics are widely used. Strains of *Bacillus* sp. are the most evident, due to its sporulation ability and other traits which a probiotic bacterial strain must have. Probiotics are considered as an alternative to the antibiotics. The animal's metabolism and altered intestinal function can be influenced by sub-therapeutic antibiotics. There are several therapeutic uses of probiotics in the livestock sector. The impact of probiotic food supplementation (Lacto-Sacc from Alltech) on broiler chicken growth was studied by Singh et al. (2009). *Streptococcus faecium* and *Lactobacillus acidophilus* were present in the live yeast culture supplement. The findings suggested that broiler growth performance was positively impacted by supplementation at various levels, with the most benefit occurring at a level of 0.025%. With a weight gain of

3.28–4.03%, broilers outperformed the control group in terms of body weight gain, feed intake efficiency, protein efficiency ratio, and performance index (Lin et al., 2023).

In a study conducted by (Dutt et al., 2022) probiotics have demonstrated intriguing potential when employed as animal feed, especially for cattle, pigs, and poultry. Lactic acid bacteria are often employed in probiotic preparations for non-ruminants, but *Saccharomyces cerevisiae*, also known as yeast culture or YC, has been demonstrated to be a promising probiotic culture for ruminants, and consequently for microbial populations, microbial protein synthesis, and the production of volatile fatty acids (VFAs). YC's impact on animal productivity differs based on the particular technique employed. Studies indicate that introducing *Enterococcus faecium* strains to dairy cattle diets enhances the production of milk. This effect is not due to body stores building up, but rather to the mammary gland receiving more nutrients. However, probiotic consumption as well as processing specificity certainly play a role.

DISCUSSION

Livestock infection is a threat to both humans and animals, it is a challenge to eradicate them and it requires novel and effective therapeutic strategies. As we have discussed biofilm infection shows resistance to conventional treatment so developing an effective treatment against them is crucial to ensure animal health and prevent economic losses in the agricultural setting. The infected animals can spread the infection to humans which is referred to as zoonotic disease. The conventional treatments do not have much efficiency against this biofilm-based infection due to the protective matrix the biofilm has to resist the treatment. Several new generation techniques have been discussed in this chapter like phage therapy, antimicrobial peptides, and combinational therapies are used as therapeutics. It is better to prevent biofilm infection than to treat them. Good hygiene practices should be implemented to minimise the favorable conditions for biofilm formation along with this biosecurity measures should also be followed. It is highly recommended to prevent the initial attachment, which subsequently progresses to biofilm formation.

Probiotics are the best possible approach to treat the biofilm-based infection as we have discussed, it is safe and convenient to use. As in this paper along with Lambo, Modinat Tolani, Xiaofeng Chang, and Dasen Liu. "The recent trend in the use of multistrain probiotics in livestock production: an overview." *Animals* 11.10 (2021): 2805. Has demonstrated how probiotics can help treat the biofilm infection as it has antimicrobial properties, but they do not allow the colonization of the pathogen. Probiotics contribute to a more healthier and resilient environment within the host. Additionally, strategies to strengthen the host's immune response to infections linked with biofilms include immunomodulation and immunization. It may be

possible to lower infection rates and enhance the overall well-being of livestock by creating vaccinations that specifically target antigens expressed during biofilm development.

Despite being optimistic strategies, still hurdles are there to translate the lab result into the agricultural setting. Due to the dynamic character of the agricultural system and the heterogeneity of the biofilm, the approaches are not highly successful in the farms. To treat biofilm-based infection in livestock it needs to be ensured the approach should be helpful in the agricultural setting as well.

FUTURE PROSPECT

Biofilm associated infections threaten cattle outcome, creating animal welfare issues. Targeted antimicrobials can cease biofilms or kill entrenched bacteria, minimizing broad-spectrum antibiotic use and resistance. Combination of antibiotics with biofilm-degrading enzymes, phage therapy, or immunomodulators increases biofilm treatment. Nanoparticles may transport medications directly to biofilms, enhancing potency and lowering systemic exposure. Animal biofilm associated infections defense can be improved by vaccinations or immunostimulants. Beneficial microorganisms or bacteriophages can cease biofilms or kill pathogens. Biofilm associated infections rapid detection and identification methods allow timely treatments. Successful immunization regimens lower bacterial load and biofilm development. Optimizing animal nutrition boosts immunity and infection resistance.

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CONFLICT OF INTEREST

There is no conflict of interest among the author.

DATA AVAILABILITY

All data generated during this study are available through a reasonable request to the corresponding authors.

AUTHOR'S CONTRIBUTION

The authors of the manuscript have contributed in various sections. Risav Banerjee has designed the work, scripted the manuscript. Trisha Bhattacharya has compiled the data, executed and proof read the manuscript.

REFERENCES

1. AbduLLahi, U. F., Igwenagu, E., Mu'azu, A., Aliyu, S., & Umar, M. I. (2016). Intrigues of biofilm: A perspective in veterinary medicine. *Veterinary world*, 9(1): 12.
2. Algharib, S. A., Dawood, A., & Xie, S. (2020). Nanoparticles for treatment of bovine *Staphylococcus aureus* mastitis. *Drug delivery*, 27(1): 292-308.
3. Ashraf, A., & Imran, M. (2020). Causes, types, etiological agents, prevalence, diagnosis, treatment, prevention, effects on human health and future aspects of bovine mastitis. *Animal health research reviews*, 21(1): 36-49.
4. Atshan, S. S., Hamat, R. A., Aljaberi, M. A., Chen, J. S., Huang, S. W., Lin, C. Y., & Kicic, A. (2023). Phage Therapy as an Alternative Treatment Modality for Resistant *Staphylococcus aureus* Infections. *Antibiotics*, 12(2): 286.
5. Avershina, E., Shapovalova, V., & Shipulin, G. (2021). Fighting antibiotic resistance in hospital-acquired infections: current state and emerging technologies in disease prevention, diagnostics and therapy. *Frontiers in microbiology*, 12: 707330.
6. Barzegari, A., Kheyrolahzadeh, K., Hosseiniyan Khatibi, S. M., Sharifi, S., Memar, M. Y., & Zununi Vahed, S. (2020). The battle of probiotics and their derivatives against biofilms. *Infection and drug resistance*, 659-672.
7. Becker, K., Both, A., Weißelberg, S., Heilmann, C., & Rohde, H. (2020). Emergence of coagulase-negative staphylococci. Expert review of anti-infective therapy, 18(4): 349-366.
8. Brady, R. A., O'May, G. A., Leid, J. G., Prior, M. L., Costerton, J. W., & Shirliff, M. E. (2011). Resolution of *Staphylococcus aureus* biofilm infection using vaccination and antibiotic treatment. *Infection and immunity*, 79(4): 1797-1803.
9. Cao, C., Ge, W., Yin, J., Yang, D., Wang, W., Song, X., & Dong, X. (2020). Mesoporous silica supported silver-bismuth nanoparticles as photothermal agents for skin infection synergistic antibacterial therapy. *Small*, 16(24): 2000436.
10. Cheung, G. Y., Bae, J. S., & Otto, M. (2021). Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence*, 12(1): 547-569.
11. Chlebicz, A., & Śliżewska, K. (2018). Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne diseases: a review. *International journal of environmental research and public health*, 15(5): 863.
12. de Barros, P. P., Rossoni, R. D., de Souza, C. M., Scorzoni, L., Fenley, J. D. C., & Junqueira, J. C. (2020). *Candida* biofilms: an update on developmental mechanisms and therapeutic challenges. *Mycopathologia*, 185(3): 415-424.
13. De Silva, L. A. D. S., & Heo, G. J. (2023). Biofilm formation of pathogenic bacteria isolated from aquatic animals. *Archives of Microbiology*, 205(1): 36.
14. Depypere, M., Morgenstern, M., Kuehl, R., Senneville, E., Moriarty, T. F., Obremskey, W. T., & Metsemakers, W. J. (2020). Pathogenesis and management of fracture-related infection. *Clinical Microbiology and Infection*, 26(5): 572-578.
15. Dos Santos, G. M. P., Borba-Santos, L. P., Vila, T., Ferreira Gremião, I. D., Pereira, S. A., De Souza, W., & Rozental, S. (2022). *Sporothrix* spp. biofilms

- impact in the zoonotic transmission route: Feline claws associated biofilms, itraconazole tolerance, and potential repurposing for miltefosine. *Pathogens*, 11(2): 206.
16. Dutt, Y., Dhiman, R., Singh, T., Vibhuti, A., Gupta, A., Pandey, R. P., & Priyadarshini, A. (2022). The association between biofilm formation and antimicrobial resistance with possible ingenious bio-remedial approaches. *Antibiotics*, 11(7): 930.
 17. Effah, C. Y., Sun, T., Liu, S., & Wu, Y. (2020). *Klebsiella pneumoniae*: an increasing threat to public health. *Annals of clinical microbiology and antimicrobials*, 19(1): 1-9.
 18. Eid, S., Tolba, H. M., Hamed, R. I., & Al-Atfeehy, N. M. (2022). Bacteriophage therapy as an alternative biocontrol against emerging multidrug resistant *E. coli* in broilers. *Saudi Journal of Biological Sciences*, 29(5): 3380-3389.
 19. El-Kafrawy, S. A., Abbas, A. T., Oelkrug, C., Tahoon, M., Ezzat, S., Zumla, A., & Azhar, E. I. (2023). IgY antibodies: The promising potential to overcome antibiotic resistance. *Frontiers in Immunology*, 14: 1065353.
 20. Elmberg, J., Berg, C., Lerner, H., Waldenström, J., & Hessel, R. (2017). Potential disease transmission from wild geese and swans to livestock, poultry and humans: a review of the scientific literature from a One Health perspective. *Infection ecology & epidemiology*, 7(1): 1300450.
 21. Fan, Q., Zuo, J., Wang, H., Grenier, D., Yi, L., & Wang, Y. (2022). Contribution of quorum sensing to virulence and antibiotic resistance in zoonotic bacteria. *Biotechnology Advances*, 59: 107965.
 22. Ferriol-González, C., & Domingo-Calap, P. (2020). Phages for biofilm removal. *Antibiotics*, 9(5): 268.
 23. Ferriol-González, C., & Domingo-Calap, P. (2021). Phage therapy in livestock and companion animals. *Antibiotics*, 10(5): 559.
 24. França, A., Gaio, V., Lopes, N., & Melo, L. D. (2021). Virulence factors in coagulase-negative staphylococci. *Pathogens*, 10(2): 170.
 25. Gao, Y., Wang, J., Chai, M., Li, X., Deng, Y., Jin, Q., & Ji, J. (2020). Size and charge adaptive clustered nanoparticles targeting the biofilm microenvironment for chronic lung infection management. *ACS nano*, 14(5): 5686-5699.
 26. Garcia, A. B., & Percival, S. L. (2011). Zoonotic infections: the role of biofilms. *Biofilms and Veterinary Medicine*, 6: 69.
 27. García, A. B., & Percival, S. L. (2011). Zoonotic infections: the role of biofilms. In *Biofilms and veterinary medicine* (pp. 69-110). Berlin, Heidelberg: Springer Berlin Heidelberg.
 28. Gharieb, R. M. A., Saad, M. F., Mohamed, A. S., & Tartor, Y. H. (2020). Characterization of two novel lytic bacteriophages for reducing biofilms of zoonotic multidrug-resistant *Staphylococcus aureus* and controlling their growth in milk. *LWT*, 124: 109145.
 29. Ghose, C., & Euler, C. W. (2020). Gram-negative bacterial lysins. *Antibiotics*, 9(2): 74.
 30. Giaouris, E. E., & Simões, M. V. (2018). Pathogenic biofilm formation in the food industry and alternative control strategies. In *Foodborne diseases* (pp. 309-377). Academic Press.
 31. Gildea, L., Ayariga, J. A., & Robertson, B. K. (2022). Bacteriophages as biocontrol agents in livestock food production. *Microorganisms*, 10(11): 2126.
 32. González-Martín, M., Silva, V., Poeta, P., Corbera, J. A., & Tejedor-Junco, M. T. (2022). Microbiological aspects of osteomyelitis in veterinary medicine: drawing parallels to the infection in human medicine. *Veterinary Quarterly*, 42(1): 1-11.
 33. Guéneau, V., Plateau-Gonthier, J., Arnaud, L., Piard, J. C., Castex, M., & Briandet, R. (2022). Positive biofilms to guide surface microbial ecology in livestock buildings. *Biofilm*, 4: 100075.
 34. Guo, Y., Song, G., Sun, M., Wang, J., & Wang, Y. (2020). Prevalence and therapies of antibiotic-resistance in *Staphylococcus aureus*. *Frontiers in cellular and infection microbiology*, 10: 107.
 35. Hancock, R. E., Alford, M. A., & Haney, E. F. (2021). Antibiofilm activity of host defence peptides: Complexity provides opportunities. *Nature Reviews Microbiology*, 19(12): 786-797.
 36. Horiuk, Y., Kukhtyn, M., Kovalenko, V., Kornienko, L., Horiuk, V., & Liniichuk, N. (2019). Biofilm formation in bovine mastitis pathogens and the effect on them of antimicrobial drugs. *Independent Journal of Management & Production*, 10(7): 897-910.
 37. Hotinger, J. A., Morris, S. T., & May, A. E. (2021). The case against antibiotics and for anti-virulence therapeutics. *Microorganisms*, 9(10): 2049.
 38. Hou, Z., Liu, L., Wei, J., & Xu, B. (2023). Progress in the Prevalence, Classification and Drug Resistance Mechanisms of Methicillin-Resistant *Staphylococcus aureus*. *Infection and Drug Resistance*, 3271-3292.
 39. Howden, B. P., Giulieri, S. G., Wong Fok Lung, T., Baines, S. L., Sharkey, L. K., Lee, J. Y., & Stinear, T. P. (2023). *Staphylococcus aureus* host interactions and adaptation. *Nature Reviews Microbiology*, 1-16.
 40. Huemer, M., Mairpady Shambat, S., Brugger, S. D., & Zinkernagel, A. S. (2020). Antibiotic resistance and persistence—Implications for human health and treatment perspectives. *EMBO reports*, 21(12): e51034.
 41. Jacques, M., Aragon, V., & Tremblay, Y. D. (2010). Biofilm formation in bacterial pathogens of veterinary importance. *Animal Health Research Reviews*, 11(2): 97-121.
 42. Kabelitz, T., Aubry, E., van Vorst, K., Amon, T., & Fulde, M. (2021). The role of *Streptococcus* spp. in bovine mastitis. *Microorganisms*, 9(7): 1497.
 43. Karygianni, L., Ren, Z., Koo, H., & Thurnheer, T. (2020). Biofilm matrixome: extracellular components in structured microbial

- communities. Trends in microbiology, 28(8): 668-681.
44. Kaspar, U., de Haro Sautto, J. A., Molinaro, S., Peters, G., Idelevich, E. A., & Becker, K. (2018). The novel phage-derived antimicrobial agent HY-133 Is active against livestock-associated methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, 62(7): 10-1128.
 45. Katrin Schilcher., Alexander R. Horswill. (2020). Staphylococcal Biofilm Development: Structure, Regulation, and Treatment Strategies. *ASM Journals Microbiology and Molecular Biology Reviews*, 84(3).
 46. Khan, F., Pham, D. T. N., Oloketuyi, S. F., Manivasagan, P., Oh, J., & Kim, Y. M. (2020). Chitosan and their derivatives: Antibiofilm drugs against pathogenic bacteria. *Colloids and Surfaces B: Biointerfaces*, 185: 110627.
 47. Khan, F., Pham, D. T., Oloketuyi, S. F., & Kim, Y. M. (2020). Antibiotics application strategies to control biofilm formation in pathogenic bacteria. *Current pharmaceutical biotechnology*, 21(4): 270-286.
 48. Kirtane, A. R., Verma, M., Karandikar, P., Furin, J., Langer, R., & Traverso, G. (2021). Nanotechnology approaches for global infectious diseases. *Nature Nanotechnology*, 16(4): 369-384.
 49. Komodromos, D., Kotzamanidis, C., Giantzi, V., Angelidis, A. S., Zdragas, A., & Sergelidis, D. (2022). Prevalence and biofilm-formation ability of *Staphylococcus aureus* isolated from livestock, carcasses, the environment, and workers of three abattoirs in Greece. *Journal of the Hellenic Veterinary Medical Society*, 73(2): 4097-4104.
 50. Koo, H., Allan, R. N., Howlin, R. P., Stoodley, P., & Hall-Stoodley, L. (2017). Targeting microbial biofilms: current and prospective therapeutic strategies. *Nature Reviews Microbiology*, 15(12): 740-755.
 51. Lemos, J. A., Palmer, S. R., Zeng, L., Wen, Z. T., Kajfasz, J. K., Freires, I. A., ... & Brady, L. J. (2019). The biology of *Streptococcus mutans*. *Microbiology spectrum*, 7(1): 10-1128.
 52. Li, P., Yin, R., Cheng, J., & Lin, J. (2023). Bacterial biofilm formation on biomaterials and approaches to its treatment and prevention. *International Journal of Molecular Sciences*, 24(14): 11680.
 53. Li, X., Chen, Y., Wang, S., Duan, X., Zhang, F., Guo, A., ... & Qian, P. (2022). Exploring the benefits of metal ions in phage cocktail for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infection. *Infection and Drug Resistance*, 2689-2702.
 54. Li, X., Wang, S., Nyaruaba, R., Liu, H., Yang, H., & Wei, H. (2021). A highly active chimeric lysin with a calcium-enhanced bactericidal activity against *Staphylococcus aureus* in vitro and in vivo. *Antibiotics*, 10(4): 461.
 55. Lieke, T., Meinelt, T., Hoseinifar, S. H., Pan, B., Straus, D. L., & Steinberg, C. E. (2020). Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. *Reviews in Aquaculture*, 12(2): 943-965.
 56. Lin, W. C., Hsu, K. C., You, M. F., Lee, K. H., Chi, C. H., & Chen, J. Y. (2023). Octanoic acid promotes clearance of antibiotic-tolerant cells and eradicates biofilms of *Staphylococcus aureus* isolated from recurrent bovine mastitis. *Biofilm*, 6: 100149.
 57. Ling, H., Lou, X., Luo, Q., He, Z., Sun, M., & Sun, J. (2022). Recent advances in bacteriophage-based therapeutics: Insight into the post-antibiotic era. *Acta Pharmaceutica Sinica B*, 12(12): 4348-4364.
 58. Liu, H., Hu, Z., Li, M., Yang, Y., Lu, S., & Rao, X. (2023). Therapeutic potential of bacteriophage endolysins for infections caused by Gram-positive bacteria. *Journal of Biomedical Science*, 30(1): 29.
 59. Liu, X., Yao, H., Zhao, X., & Ge, C. (2023). Biofilm Formation and Control of Foodborne Pathogenic Bacteria. *Molecules*, 28(6): 2432.
 60. Liu, X., Yao, H., Zhao, X., & Ge, C. (2023). Biofilm Formation and Control of Foodborne Pathogenic Bacteria. *Molecules*, 28(6): 2432.
 61. Looft, T., & Allen, H. K. (2012). Collateral effects of antibiotics on mammalian gut microbiomes. *Gut microbes*, 3(5): 463-467.
 62. Luong, T., Salabarria, A. C., & Roach, D. R. (2020). Phage therapy in the resistance era: where do we stand and where are we going?. *Clinical therapeutics*, 42(9): 1659-1680.
 63. Ma, Y. X., Wang, C. Y., Li, Y. Y., Li, J., Wan, Q. Q., Chen, J. H., ... & Niu, L. N. (2020). Considerations and caveats in combating ESKAPE pathogens against nosocomial infections. *Advanced Science*, 7(1): 1901872.
 64. MacKenzie, K. D., Palmer, M. B., Köster, W. L., & White, A. P. (2017). Examining the link between biofilm formation and the ability of pathogenic *Salmonella* strains to colonize multiple host species. *Frontiers in veterinary science*, 4: 138.
 65. Makabenta, J. M. V., Nabawy, A., Li, C. H., Schmidt-Malan, S., Patel, R., & Rotello, V. M. (2021). Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. *Nature Reviews Microbiology*, 19(1): 23-36.
 66. Mala, L., Lalouckova, K., & Skrivanova, E. (2021). Bacterial skin infections in livestock and plant-based alternatives to their antibiotic treatment. *Animals*, 11(8): 2473.
 67. Menzies, F. D., & Neill, S. D. (2000). Cattle-to-cattle transmission of bovine tuberculosis. *The Veterinary Journal*, 160(2): 92-106.
 68. 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.
69. Mingmongkolchai, S., & Panbangred, W. (2018). *Bacillus* probiotics: an alternative to antibiotics for livestock production. *Journal of applied microbiology*, 124(6): 1334-1346.
 70. Mishra, R., Panda, A. K., De Mandal, S., Shakeel, M., Bisht, S. S., & Khan, J. (2020). Natural anti-biofilm agents: strategies to control biofilm-forming pathogens. *Frontiers in microbiology*, 11, 566325.
 71. Mohamed, H., Nayak, G., Rendine, N., Wigdahl, B., Krebs, F. C., Bruggeman, P. J., & Miller, V. (2021). Non-thermal plasma as a novel strategy for treating or preventing viral infection and associated disease. *Frontiers in Physics*, 9: 683118.
 72. Mohan, A., Nisha, A. R., Sujith, S., Rani, S. S., & Thomas, N. (2022). Antibiofilm activity of berberine and capsaicin in combination with quinolones against *Staphylococcus aureus* from bovine mastitis. *J. Vet. Anim. Sci*, 53(2): 253-261.
 73. Mohseni, P., Ghorbani, A., & Fariborzi, N. (2023). Exploring the potential of cold plasma therapy in treating bacterial infections in veterinary medicine: Opportunities and challenges. *Frontiers in Veterinary Science*, 10.
 74. Mourenza, Á., Gil, J. A., Mateos, L. M., & Letek, M. (2020). Alternative anti-infective treatments to traditional antibiotherapy against staphylococcal veterinary pathogens. *Antibiotics*, 9(10): 702.
 75. Mousavi, S. M., Babakhani, S., Moradi, L., Karami, S., Shahbandeh, M., Mirshekar, M., & Moghadam, M. T. (2021). Bacteriophage as a novel therapeutic weapon for killing colistin-resistant multi-drug-resistant and extensively drug-resistant gram-negative bacteria. *Current microbiology*, 1-14.
 76. Murugaiyan, J., Kumar, P. A., Rao, G. S., Iskandar, K., Hawser, S., Hays, J. P., ... & van Dongen, M. B. (2022). Progress in alternative strategies to combat antimicrobial resistance: Focus on antibiotics. *Antibiotics*, 11(2): 200.
 77. Nale, J. Y., & McEwan, N. R. (2023). Bacteriophage Therapy to Control Bovine Mastitis: A Review. *Antibiotics*, 12(8): 1307.
 78. Nandhini, P., Kumar, P., Mickymaray, S., Alothaim, A. S., Somasundaram, J., & Rajan, M. (2022). Recent developments in methicillin-resistant *Staphylococcus aureus* (MRSA) treatment: A review. *Antibiotics*, 11(5): 606.
 79. Nawaz, S., Khan, M. M., Noack, J., Awan, A. B., Schiebel, J., Roggenbuck, D., & Ali, A. (2020). Rapid detection of biofilm formation by zoonotic serovars of *Salmonella enterica* and avian pathogenic *E. coli* isolates from poultry. *Biofilms*.
 80. Ndlovu, T., Kgosietsile, L., Motshwarakgole, P., & Ndlovu, S. I. (2023). Evaluation of Potential Factors Influencing the Dissemination of Multidrug-Resistant *Klebsiella pneumoniae* and Alternative Treatment Strategies. *Tropical Medicine and Infectious Disease*, 8(8): 381.
 81. 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.
 82. Nie, D., Hu, Y., Chen, Z., Li, M., Hou, Z., Luo, X., ... & Xue, X. (2020). Outer membrane protein A (OmpA) as a potential therapeutic target for *Acinetobacter baumannii* infection. *Journal of biomedical science*, 27: 1-8.
 83. Nocera, F. P., Attili, A. R., & De Martino, L. (2021). *Acinetobacter baumannii*: its clinical significance in human and veterinary medicine. *Pathogens*, 10(2): 127.
 84. Olivares, E., Badel-Berchoux, S., Provot, C., Prévost, G., Bernardi, T., & Jehl, F. (2020). Clinical impact of antibiotics for the treatment of *Pseudomonas aeruginosa* biofilm infections. *Frontiers in microbiology*, 2894.
 85. Olson, M. E., Ceri, H., Morck, D. W., Buret, A. G., & Read, R. R. (2002). Biofilm bacteria: formation and comparative susceptibility to antibiotics. *Canadian journal of veterinary research*, 66(2): 86.
 86. Omoigberale, M. (2021). Evaluating the impact of alternative antimicrobials on biofilms formed by *Clostridium perfringens* (Doctoral dissertation, University of Lincoln).
 87. Onsea, J., Wagemans, J., Pirnay, J. P., Di Lucas, M., Gonzalez-Moreno, M., Lavigne, R., & Metsemakers, W. J. (2020). Bacteriophage therapy as a treatment strategy for orthopaedic-device-related infections: where do we stand?. *European Cells & Materials*, 39: 193-210.
 88. Osman, A. H., Kotey, F. C., Odoom, A., Darkwah, S., Yeboah, R. K., Dayie, N. T., & Donkor, E. S. (2023). The potential of bacteriophage-antibiotic combination therapy in treating infections with multidrug-resistant bacteria. *Antibiotics*, 12(8): 1329.
 89. Osuoha, J. O., Anyanwu, B. O., & Ejileugha, C. (2023). Pharmaceuticals and personal care products as emerging contaminants: Need for combined treatment strategy. *Journal of hazardous materials advances*, 9: 100206.
 90. Parai, D., Banerjee, M., Dey, P., & Mukherjee, S. K. (2020). Reserpine attenuates biofilm formation and virulence of *Staphylococcus aureus*. *Microbial Pathogenesis*, 138: 103790.
 91. Parasana, D. K., Javia, B. B., Fefar, D. T., Barad, D. B., Ghodasara, S. N., & Kalyani, I. H. (2022). Bacterial Biofilms-A Therapeutic Challenge. *INTERNATIONAL JOURNAL OF PLANT AND ENVIRONMENT*, 8(04): 44-47.
 92. Park, S. (2022). Understanding «*Staphylococcus Aureus*» and Associated Inter-Bacterial Interactions to Develop Prophylactics and Therapeutics for Bovine Clinical Mastitis. McGill University (Canada).
 93. Pires, D. P., Costa, A. R., Pinto, G., Meneses, L., & Azeredo, J. (2020). Current challenges and future

- opportunities of phage therapy. FEMS microbiology reviews, 44(6): 684-700.
94. Raheel, I., Mohammed, A. N., & Mohamed, A. A. (2023). The Efficacy of Bacteriocins against Biofilm-Producing Bacteria Causing Bovine Clinical Mastitis in Dairy Farms: A New Strategy. *Current Microbiology*, 80(7): 1-11.
 95. Richter, A., Feßler, A. T., Böttner, A., Köper, L. M., Wallmann, J., & Schwarz, S. (2020). Reasons for antimicrobial treatment failures and predictive value of in-vitro susceptibility testing in veterinary practice: An overview. *Veterinary microbiology*, 245: 108694.
 96. Royer, S., Morais, A. P., & da Fonseca Batistão, D. W. (2021). Phage therapy as strategy to face post-antibiotic era: a guide to beginners and experts. *Archives of microbiology*, 203: 1271-1279.
 97. Rudenko, P., Sachivkina, N., Vatnikov, Y., Shabunin, S., Engashev, S., Kontsevaya, S., ... & Vasilieva, E. (2021). Role of microorganisms isolated from cows with mastitis in Moscow region in biofilm formation. *Veterinary World*, 14(1): 40.
 98. Rumbaugh, K. P., & Sauer, K. (2020). Biofilm dispersion. *Nature Reviews Microbiology*, 18(10): 571-586.
 99. Russell, K. A., Garbin, L. C., Wong, J. M., & Koch, T. G. (2020). Mesenchymal stromal cells as potential antimicrobial for veterinary use—a comprehensive review. *Frontiers in Microbiology*, 11: 606404.
 100. Saeed, S. I., Vivian, L., Zalati, C. S. C., Sani, N. I. M., Aklilu, E., Mohamad, M., ... & Kamaruzzaman, N. F. (2023). Antimicrobial activities of graphene oxide against biofilm and intracellular *Staphylococcus aureus* isolated from bovine mastitis. *BMC Veterinary Research*, 19(1): 10.
 101. Samuel Jawahar, B., & Princess Rajendran, A. (2021). Causes, symptoms, prevention and treatment of mastitis (MAST) in dairy cows. *Intern. J. Zool. Invest.* 7(2): 1041-1067.
 102. Schleimer, N., Kaspar, U., Knaack, D., von Eiff, C., Molinaro, S., Grallert, H., ... & Becker, K. (2019). In vitro activity of the bacteriophage endolysin HY-133 against *Staphylococcus aureus* small-colony variants and their corresponding wild types. *International Journal of Molecular Sciences*, 20(3): 716.
 103. Sharma, G., Sharma, S., Sharma, P., Chandola, D., Dang, S., Gupta, S., & Gabrani, R. (2016). *Escherichia coli* biofilm: development and therapeutic strategies. *Journal of applied microbiology*, 121(2): 309-319.
 104. Sharun, K., Dhama, K., Tiwari, R., Gugjoo, M. B., Iqbal Yatoo, M., Patel, S. K., & Chaicumpa, W. (2021). Advances in therapeutic and managemental approaches of bovine mastitis: a comprehensive review. *Veterinary Quarterly*, 41(1): 107-136.
 105. Shein, A. M. S., Wannigama, D. L., Higgins, P. G., Hurst, C., Abe, S., Hongsing, P., & Chatsuwat, T. (2022). High prevalence of mgrB-mediated colistin resistance among carbapenem-resistant *Klebsiella pneumoniae* is associated with biofilm formation, and can be overcome by colistin-EDTA combination therapy. *Scientific Reports*, 12(1): 12939.
 106. Shin, B., & Park, W. (2018). Zoonotic diseases and phytochemical medicines for microbial infections in veterinary science: current state and future perspective. *Frontiers in veterinary science*, 5: 166.
 107. Shoji, M., Sloan, M., Premkumar, A., Sheth, N., Phillips, J., Crane, T., & Auguściak-Duma, A. (2020). Biofilms in periprosthetic joint infections: a review of diagnostic modalities, current treatments, and future directions. *The Journal of Knee Surgery*, 33(02): 119-131.
 108. Silva, D. R., Sardi, J. D. C. O., de Souza Pitangui, N., Roque, S. M., da Silva, A. C. B., & Rosalen, P. L. (2020). Probiotics as an alternative antimicrobial therapy: Current reality and future directions. *Journal of Functional Foods*, 73: 104080.
 109. 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.
 110. Smith, T. C. (2015). Livestock-associated *Staphylococcus aureus*: the United States experience. *PLoS pathogens*, 11(2): e1004564.
 111. Stachelek, M., Zalewska, M., Kawecka-Grochowska, E., Sakowski, T., & Bagnicka, E. (2021). Overcoming bacterial resistance to antibiotics: the urgent need—a review. *Annals of Animal Science*, 21(1): 63-87.
 112. Stecher, B., Denzler, R., Maier, L., Bernet, F., Sanders, M. J., Pickard, D. J., & Hardt, W. D. (2012). Gut inflammation can boost horizontal gene transfer between pathogenic and commensal *Enterobacteriaceae*. *Proceedings of the National Academy of Sciences*, 109(4): 1269-1274.
 113. Sun, F., Qu, F., Ling, Y., Mao, P., Xia, P., Chen, H., & Zhou, D. (2013). Biofilm-associated infections: antibiotic resistance and novel therapeutic strategies. *Future microbiology*, 8(7): 877-886.
 114. Thandar, M., Lood, R., Winer, B. Y., Deutsch, D. R., Euler, C. W., & Fischetti, V. A. (2016). Novel engineered peptides of a phage lysin as effective antimicrobials against multidrug-resistant *Acinetobacter baumannii*. *Antimicrobial agents and chemotherapy*, 60(5): 2671-2679.
 115. Thi, M. T. T., Wibowo, D., & Rehm, B. H. (2020). *Pseudomonas aeruginosa* biofilms. *International journal of molecular sciences*, 21(22): 8671.
 116. Tong, C., Zhong, X., Yang, Y., Liu, X., Zhong, G., Xiao, C., & Yang, X. (2020). PB@ PDA@ Ag nanosystem for synergistically eradicating MRSA and accelerating diabetic wound healing assisted with laser irradiation. *Biomaterials*, 243: 119936.
 117. Tremblay, Y. D., Caron, V., Blondeau, A., Messier, S., & Jacques, M. (2014). Biofilm formation by coagulase-negative staphylococci: impact on the efficacy of antimicrobials and disinfectants

- commonly used on dairy farms. *Veterinary Microbiology*, 172(3-4): 511-518.
118. Uddin, T. M., Chakraborty, A. J., Khushro, A., Zidan, B. R. M., Mitra, S., Emran, T. B., ... & Koirala, N. (2021). Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. *Journal of infection and public health*, 14(12): 1750-1766.
 119. 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.
 120. Versey, Z., da Cruz Nizer, W. S., Russell, E., Zigic, S., DeZeeuw, K. G., Marek, J. E., & Cassol, E. (2021). Biofilm-innate immune interface: contribution to chronic wound formation. *Frontiers in immunology*, 12, 648554.
 121. Vila, T., Sultan, A. S., Montelongo-Jauregui, D., & Jabra-Rizk, M. A. (2020). Oral candidiasis: a disease of opportunity. *Journal of Fungi*, 6(1): 15.
 122. Vlaeminck, J., Raafat, D., Surmann, K., Timbermont, L., Normann, N., Sellman, B., ... & Malhotra-Kumar, S. (2020). Exploring virulence factors and alternative therapies against *Staphylococcus aureus* pneumonia. *Toxins*, 12(11): 721.
 123. Von Borowski, R. G., & Trentin, D. S. (2021). Biofilms and coronavirus reservoirs: A perspective review. *Applied and Environmental Microbiology*, 87(18): e00859-21.
 124. Vrancianu, C. O., Gheorghe, I., Czobor, I. B., & Chifiriuc, M. C. (2020). Antibiotic resistance profiles, molecular mechanisms and innovative treatment strategies of *Acinetobacter baumannii*. *Microorganisms*, 8(6): 935.
 125. Wang, C., Zhao, W., Cao, B., Wang, Z., Zhou, Q., Lu, S., & Hu, X. (2020). Biofilm-responsive polymeric nanoparticles with self-adaptive deep penetration for in vivo photothermal treatment of implant infection. *Chemistry of Materials*, 32(18): 7725-7738.
 126. Werkneh, A. A., Gebretsadik, G. G., & Gebru, S. B. (2023). Review on environmental selenium: occurrence, public health implications and biological treatment strategies. *Environmental Challenges*, 100698.
 127. Wollanke, B., Gerhards, H., & Ackermann, K. (2022). Infectious uveitis in horses and new insights in its leptospiral biofilm-related pathogenesis. *Microorganisms*, 10(2): 387.
 128. Wu, C., Labrie, J., Tremblay, Y. D. N., Haine, D., Mourez, M., & Jacques, M. (2013). Zinc as an agent for the prevention of biofilm formation by pathogenic bacteria. *Journal of Applied Microbiology*, 115(1), 30-40.
 129. Wu, H., Moser, C., Wang, H. Z., Høiby, N., & Song, Z. J. (2015). Strategies for combating bacterial biofilm infections. *International journal of oral science*, 7(1): 1-7.
 130. Yang, B., Fang, D., Lv, Q., Wang, Z., & Liu, Y. (2021). Targeted therapeutic strategies in the battle against pathogenic bacteria. *Frontiers in pharmacology*, 12: 673239.
 131. Yang, X., Xia, P., Zhang, Y., Lian, S., Li, H., Zhu, G., & Wang, P. (2020). Photothermal nano-antibiotic for effective treatment of multidrug-resistant bacterial infection. *ACS Applied Bio Materials*, 3(8): 5395-5406.
 132. Yuan, Z., Lin, C., He, Y., Tao, B., Chen, M., Zhang, J., & Cai, K. (2020). Near-infrared light-triggered nitric-oxide-enhanced photodynamic therapy and low-temperature photothermal therapy for biofilm elimination. *ACS nano*, 14(3): 3546-3562.
 133. Zhang, L., Cai, Y., Li, L., Chen, C., Zhao, H., Zhang, Z., & Liu, M. (2022). Effects of Luteolin on Biofilm of *Trueperella pyogenes* and Its Therapeutic Effect on Rat Endometritis. *International Journal of Molecular Sciences*, 23(22): 14451.
 134. Zhang, Y., Lin, Y., Galgano, S., Houdijk, J., Xie, W., Jin, Y., ... & Li, T. (2022). Recent progress in phage therapy to modulate multidrug-resistant *Acinetobacter baumannii*, including in human and poultry. *Antibiotics*, 11(10): 1406.
 135. Zia, S., & Alkheraije, K. A. (2023). Recent trends in the use of bacteriophages as replacement of antimicrobials against food-animal pathogens. *Frontiers in Veterinary Science*, 10: 1162465.
 136. Ammar, A. M., El-Aziz, A., Norhan, K., & Mohamed, S. S. (2020). Biofilm formation and its correlation with antimicrobial resistance in *Klebsiella pneumoniae*. *Zagazig Veterinary Journal*, 48(4): 366-377.
 137. Donlan, R. M. (2002). Biofilms: microbial life on surfaces. *Emerging infectious diseases*, 8(9): 881.
 138. Varshney, J. P., & Naresh, R. (2004). Evaluation of a homeopathic complex in the clinical management of udder diseases of riverine buffaloes. *Homeopathy*, 93(01): 17-20.
 139. Sadeghzadeh, R., Esfandiari, Z., Khaneghah, A. M., & Rostami, M. (2024). A Review of Challenges and Solutions of Biofilm Formation of *Escherichia coli*: Conventional and Novel Methods of Prevention and Control. *Food and Bioprocess Technology*, 1-36.
 140. Thallinger, B., Prasetyo, E. N., Nyanhongo, G. S., & Guebitz, G. M. (2013). Antimicrobial enzymes: an emerging strategy to fight microbes and microbial biofilms. *Biotechnology journal*, 8(1): 97-109.
 141. Szczepanski, S., & Lipski, A. (2014). Essential oils show specific inhibiting effects on bacterial biofilm formation. *Food Control*, 36(1): 224-229.
 142. Fernández-Gómez, P., Muro-Fraguas, I., Múgica-Vidal, R., Sainz-García, A., Sainz-García, E., González-Raurich, M., & Alba-Elías, F. (2022). Development and characterization of anti-biofilm coatings applied by Non-Equilibrium Atmospheric Plasma on stainless steel. *Food research international*, 152: 109891.

143. Kostakioti, M., Hadjifrangiskou, M., & Hultgren, S. J. (2013). Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. *Cold Spring Harbor perspectives in medicine*, 3(4).
144. Anter Saad, A. N. (2021). Bacteriological studies on methicillin-resistance *Staphylococcus Aureus* (MRSA) isolated from cow's mastitis milk. *Benha Veterinary Medical Journal*, 40(2): 154-158.
145. Manaia, C. M. (2017). Assessing the risk of antibiotic resistance transmission from the environment to humans: non-direct proportionality between abundance and risk. *Trends in microbiology*, 25(3): 173-181.
146. Adegbeye, M. J., Elghandour, M. M., Reddy, P. R. K., Alqaisi, O., Oloketuyi, S., Salem, A. Z., & Asaniyan, E. K. (2021). Potential of silver nanoparticles for veterinary applications in livestock performance and health. In *Silver Nanomaterials for Agri-Food Applications* (pp. 657-683). Elsevier.