# Emerging Role of Gram-Negative Bacteria in Human Mastitis Infection

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**Abstract:** Mastitis, a prevalent inflammatory condition primarily affecting lactating women, has long been associated with gram-positive bacteria like *Staphylococcus aureus*. However, emerging evidence underscores the growing role of gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* in its pathogenesis. These pathogens exhibit unique virulence factors, including endotoxins, biofilm formation, and antimicrobial resistance mechanisms, leading to more severe clinical manifestations and significant therapeutic challenges. Despite their rising clinical importance, gram-negative bacteria remain underexplored in mastitis research, leaving critical gaps in diagnostics, pathogen-specific treatment, and effective disease management. This review highlights the need for intensified research on gram-negative bacteria in mastitis, emphasizing their contribution to disease severity and the impact of multidrug resistance. Addressing these gaps is essential to advancing diagnostic accuracy, developing targeted therapeutics, and improving patient outcomes in this increasingly complex condition.

**Key Words:** mastitis, breast infection, gram-negative bacteria, antimicrobial resistant, pathogenesis

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Human mastitis is the inflammation of breast tissue, commonly occurring in lactating women, primarily during the initial months postpartum, but it can develop at any time during breastfeeding.[1] Some of the symptoms of this serious condition include a high fever and flu-like signs, such as chills, body aches, redness, tenderness, heat, and swelling in the breast area.[2] Inflammation of the connective tissue within the mammary gland is a common yet significant factor contributing to early weaning among breastfeeding women.[3] The primary causes of human mastitis are milk stasis, which typically occurs because of improper milk removal from the breast duct, often caused by the infant's poor latching, inefficient sucking, or blocking the duct of the breast.[4] The World Health Organization claims that 2%–33% of lactating mothers suffer from human mastitis. Microorganisms that accept opportunities, such as *Staphylococcus epidermis, Staphylococcus aureus, Streptococcus agalactiae, Enterococcus faecalis, Klebsiella pneumonia, Proteus spp.,* and *Pseudomonas aeruginosa.*[5] Currently, bacteriological cultures used for diagnosing human mastitis indicate that infectious mastitis is specifically applied to describe acute cases caused by *Staphylococcus aureus* and particular *Streptococcal species.*[6] Mastitis can lead to various health issues, from mild inflammation to the formation of a severe abscess. The involvement of gram-negative organisms triggers a strong immune reaction via endotoxins, such as lipopolysaccharides (LPS).[7] Increasing antimicrobial resistance among gram-negative bacteria also challenges effective treatment, emphasizing the need for targeted therapeutic and diagnostic approaches. Research on gram-negative bacteria in human mastitis is limited, creating gaps in clinical practice, treatments, and diagnostic protocols.

Mastitis is of 2 types that are acute mastitis and subacute mastitis:

Acute mastitis: Acute mastitis, though less common, is usually the only type accurately diagnosed due to its severe symptoms like breast redness, fever, pain, and malaise.[8] Women with acute mastitis had higher levels of *S. aureus* (about 106 CFU/mL) in their milk than those with subacute mastitis.[9] Once in the mammary gland, it can multiply and produce toxins that severely inflame the tissue, leading to intense pain, heat, and redness in the breasts.[8] Other reports consider bacteria such as *Klebsiella* and *Proteus* to be prevalent in acute mastitis.[5]

Subacute mastitis: It is unclear whether women with subacute mastitis fail to recognize the symptoms or if the symptoms are milder than acute mastitis, resulting in a lower diagnostic rate.[8] When diagnosed, subacute mastitis typically presents as a burning feeling and sharp, needle-like irritation in the breast. A further study of 20 women with subacute mastitis also identified *S. epidermidis* as the most prevalent species.[4] Under normal conditions, bacteria form thin biofilms along the mammary duct epithelium, supporting regular milk production. A small number of these bacteria are carried by the milk's pressure during ejection and transferred to the baby.[8] Other gram-negative bacteria, such as *Pseudomonas*, are also prevalent in subacute mastitis.

Mastitis can be caused by bacterial infections such as *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus species, Klebsiella pneumoniae,* and *Escherichia coli.* These bacteria can enter the breast through cracks or damage to the nipple during breastfeeding and cause inflammation of the breast tissue.[10] Other causes of mastitis are blocked milk duck due to improper drainage, engorgement from delayed or insufficient feeding, tight clothing that restricting the milk flow, and underlying conditions like diabetes that weakens the immune function.[11] The symptoms may consist of localized or extensive sharp pain in the breast that causes pain, redness, swelling, lumps on clogged ducts, and form abscesses and alterations in milk appearance, such as thickened or discolored milk. Common signs also include fever exceeding 101°F, chills, and decreased milk supply.[2]

## PREVALENCE OF MICROORGANISMS ASSOCIATED WITH HUMAN MASTITIS

The most common bacteria associated with human mastitis is *Staphylococcus aureus*. *Corynebacterium* species*, Enterococcus*

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*faecalis, Streptococcus agalactiae,* and *Staphylococcus epidermidis* are other gram-positive bacteria that are frequently associated with this condition. *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Escherichia coli* are less commonly found bacteria in human mastitis. Depending on their unique risk factors, these organisms have the potential to cause the infection.[10]

The bacterial species *Staphylococcus aureus* is the most prevalent gram-positive bacterium associated with human mastitis, especially in breastfeeding women, and it frequently results in abscesses.[12] Resistant to methicillin, antibiotic resistance in *Staphylococcus aureus* (MRSA) is a growing concern. Another important pathogen is *Streptococcus agalactiae,* sometimes known as group B *Streptococcus,* which can cause mastitis during lactation and can cause severe infections in women who are not breastfeeding.[13] Although less prevalent, *Streptococcus pneumoniae* can cause mastitis, especially in people with respiratory illnesses or impaired immune systems.[14] *Enterococcus faecalis* is the cause of the nonpuerperal breast abscess.

The microbe *Enterococcus faecalis*, which frequently occurs in the gut, can cause mastitis in older women or people with diabetes. Furthermore, skin exposures, such as cracked nipples, could result in mastitis caused by *Corynebacterium* species, a common skin microbiota.[6]

*Escherichia coli* is one of the gram-negative bacteria that is frequently associated with mastitis. This bacterium is commonly linked to hospital-acquired illnesses and is generally associated with nonlactating women or those with diseases like diabetes. The 10%–20% of mastitis cases are caused by *E. coli.*[15] *Klebsiella pneumoniae*, responsible for 3% of mastitis cases, is particularly problematic in women with diabetes, as high glucose levels and impaired immune function facilitate bacterial growth.[16,17] This pathogen is resistant to some antibiotics and may produce extended-spectrum beta-lactamases (ESBLs), complicating treatment.[18] *Pseudomonas aeruginosa*, though rarer, can cause aggressive infections in immunocompromised individuals or following nipple trauma and accounts for 1%–5% of mastitis cases.[4] *Proteus* species, including *Proteus mirabilis*, which is responsible for about 5% of cases, are also implicated in mastitis[16,17]. Effective treatment for these infections often requires early detection, targeted antibiotics, and sometimes surgical intervention, particularly in cases involving resistant organisms.

## Key Factors of Gram-Negative Bacteria Affecting Women's Health and Contributing to Human Mastitis

Various host and environmental factors can significantly increase women's susceptibility to gram-negative bacterial infections, which may lead to the development of mastitis.[19] One key factor is milk stasis, which happens when milk flow is obstructed because of insufficient drainage of the mammary glands.[20] The stagnation of milk creates an ideal environment for bacterial growth, as it provides essential nutrients that promote the proliferation of pathogens, such as *Escherichia coli* and *Klebsiella pneumoniae.*[21] Bacteria can quickly create infections in stagnant ducts, causing inflammation and tissue damage. Cracked or damaged nipples are another concern. These often occur because of improper latching during breastfeeding or long nursing sessions. It is important to address these issues to support a better breastfeeding experience.[10] *Pseudomonas aeruginosa* is associated with various skin infections, which can range from localized conditions to severe systemic infections, particularly in individuals with weakened immune systems.[22] In mammary duct ectasia, the presence of both aerobic and anaerobic bacteria in nipple discharge and peri-areolar abscesses indicates their potential role in the condition's development.[23] Once inside, these bacteria release virulence factors that exacerbate tissue inflammation and infection.

A weakened immune system during the postpartum period also significantly increases susceptibility to gram-negative bacterial infections.[24] Hormonal changes after childbirth can temporarily suppress the immune response, reducing the body's ability to fight opportunistic pathogens.[25] This immunosuppression provides an advantage to gram-negative bacteria like *E. coli*, which can evade host defenses through mechanisms, such as biofilm formation and resistance to phagocytosis.[26] Lastly, the hospital environment, particularly exposure to healthcare-associated pathogens, poses an additional risk. Women who have undergone procedures like cesarean sections or whose infants are admitted to neonatal intensive care units are often exposed to antibiotic-resistant strains of gram-negative bacteria, including multidrug-resistant *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*[27] These bacteria can spread from contaminated surfaces, medical devices, or healthcare workers, making infections more difficult to treat and manage.[28] Together, these host and environmental factors highlight the need for preventive measures, including proper breastfeeding techniques, enhanced hygiene, and prompt treatment of any early signs of mastitis to minimize the impact of gram-negative bacterial infections.

## Prevalence of Gram-Negative Bacteria in Mastitis Cases

The most frequently encountered gram-negative bacteria associated with mastitis present a significant challenge in diagnosis, treatment, and understanding their pathogenic mechanisms, particularly given their varying prevalence across different populations and geographical regions. *Escherichia coli* is a major contributor among these pathogens, particularly in nonlactating women or those with underlying conditions like diabetes. This bacterium is commonly linked to hospital-acquired infections and is responsible for 10%–20% of mastitis cases.[15] The ability of *E. coli* to cause severe infections underscores its relevance in mastitis research, especially in immunocompromised individuals. Similarly, *Klebsiella pneumoniae*, which accounts for 8.7% of mastitis cases,[18] poses a significant challenge, particularly in diabetic women, where elevated glucose levels and weakened immune defenses create a conducive environment for bacterial proliferation. Furthermore, this pathogen is often resistant to antibiotics due to its ability to produce ESBLs, complicating treatment efforts.

*Pseudomonas aeruginosa* is another notable gram-negative bacterium, although it is less frequently associated with mastitis, accounting for 1%–5% of cases.[4] This pathogen tends to cause more aggressive infections in immunocompromised individuals or following nipple trauma, making it a critical focus for study in high-risk populations. *Proteus species*, including *Proteus mirabilis*, are also implicated in breast abscess, with an estimated prevalence of about 5%.[29] These bacteria often require targeted therapies, and delays in appropriate treatment can lead to complications such as abscess formation. Table 1 presents the prevalence of gram-negative organisms associated with lactational mastitis

## Correlation of Gram-Positive and Gram-Negative bacteria Associated With Human Mastitis

Human mastitis is a complex condition caused by gram-positive and gram-negative bacteria, each contributing to infection through unique mechanisms and risk factors. Gram-positive bacteria, such as *Staphylococcus aureus*, dominate in lactating women, often causing abscesses, with MRSA posing a particular challenge due to its antibiotic resistance.[4] Other gram-positive pathogens like S*treptococcus agalactiae* and *Enterococcus faecalis* exploit

**TABLE 1.** Prevalence of Gram-Negative Organisms Associated With Lactational Mastitis

| Sr. No. | Year | Study Title | Country | Common Gram-Negative bacteria *Spp.* | Percentage (%) | Reference |
|---|---|---|---|---|---|---|
| 1 | 2007 | Choice of initial antibacterial drug therapy | USA | *Pseudomonas aeruginosa, Proteus mirabilis* | 8% & 5% | 27 |
| 2 | 2009 | Surgically treated for a primary breast abscess | USA | *Proteus* | 9.1% | 28 |
| 3 | 2009 | Development of primary breast abscesses and subsequent recurrence | USA | *Proteus* | 9.1% | 28 |
| 4 | 2011 | Lactational mastitis cases | USA | *Escherichia coli, Klebsiella pneumoniae* | <3% | 5 |
| 5 | 2015 | Taxonomic assignment of the bacterial sequences | Spain | *Pseudomonas* | 17.67% | 29 |
| 6 | 2016 | Gram-stained and aerobic cultures were performed | Turkey | *Klebsiella pneumoniae* | 1% | 30 |
| 7 | 2017 | Microbiological findings | Spain | *Enterobacteriaceae* | 4% | 31 |
| 8 | 2017 | Based on local signs and symptoms of mastitis | India | *Klebsiella pneumonia, Pseudomonas aeruginosa* | (AM 10.99% & SAM 6.84%) (AM 1.57% & SAM 5.21%) | 3 |
| 9 | 2020 | Antimicrobial resistance of responsible pathogens | Ukraine | *Klebsiella spp., Pseudomonas aeruginosa, Proteus mirabilis* | 8.7% & 4.8% & 4.6% | 16 |
| 10 | 2023 | Gram staining revealed detection rates | China | Gram-negative bacteria | 11.29% | 32 |

weakened immunity or skin breaches to establish infections.[30] Similarly, gram-negative bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*, are prevalent in nonlactating women, especially those with diabetes or immune suppression, where their ability to produce ESBLs complicates treatment.[15] Both groups share common predisposing factors, such as cracked nipples, milk stasis, and hospital environments, which create opportunities for bacterial colonization and infection.[19]

Despite differences in pathogenic strategies, both gram-positive and gram-negative bacteria present overlapping clinical symptoms, making accurate diagnosis crucial. While gram-positive bacteria primarily rely on toxin secretion and biofilm formation, gram-negative bacteria utilize endotoxins and antimicrobial resistance mechanisms to evade treatment. Antibiotic resistance is a growing concern in both groups, as seen with MRSA and ESBL-producing *Klebsiella pneumoniae*, complicating therapy and increasing the need for precise microbial identification. Understanding the correlation between these bacterial groups and their shared environmental and host risk factors is essential to developing targeted treatments, improving diagnostic tools, and mitigating the burden of mastitis in affected women.

## Mechanism of Pathogenesis

The pathogenicity of gram-negative bacteria in the mammary gland is closely linked to their ability to adhere to epithelial cells, invade host tissues, and evade immune defenses Figure 1. Adhesion is the initial and crucial step in establishing infection, facilitated by bacterial surface components.[31] Fimbriae and pili, such as type 1 fimbriae in *Escherichia coli* and type 4 pili in *Pseudomonas aeruginosa*, enable bacterial attachment to epithelial cells through interactions with specific surface molecules, like mannose residues.[32] Additionally, LPS in the bacterial outer membrane contribute to adhesion while simultaneously activating host immune responses. Outer membrane proteins, including *OmpA* in *E. coli* and *OmpX* in *Klebsiella pneumoniae*, further promote adhesion by binding to host receptors, such as heparan sulfate and integrins, facilitating bacterial colonization and tissue invasion[33].

Once adhered, gram-negative bacteria employ several mechanisms to penetrate host tissues and establish infection. A key strategy is the formation of biofilms, which are complex bacterial communities encased in a self-produced extracellular matrix.[34] Biofilms enhance bacterial survival by protecting them from phagocytosis, antimicrobial peptides, and the effects of antibiotics.[3] Bacteria, such as *E. coli* and *Klebsiella pneumoniae,* are known to form biofilms on mammary epithelial cells, which allows them to persist within the host environment despite immune system activation and therapeutic interventions.[35]

The ability of biofilms to shield bacteria from host defenses and antimicrobial treatments significantly contributes to the chronic and recurrent nature of infection. Within biofilms, bacteria exhibit increased resistance, making eradication challenging and prolonging the inflammatory response in the mammary gland. This persistence exacerbates tissue damage and complicates treatment efforts, highlighting the importance of understanding adhesion and invasion mechanisms to develop effective strategies for managing mastitis caused by gram-negative bacteria.

Figure 1 shows the difference between a healthy mammary gland and one affected by infectious mastitis, highlighting inflammation, bacterial invasion, and immune response changes in the infected tissue.

## Mechanisms of Antimicrobial Resistance

Bacteria employ several mechanisms to resist antibiotics. Efflux pumps actively expel antibiotics from the cell, decreasing their effectiveness by lowering their intracellular concentration The concept of efflux-mediated antibiotic resistance originated with the discovery that *Escherichia coli* could use efflux pumps to resist the effects of tetracycline.[36] Gram-negative bacteria, including *Escherichia coli* and *Klebsiella pneumoniae,* exhibit antibiotic resistance through porin channel modifications that reduce outer membrane permeability.[37] Mutations in porin channels, which control the entry of antibiotics, can limit drug uptake into the cell.[38] Gram-negative bacteria, such as *E. coli* and *Klebsiella pneumonia*, produce beta-lactamases that break down beta-lactam

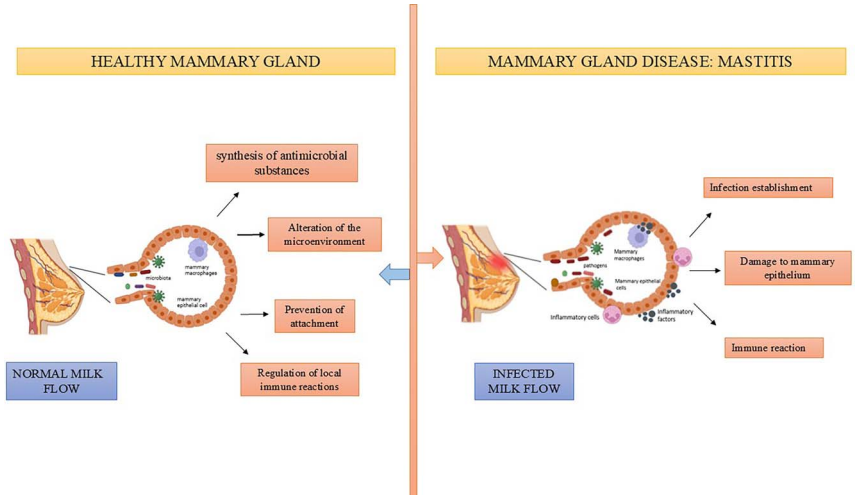


**FIGURE 1.** Difference between healthy and infectious mammary gland in human mastitis.

antibiotics, including penicillin and cephalosporins.[39] Extended-spectrum beta-lactamases are particularly concerning, which provide resistance to a broad range of these drugs.[40] Additionally, some bacteria produce carbapenemases, enzymes that can degrade carbapenems, which are often the last line of defense against resistant infections. Finally, bacteria may modify the target site of an antibiotic, altering the structure of the enzyme or molecule the drug attacks, rendering it ineffective.[41,42]

Gram-negative bacteria are increasingly recognized as significant pathogens in mastitis, contributing to severe infections and posing considerable therapeutic challenges due to their intrinsic and acquired antibiotic resistance mechanisms. *Escherichia coli* is a frequent gram-negative bacterium associated with human mastitis, particularly in nonlactating women or those with conditions, such as diabetes, and many strains exhibit resistance to beta-lactam antibiotics, fluoroquinolones, and aminoglycosides due to the production of ESBLs.[18] Its pathogenicity is driven by an array of virulence factors, such as adhesins that enable adherence to mammary epithelial cells, hemolysins that damage host tissues, and lipopolysaccharides that trigger intense inflammatory responses.[43] Compounding its impact is the alarming prevalence of multidrug-resistant strains. Many *E. coli* isolates associated with mastitis produce ESBLs, rendering beta-lactam antibiotics ineffective, while simultaneously demonstrating resistance to critical antibiotic classes like fluoroquinolones and aminoglycosides.[18] This multidrug resistance complicates treatment and contributes to persistent infections, abscess formation, and delayed recovery, making *E. coli* a formidable antagonist in mastitis cases.

*Klebsiella pneumonia*, an emerging pathogen in human mastitis, poses significant concerns due to its production of ESBLs and carbapenemases, which confer resistance to critical antibiotics, including third-generation cephalosporins and carbapenems[18]. This resistance severely limits treatment options, often necessitating combination therapies with toxic alternatives like colistin or aminoglycosides. Mastitis caused by this multidrug-resistant organism can lead to severe breast tissue inflammation, abscess formation, and systemic infections if untreated or inadequately managed.[44] The presence of resistant *K. pneumoniae* in breast milk raises public health concerns, as it can expose infants to antibiotic-resistant strains, potentially disrupting their microbiome and increasing the risk of neonatal infections.[45] Additionally, the toxicity of second-line antibiotics and limited safety data in lactating individuals may force the suspension of breastfeeding, affecting maternal-infant bonding and infant nutrition.[46] Early diagnosis through molecular methods, antibiotic stewardship, and strict infection control measures are critical to managing this challenging clinical scenario while preventing the dissemination of resistance genes in healthcare and community settings.

*Pseudomonas aeruginosa*, a well-known opportunistic pathogen, is increasingly implicated in cases of human mastitis, particularly in hospital or healthcare-associated infections.[47] Its inherent resistance to many antibiotics, combined with its ability to acquire additional resistance through the production of beta-lactamases and efflux pumps, makes it a formidable pathogen.[48] *P. aeruginosa* frequently exhibits resistance to major antibiotic classes, including beta-lactams, aminoglycosides, and fluoroquinolones, with multidrug-resistant and extensively drug-resistant strains becoming more prevalent.[49] Mastitis caused by these resistant strains can lead to severe breast inflammation, abscess formation, and systemic complications, posing significant treatment challenges. In lactating individuals, limited antibiotic options compatible with breastfeeding exacerbate the concern, as effective drugs like colistin and ceftolozane-tazobactam may not be safe for infants or readily available. Furthermore, the presence of *P. aeruginosa* in breast milk can increase the risk of infant colonization with resistant strains, potentially leading to infections or the transfer of resistance genes.[50] Early and accurate diagnosis using advanced molecular techniques, strict infection control measures, and judicious antibiotic use are essential to manage *P. aeruginosa* mastitis and reduce its broader public health implications.

Although less frequently associated with mastitis, *Enterobacter* species can cause infections in hospitalized patients or those with weakened immune systems. These bacteria can produce beta-lactamases, making them resistant to beta-lactam antibiotics, and some strains are also resistant to carbapenems[18].

## Mobile Genetic Element

A mobile genetic element (MGE) is a DNA sequence that can move within a genome or between genomes. These elements play a significant role in genetic variation, horizontal gene transfer, and the evolution of organisms. Mobile genetic elements, such as transposons, plasmids, and bacteriophages, are critical in bacterial genetic variation. Transposons can disrupt genes by "jumping" within a genome, plasmids carry antibiotic resistance genes, and bacteriophages can transfer virulence or resistance traits to host

bacteria.[51] These MGEs contribute to the adaptability and pathogenicity of bacteria. MGEs can enhance the virulence and antibiotic resistance of bacteria causing breast infections. Plasmids may carry resistance genes, while transposons and bacteriophages can transfer virulence factors, making infections harder to treat and potentially more severe. These genetic exchanges contribute to bacterial adaptation and persistence in the host.[52] Exploring inhibitors of plasmid transfer, transposase activity, and bacteriophage-mediated gene exchange of gram-negative bacteria holds significant promise for developing novel therapeutic approaches to combat antibiotic resistance and virulence in breast infections, potentially leading to more effective treatments for breast infections.

## Gene Transfer Dynamics

Horizontal gene transfer (HGT) significantly influences the evolution and severity of infections, including mastitis, which affects women's health during lactation. Through mechanisms, such as conjugation, transformation, and transduction, HGT facilitates the exchange of genetic material, including antibiotic resistance and virulence genes, between gram-negative and gram-positive bacteria. For instance, mobile genetic elements like integrons and transposons have been identified in mastitis-associated pathogens, transferring resistance genes, such as *blaCTX-M* and *blaTEM*, which compromise the effectiveness of critical antibiotics like beta-lactams.[53] Additionally, the horizontal transfer of virulence genes, including those encoding toxins and adhesins, increases the pathogenicity of bacteria like *Escherichia coli* and *Staphylococcus aureus*, contributing to persistent infections and complicating treatment strategies during breastfeeding.[54]

In *Neisseria gonorrhoeae*, HGT has led to the acquisition of resistance to cephalosporins, highlighting the growing threat of multidrug-resistant gonorrhea.[55] In *Staphylococcus aureus*, including MRSA, HGT is responsible for spreading the *mecA* gene, contributing to the persistence of these pathogens in healthcare and community settings.[56] *Pseudomonas aeruginosa*, a common cause of hospital-acquired infections, also utilizes HGT to acquire resistance to a wide array of antibiotics, including carbapenems, which poses significant challenges in treating chronic infections, especially in immunocompromised individuals.[57] These examples demonstrate how HGT not only accelerates the spread of antibiotic resistance but also increases the pathogenic potential of bacteria, complicating treatment options and posing a growing threat to public health.

The implications of HGT on women's health are profound, particularly concerning mastitis, where it exacerbates infection severity and limits treatment options. Co-infections involving gram-negative and gram-positive bacteria, facilitated by HGT, promote biofilm formation, reducing antibiotic efficacy and leading to chronic and recurrent infections.[58] This can result in prolonged pain, breastfeeding difficulties, and an increased risk of discontinuing breastfeeding, adversely affecting maternal and infant health. Understanding the mechanisms and consequences of HGT in mastitis pathogens is essential for developing effective prevention and treatment strategies, ultimately supporting women's health and well-being during lactation.[56] It has been observed in cases of bovine mastitis, and similar mechanisms can also be seen in human mastitis infections.

## Synergistic Interactions Between Gram-Positive and Gram-Negative Bacteria: Implications for Human Breast Infections

In human breast infections, the synergistic behavior between gram-negative and gram-positive bacteria contributes significantly to the onset and persistence of infection. *Staphylococcus aureus* can trigger an imbalanced immune response by suppressing the host's defenses and facilitating cell invasion, enabling it to colonize during gram-negative bacterial infections that weaken the immune system and lead to udder infections in cows.[59] This creates a potential risk for similar infections in the human breast. Co-infection of *S. aureus* and *P. aeruginosa* in chronic wounds is more virulent than single infection, producing virulence factors that degrade host tissue.[60] Furthermore, gram-negative bacteria may release enzymes like elastases, which facilitate the invasion of gram-positive species into deeper tissues, thereby exacerbating the infection. Gram-negative *Pseudomonas aeruginosa* uses peptidoglycan shed by gram-positive bacteria as a cue to stimulate the production of virulence factors that enhance its pathogenicity in polymicrobial infections.[61] Both types of bacteria may also share nutrients or metabolic byproducts, such as amino acids or short-chain fatty acids, which help sustain their growth and further establish the infection.

Biofilm formation is another crucial aspect of synergistic behavior in breast infections. Gram-positive and gram-negative bacteria contribute to the biofilm matrix, which acts as a protective shield against host immune cells and antibiotic treatments.[62] Biofilms on mammary duct walls can provide a resilient environment for bacteria to persist, leading to recurrent human breast infections.[63] Moreover, bacteria within the biofilm exchange genetic material, including antibiotic-resistance genes, further complicating treatment options. In some cases, biofilms can alter the local pH or nutrient availability, creating a microenvironment that favors specific bacterial species. For example, while gram-negative *Pseudomonas aeruginosa* might break down complex substrates,[64] gram-positive bacteria, such as *Lactobacillus,* could contribute to a shift in local immunity, facilitating bacterial colonization. Polymicrobial biofilms with synergistic and antagonistic cross-species interactions are a significant challenge in treating chronic infections. The role of synergistic behaviors in these infections underscores the complexity of microbial dynamics in the mammary gland. It highlights the need for multi-faceted therapeutic strategies targeting bacterial species and their interactions within biofilms.

## Future Direction

Future research and therapeutic strategies for human mastitis will focus on rapid diagnostic tools to differentiate pathogens and enable targeted treatments. Advanced molecular techniques, such as next-generation sequencing, will uncover genetic drivers of resistance. Innovative therapies, including nanoparticle-based drug delivery and biofilm disruption, promise to enhance antibiotic efficacy. Vaccines targeting prevalent gram-negative pathogens like *E. coli* and *Klebsiella*, alongside alternative approaches like bacteriophage therapy, offer transformative potential for combating antibiotic resistance and improving mastitis prevention and management. These advancements are key to addressing the rising threat of gram-negative bacteria in mastitis and safeguarding maternal health.

# CONCLUSIONS

The emerging role of gram-negative bacteria in human mastitis signals a shift in the condition's microbial landscape, with pathogens like *Escherichia coli* and *Klebsiella pneumoniae* becoming more prominent. These bacteria often exhibit antibiotic resistance, complicating treatment, particularly in vulnerable populations. Their ability to form biofilms and evade immune responses adds to the challenge of effective management. To address this, clinicians must adopt comprehensive diagnostic and treatment strategies, focusing on susceptibility testing, biofilm-targeting therapies, and

careful antibiotic resistance monitoring, ensuring better patient outcomes.

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