Chapter

Etiology of Bovine Mastitis

Muhammad Shoaib, Amjad Islam Aqib, Muhammad Aamir Naseer, Zeeshan Ahmad Bhutta, Wanxia PU, Qaisar Tanveer, Iqra Muzammil, Muhammad Fakhar-e-Alam Kulyar, Muhammad Salman Younas and Muhammad Hammad

Abstract

Mastitis in dairy animals is the primary concern of dairy farmers, which is the most common disease that causes huge economic losses in the dairy industry. The economic losses due to mastitis are from a reduction in milk yield, condemnation of milk with antibiotic residues, veterinary treatment costs, and death. In addition, some mastitis pathogens also cause serious human diseases associated with the contamination of milk or milk products with bacteria or their toxins. Bovine mastitis is mainly caused by a wide range of environmental and contagious bacterial mastitis pathogens. Contagious pathogens are those whose main reservoir is the infected udder. Contagious pathogens mainly spread among animals during milking process whereas environmental pathogens spread from environment to udder at any time. The source of the environmental pathogens is the surrounding environment of an animal. The major contagious pathogens include *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma* spp. and the minor contagious pathogens include *Corynebacterium bovis* and others. Major environmental pathogens include coliform bacteria (*Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp.), environmental streptococci (*Strep. dysgalactiae, Strep. uberis*). This chapter covers detailed review of published data on contagious and environmental pathogens responsible for bovine mastitis.

Keywords: Bovine mastitis, Etiology of mastitis, Microorganism, Contagious pathogen, Environmental pathogen

1. Introduction

Mastitis is an inflammation of the mammary gland caused by microorganisms or trauma. Its purpose is to eliminate or neutralize infectious agents or repair injury and set the stage for healing and restoring normal functioning [1]. Inflammation can be caused by many types of injuries, including infectious agents and their toxins, chemical irritation, and physical trauma [2, 3]. In dairy cows, mastitis is most often caused by microorganisms, usually bacteria that enter the udder and multiply in the milk and gland tissues, producing toxins and other virulence factors that cause direct damage to the gland tissue [4]. Mastitis is one of the main diseases of dairy animals (e.g., cattle, buffalo, sheep, goats and camels). It causes several

problems including reduction in milk production, affect quality of milk to be processed and milk and dairy products quality as well as a huge financial loss for the dairy industry [5]. Mastitis affects the physical and chemical properties, bacteriological load, and other milk qualities. In the milk of infected animals, pathogens and their toxins may present. So, the disease is also very important from the consumer's health risk point of view [6]. The presence of heat-resistant pathogenic spores and toxins in commercially available raw milk poses a serious threat to consumer's health and wellbeing [7–9].

Mastitis can be caused by a single pathogen or combination of two pathogens. According to the US National Mastitis Council Guidelines for diagnosis of mastitis, isolation of more than three pathogens in a milk sample is considered contamination. About 137 microbes have been isolated from milk [4]. Environmental microorganisms that can cause mastitis include *Strep. uberis, Strep. agalactiae, Trueperella pyogenes, Enterobacter aerogenes, Klebsiella* spp., *E. coli,* some yeast, and fungi [10]. In herds that lack an effective mastitis control program, infectious agents such as *Staphylococcus aureus* and *Streptococcus agalactiae* are generally considered to be the main organisms causing mastitis [11]. The incidence rates of these pathogens were significantly reduced with strict adoption of mastitis control programs in countries with well-established dairy farming systems. However, in well managed dairy farms with strict application of mastitis control programs environmental pathogens are of more concern in well-established dairies [12, 13]. Prior to the implementation of mastitis control strategies such as 5-point mastitis control program and later on 10-point mastitis control program by National Mastitis council, contagious mastitis pathogens were considered as the main causative agents of mastitis in dairy cows, even in developed countries [14–16]. The epidemiological field study of mastitis concluded that agents such as *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli* account for over 75% of mastitis cases, and *Staphylococcus aureus* is the most prevalent, resistant and challenging candidate among them [8, 15, 17]. The bacterial entry into mammary glands leads to bacteria interaction with the mammary epithelial cells, resulting in local inflammatory signs and deteriorated milk quality. Environmental microorganisms can accidentally enter the udder during intramammary injection [18]. Moreover, contagious intramammary infection can be transmitted by milker's hands, cleaning towels, flies, and milking machines [19, 20]. *Streptococcus dysgalactiae, Strep. uberis, Klebsiella* and *E. coli* are the most common environmental pathogens, gaining access to udder at any time including during milking process. Clinical mastitis manifest symptoms such as udder/quarter swelling, abnormal milk quality and quantity, and anorexia [21–23].

2. Contagious mastitis pathogens

2.1 Major pathogens

2.1.1 Staphylococcus aureus

Staphylococcus aureus is major pathogen causing infectious mastitis in dairy cows, with prevalence of 43–74% [24]. It is a gram-positive, catalase and coagulase-positive, non-spore-forming, oxidase-negative, immobile, and facultative anaerobic bacteria [25]. *Staphylococcus aureus* is the most common mastitis pathogen [26]. While it is possible to reduce the incidence of *S. aureus* mastitis through hygienic milking and proper management systems, it remains a major challenge for dairy farms with a prevalence rate higher than 60% [8, 27]. The incidence of *S. aureus* mastitis differs due to changes in hygienic milking practices and general differences in the management of infectious

mastitis on farm [20, 28]. Optimal milking parlor hygiene can considerably decrease the incidence of new *S. aureus* mastitis in the herd but cannot exclude existing cases in the herd [29]. Based on early observations by Neave et al. [29], numerous studies have reported that treatments can decrease the number of new cases of mastitis [12] but cannot eliminate persistent infections in the herd. In the United States, the occurrence of clinical and subclinical *S. aureus* mastitis is 10–45% and 15–75%, respectively [30]. Its virulence is due to its ability of producing wide array of virulence factors that enhance its pathogenicity and persistence in epithelial linings of udder. These virulence factors contribute to microbial attachment, colonization, longer persistence and escaping the immune response. Such abilities make *S. aureus* one of the most important challenging pathogen for animal and human health [31, 32]. *Staphylococcus aureus* isolated from udder infections in ruminants are found producing a layer of slime around them, which enables them to resist host immune system and antibiotics [8]. This slime layer also helps in adherence and colonization of pathogen in udder glandular cells [33]. *Staphylococcus aureus* virulence factors and pathogenicity associated mechanisms such as resistance to phagocytosis, adherence and biofilm formation enable it to cause persistent and chronic infections [34].

Staphylococcus aureus has numerous virulence factors, that can be divided into two categories. These include non-secretory factors which are surface restricted structural component that acts as virulence factors, and secretory factors that are produced by bacterial cells, and act on a variety of target sites in the host. Both secretory and non-secretory factors enable this pathogen to evade host's defenses and colonize the udder [35–37]. Microbial membrane proteins, including fibrinogen-binding protein, collagen-binding protein, penicillin-binding protein, elastin-binding protein, and lipoteichoic acid can act as non-secretory virulence factors [36, 38, 39]. Cell wall binding factors such as lipoprotein, peptidoglycans, protein A, phthalic acid, protease, and β-lactamase can act as secretory virulence factors [39, 40]. Other virulence factors related with the cell surface include exopolysaccharides, biofilms, and capsules [37, 41, 42]. Overall, *Staphylococcus aureus* has more than 13 secreted proteins and 24 surface proteins involved in immune evasion [43], as well as about 15–26 proteins involved in biofilm formation [44]. The most familiar secretory virulence factors are toxins, including non-enteric exfoliative toxins, staphylococcal enterotoxins, leucocidin, toxic shock syndrome toxin 1, and hemolysins (α, β, δ, and γ) [45]. Likewise, enzymes like staphylokinase, coagulase, phosphatase, DNase, phospholipase, lipase, and hyaluronidase are also virulence factors of *Staphylococcus aureus* [7, 46, 47].

2.1.2 Streptococcus agalactiae

Streptococcus agalactiae is the contagious mastitis pathogen and the infected mammary gland acts as reservoir of the bacterium in the herd. Transmission of the bacterium is mainly through milking equipment, milker's hands, and regular towels [48]. Developed dairy sectors have overcome this challenge by optimal managemental and biosecurity practices but *Streptococcus agalactiae* is still an important cause of intramammary infections (IMI) around the globe [16, 49–51]. A study from dairy farms in Colombia indicated a higher prevalence of *Streptococcus agalactiae* induced IMI in cattle ranging from 28–35% [52]. Moreover, *Streptococcus agalactiae* reemergence has also been reported in Northern Europe [53]. Non-dairy sources (e.g., humans) have been reported to be the main cause of reintroduction of this pathogen into dairy herds [54].

Capsular polysaccharide is the most important virulence factor of *Streptococcus agalactiae* [55], which protects bacteria from phagocytosis by macrophages and subsequent depletion [55]. An additional virulence factor for *S. agalactiae* is the

surface protein, which provides resistance to proteases. Emaneini et al. [55] discovered that 89% of cattle mastitis causing *Streptococcus agalactiae* isolates possess gene encoding (*rib*). *Streptococcus agalactiae* is extremely contagious but responds well to antibiotic treatment, allowing its removal from the herd with effective mastitis control programs [56]. As a result of standard managemental practices, *Streptococcus agalactiae* mastitis has been significantly reduced and is now rare in developed dairy systems [57].

2.1.3 Mycoplasma *spp.*

Mycoplasma is a highly contagious microorganism, but not to the same extent as *Streptococcus agalactiae* or *Staphylococcus aureus*. However, *Mycoplasma* damages the secretory tissue and causes abscess and lymph node fibrosis as well as gland fibrosis [4, 16]. Animals of any age and at any time of lactation are sensitive to *Mycoplasma* infection. Those in the early stages of lactation are susceptible to *Mycoplasma* infection and may be isolated from asymptomatic high producing animals. Mycoplasmosis is usually associated with the appearance of mastitis, the appearance of new animals, previous respiratory or joint diseases, and herds of cattle that have not responded to antibiotic treatment [18, 58]. *Mycoplasma* infection is suspected if there is at least one recurrence of mastitis, asymptomatic disease, and no response to treatment [59].

The species detection in *Mycoplasma* mastitis is usually carried out by PCR with defined endpoints for *Mycoplasma bovis*, *Mycoplasma bovigenitelium*, *Mycoplasma californicum*, and *Mycoplasma alkalescens.* Laboratory monitoring of dairy herds showed the presence of *Mycoplasma* spp. in at least one cow of the herd [60]. Herd-level study of 463 Northwest Dairy Association miking herds reported that 93 herds were positive for *Mycoplasma* mastitis. Cattle in milk were noted more prone to *Mycoplasma* infection. Moreover, *Mycoplasma* infection was noted indirectly related to herd size [61].

Mycoplasma mastitis is less common than other bacterial mastitis, but it can cause severe mammary infections and has unique epidemiology and risk factors [58, 61]. It can usually be distinguished from mastitis caused by staphylococci and streptococci because it is (1) highly infectious, (2) infects more than one quarter, (3) causes significant milk yield loss, (4) is often resistant to antibiotic treatment, and (5) can become purulent. In some cases, affected cows may appear normal and not show obvious clinical signs. Since *Mycoplasma* mastitis is considered incurable, culling remains the most commonly recommended control measure [58, 62].

2.2 Minor contagious pathogens

2.2.1 Mannheimia *spp.*

Mastitis, caused by *Mannheimia* (formerly known as *Pasteurella*) *haemolytica* and *Pasteurella multocida*, is common in sheep and manifests itself as peracute gangrenous, but less commonly in goats and cattle [63, 64].

2.2.2 Corynebacterium bovis

Corynebacterium bovis (C. bovis) is a common infectious agent, most associated with asymptomatic infections. However, in 7% of cases, the bacteria were isolated from cows with clinical mastitis [16]. From the herds where pathogens that cause infectious mastitis were controlled, it accounted for higher number of clinical cases. There is a continuing discussion about the importance of *Corynebacterium bovis* infection for udder health and milk production [16, 19, 21]. Studies have shown

that this bacterium has tendency for the teat canal. This characteristic is associated with lipids requirements for its growth (probably inside the keratin plug). It could be possible that *C. bovis* occlusion of the streak canal may cause competition with other ascending bacterial infections for nutrients, thus decreasing the IMI [15, 16]. Moreover, the small increase in SCC linked with *C. bovis* infection may increase the ability of the udder quarter to show response against new intramammary infections. A higher SCC than normal is caused by infection with a minor mastitis pathogens in the udder and increases the udder's resistance to invasion by other contagious pathogens [1, 65].

In herds with endemic *C. bovis* mastitis, the infection rate was noted lowest in comparison to major pathogens infected herds [15, 66]. Intramammary *C. bovis* infections are mostly associated with clinical manifestations but generally have a reasonable increase in somatic cells count. Milk in such infections is usually thicker than normal and milk loss is usually undetectable [16, 22, 23, 67].

3. Environmental pathogens

In modern dairy systems, environmental mastitis is the most common and costly challenge [59]. Environmental mastitic pathogens include various bacteria such as coliform (e.g., *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., etc.), environmental streptococci (e.g., *Streptococcus uberis*, *Streptococcus agalactiae, etc.*) [15]. In addition, farm floor, pasture and cattle manure are the main sources of environmental mastitis pathogens, especially *E. coli* and *Streptococcus uberis* [68]. Major environmental pathogens causing severe damage to bovine udder include *Streptococcus uberis, Streptococcus dysgalactiae*, coliforms, and non-aureus *staphylococci* [69]. Mixed IMI of major and environmental mastitis pathogens frequently cause severe, persistent and non-responsive mastitis, with a significant increase in somatic cell count and obvious clinical manifestations [59].

Due to emerging concern of increasing antibiotic resistance, preventive strategies for controlling environmental mastitis pathogens are needed [47, 70]. Control of significant risk factors, pasture management, optimal managemental and feeding practices is a prime goal of such strategies. There are preventive mastitis vaccines in the market that are reported to reduce the infection, but unfortunately, none of them provided promising results [53]. Understanding the transmission pathways, better diagnostic tools and implementation of mastitis control program in efficient way can lead to drastically lessen the mastitis burden in dairy industry [71–73].

3.1 Major environmental pathogens

3.1.1 Environmental streptococci

Environmental streptococcal species are considered as one of the significant pathogens that cause clinical and subclinical mastitis in dairy herds. Among these, *Streptococcus uberis* is the most common mastitis pathogen that damages the bovine udder. Mastitis control measures have minimal effect on the incidence of mastitis, caused by environmental *Streptococcus* species, coliforms and some non-aureus staphylococci [74]. Dairy environment is the key risk factor that leads to the development of mastitis particularly due to *S. uberis, S. dysgalactiae (Streptococcus dysgalactiae subsp. dysgalactiae).* Other members of *Streptococcus* species that cause mild bovine mastitis are *Streptococcus sanguis, Streptococcus salivarius* and *Streptococcus parauberis* [75].

3.1.2 Escherichia coli

Mastitis is caused by multiple bacterial etiologies, where *E. coli* is known as one of the most significant causes of clinical mastitis in dairy animals, typically occurred in high producing cows as wells as cows in the early lactation period with low somatic cell counts [76]. *Escherichia coli* (*E. coli*) is a gram-negative environmental pathogen and is positive for catalase test and negative for coagulase test [77, 78]. Many animals are the carriers, but cattle are the main carriers of *E. coli*. Pathogenic strains of *E. coli* can be differentiated from the strains of normal flora on the basis of the presence of virulence factors such as adhesin proteins, antibiotic resistance, and biofilm production [79, 80]. There are distinctive *CITED2* (Cbp/P300 Interacting Transactivator With Glu/Asp Rich Carboxy-Terminal Domain 2), *SLC40A1* (Solute Carrier Family 40 Member 1), and *LGR4* (Leucine Rich Repeat Containing G Protein-Coupled Receptor 4) genes specific to *E. coli* isolated from the bovine mastitis [81]. Moreover, *E. coli* isolates from bovine mastitis cases contain a variety of serogroups [82]. It has been reported that multiplication of *E. coli* occurs in mammary secretions without its adherence to mammary glands epithelium. A study on mastitis epidemiology has revealed that the severity of *E. coli* mastitis is mainly linked with cow factors, as well as strain characteristics [83]. *E. coli* is the udder pathogen causing mastitis in dairy animals, and its endotoxin is potential health threat at consumer end [84]. Its long persistence and associated virulence factors are more often a point of concern in the dairy farm environment [85]. Toll-like receptor-4 has major role in the pathogenesis of *E. coli* in mastitis [86]. Cephalosporins and non-steriodal anti-inflammatory drugs are commonly recommended for the treatment of *E. coli* mastitis, to which microbe has evolved the resistive character [84, 87]. The chronic nature of *E. coli* mastitis deteriorates the milk quality without notice of handlers [22]. The prevalence of subclinical mastitis in different districts of province Punjab was reported to be 32% with *E. coli* as second most common isolate from samples with incidence rate of 16.18% [88]. The *E. coli* isolation rate from subclinically infected cows was 13% with subclinical mastitis 36% [89]. 25% mastitis prevalence with *E. coli* isolation rate of 18.47% in dairy buffaloes was reported by [90].

3.1.3 Nocardia *spp.*

Mastitis caused by *Nocardia* spp. is rare in cattle and presents as mastitis with extensive granulomatous udder lesions. Nocardia is gram-positive, aerobic bacteria with filamentous branches [91]. Nocardia is an ever-present environmental saprophyte with more than 30 identified species [92].

3.1.4 Bacillus *spp.*

Bacillus cereus and *Bacillus subtilis* are saprophytes and they are the only pathogens that can cause mastitis. These are responsible for acute hemorrhagic mastitis in cattle [15, 16, 93]. *Bacillus cereus* cases are usually linked with teat injury or surgical infection. Mastitis can also occur in cattle during calving and is linked with brewing grains mixed with *Bacillus cereus* spores. Several strains of the *Bacillus* species are non-pathogenic, and the isolated strains from clinically healthy bovine teat change rapidly over time [91].

3.1.5 Klebsiella *species*

Mastitis caused by *Klebsiella pneumoniae* can be severe as it responds poorly to commonly used mastitis treatment protocols and rapid progression to toxic

shock, resulting in death [94, 95]. *Klebsiella pneumoniae* is still a challenge to dairy animals and causes udder infections even after the advancement in control of mastitis [96, 97]. Mastitis caused by *K. pneumoniae* tends to be prolonged and severe because of its low sensitivity to antibiotic treatment and can lead to animal death if left untreated. *Klebsiella* species cause more losses to the dairy industry than *E. coli* in terms of mastitis [96].

3.1.6 Pseudomonas aeruginosa

Pseudomonas aeruginosa is one of the causative agents of bovine mastitis [98, 99]. Most strains of *Pseudomonas aeruginosa* have a type III secretion system that can induce an increase in the number of somatic cells count in the mastitic milk. In addition, most *Pseudomonas aeruginosa* strains can form biofilms, reducing the effectiveness of antibiotics [100].

3.1.7 Other Pseudomonas *species*

Pseudomonas species are potential environmental pathogens, frequently associated with wet bedding and water used in milking parlor [98, 100]. Trauma to teat ends due to improper milking increases the chances of *Pseudomonas aeruginosa* infections. *P. aeruginosa* is commonly isolated from mastitic animals and possesses different virulent factors like exo-enzyme, exotoxin A and protease that initiate an inflammatory response and cellular death [51, 101]. It can survive in different environmental conditions and infect susceptible cows through teat canal. Immuno-compromised cows, due to infectious diseases and nutritional deficiencies, are more susceptible to *P. aeruginosa* infection. This microorganism is reported as extremely resistant to commonly used antimicrobials [97]; therefore, adopting the hygienic practices, isolation, and culling of infected cows are the only available control measures [100].

3.2 Minor mastitis pathogens

Minor mastitis pathogens include a range of different environmental microorganisms including some non-aureus staphylococci and *Corynebacterium* species. Some non-aureus staphylococci are opportunistic environmental bacteria that normally reside on the nasal tissue, teats, and hands of milking personnel [102]. Non-aureus staphylococci are considered as the emerging mastitis-causing bacterial pathogens [19, 103, 104]. Non-aureus staphylococci exhibit less pathogenicity as compared to other principal mastitis-causing pathogens and infections, most of the time remain subclinical. However, persistent non-aureus staphylococci infection can lead to reduced milk production and milk quality, increased somatic cell count, and severe damage to the udder [105]. *Trueperella pyogenes* causes summer mastitis and low-grade mastitis in the cows, often being clinically well but with a very enlarged and painful quarter [106]. Despite the high-frequency isolation, non-aureus staphylococci are considered minor mastitis pathogens but still a significant challenge for dairy farmers [12, 107].

4. Other mastitis pathogens

Some members of *Enterococcus* species like *Enterococcus faecalis, Enterococcus saccharolyticus* and *Enterococcus faecium* cause bovine mastitis [75]. Moreover, *Aerococcus viridans* has also been reported as a causative agent of mastitis, but its potential role has not been elucidated yet [108].

5. Conclusion

Mastitis is the most common and economically important disease for dairy industry, regarding milk quality and quantity. Microorganisms enter the udder and multiply in the glandular parenchyma, producing toxins that cause direct harm. Bovine mastitis is caused by a wide range of environmental and contagious pathogens. Contagious pathogens are those whose main reservoir is infected udder of an animal. The major contagious agents include *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma* species. On the other hand, environmental mastitis is caused by pathogens such as *Escherichia coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis, Trueperella pyogenes, Enterobacter aerogenes, Klebsiella* species, some yeast, fungi and *Pseudomonas* species. Mammary gland infections caused by these pathogens are of short duration and have severe clinical presentation. Environmental pathogens are usually linked with unsanitary managemental practices, resulting in the clinical symptoms (udder/quarter swelling, abnormal milk quality and quantity, and anorexia). Due to emerging concern of increasing antibiotic resistance, preventive strategies for controlling mastitis pathogens are needed. Control of significant risk factors, pasture management, optimal sanitary and feeding practices is a prime goal of such strategies. There are some mastitis vaccines against specific bacterial pathogen in the market that are reported to reduce the challenge, but unfortunately, none of them has provided promising results against all mastitis pathogens. Understanding the transmission pathways, better diagnostic tools and implementation of mastitis control program in efficient way can lead to drastically lessen the mastitis burden in dairy industry.

Conflict of interest

Authors declare no conflict of interest.

Author details

Muhammad Shoaib[1,2*], Amjad Islam Aqib[3], Muhammad Aamir Naseer[4], Zeeshan Ahmad Bhutta[5], Wanxia PU[2], Qaisar Tanveer[6], Iqra Muzammil[7], Muhammad Fakhar-e-Alam Kulyar[8], Muhammad Salman Younas[1] and Muhammad Hammad[1]

1 Faculty of Veterinary Science, Institute of Microbiology, University of Agriculture Faisalabad, Pakistan

2 Key Laboratory of New Animal Drug Project, Gansu Province, Key Laboratory of Veterinary Pharmaceutical Development, Ministry of Agriculture, Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS, Lanzhou, China

3 Department of Medicine, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Science, Bahawalpur, Pakistan

4 Department of Clinical Medicine and Surgery, Faculty of Veterinary Science, University of Agriculture Faisalabad, Pakistan

5 The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Midlothian, Scotland, United Kingdom

6 Faculty of Veterinary Science, Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture Faisalabad, Pakistan

7 Department of Medicine, Faculty of Veterinary Science, University of Veterinary and Animal Science, Lahore, Pakistan

8 College of Veterinary Medicine, Huazhong Agriculture University, Wuhan, China

*Address all correspondence to: shoaibsinko8@gmail.com

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