Therapeutics, Types, and Pathogenesis of *Camelus Dromedarius* Mastitis

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ABSTRACT

Camel is a milk and meat-producing multipurpose animal along with transportation utility. Camels serve as the financial reserve and a symbol of prosperity and social prestige for pastoralists. Highly nutritious camel milk is a good substitute for human milk. Udder infections are a major limitation in camel farming. These infections have been reported in various camel-producing countries of Asia and Africa (Somalia, Egypt, Sudan, Iraq, Kenya, and Saudi Arabia). Mastitis could be subclinical, chronic, or acute with distinguished clinical features. Several extrinsic or intrinsic factors contribute to camel mastitis. In addition to significant economic loss, untreated camel mastitis can also pose serious hazards to public health. The current review provides an overview of camel mastitis and discusses its various types and related bacterial pathogens along with the control measures.

Keywords: Camel, mastitis, milk, Saudi Arabia, bacterial pathogens

INTRODUCTION

Mastitis is an injury or trauma-associated bacterial infection of the udder that leads to serious economic consequences [1-4]. Bacteria in dairy products can serve as the transmission vehicle for highly resistant bacteria. Antimicrobial resistance has been documented in the bacteria isolated from milk and other dairy products [1,3]. The literature lacks reports on antimicrobial resistance in the milk bacteria of mastitis-suffering camels in Saudi Arabia. This review elaborates on the antimicrobial resistance of bacteria related to subclinical and clinical camel mastitis in Saudi Arabia. The recent advances in molecular epidemiology, host genomics, metabolomics, transcriptomics, and proteomics have facilitated a thorough understanding of mastitis biology. It could help in developing novel treatments and vaccines with better knowledge of potential risks associated with the exchange of genetic material between pathogen and host. Several risk factors (environmental, host, and pathogen) are associated with camel mastitis incidence, which are focused on while devising mastitis control programs [5] (Figure 1).

Camel mastitis is an intra-mammary infection (IMI) of different bacterial species. Based on the bacterial origin, these are classified as environmental or contagious infections [6]. Camel-to-camel mastitis transmission during milking is referred to as contagious mastitis [6]. Contagious bacteria (*Streptococcus agalactiae*, *Staphylococcus aureus*, *Corynebacterium*, and *Mycoplasma bovis*) colonize on teat and udder skin, and teat canal [7]. Such bacterial colonization causes sub-clinical infections leading to the rise in SCC. SCC consisting of epithelial cells and leukocytes (macrophages, erythrocytes, neutrophils, and lymphocytes) are important indicators of IMI infection [6]. Reduced contact between uninfected and reservoir camels can help in controlling contagious infections. Dry cow therapy (DCT), culling, teat disinfection after milking, and proper maintenance of the milking apparatus are important preventive measures to avoid contagious infections [6]. Bacteria colonizing in the bedding, soil, water, calving pads, and manure are the major source of environmental mastitis. These bacteria include Coliforms (*Klebsiella, E. coli*), *Streptococcus dysgalactiae*, and *Streptococcus uberis*. Coliforms cannot survive in the udder whereas *Streptococcus* bacteria can persist in the udder and spread during the milking process [8].

Camel mastitis can be sub-clinical or clinical (chronic or acute) (Figure 2). The symptoms of clinical mastitis include heat, swelling, pain in the mammary gland, and discolored and clotted milk. Acute mastitis is characterized by watery, blood-tinged, or yellowish mammary secretions, which could contain *E. coli*, *Klebsiella pneumoniae, Mycoplasma bovis*, and *Mycoplasma agalactiae* [7]. Keratinization and fibrosis can occur in the udder tissue during chronic mastitis whereas such symptoms do not appear in subclinical mastitis [9,10].

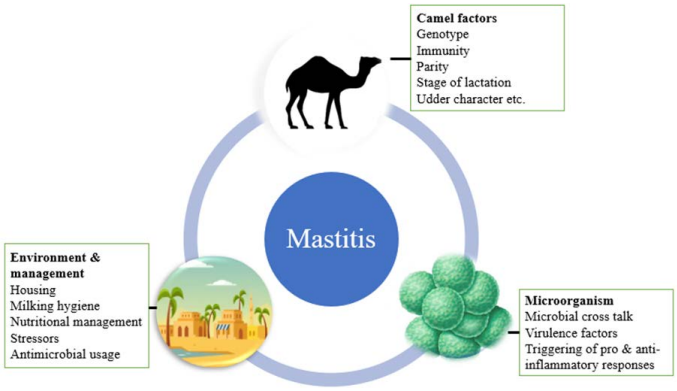

Figure 1. Schematic illustration of camel mastitis-associated factors ***Camelus dromedarius***

There are two camel species, which belong to the family Camelidae. *Camelus bactrianus* is an Asian species particularly found in cold arid regions. *Camelus dromedarius* is known as the Arabian camel that has a close association with Arab culture and history. *Camelus dromedarius* is found in almost all the semiarid, arid, and hot regions of the "old world" (Asia and Northern Africa) [11,12].

Camelus dromedarius species is a primary inhabitant of Arabian, Middle Eastern, Pakistani, and Indian regions. An estimated global camel population is 17 million and 65 % (11.2 million) belong to the species *dromedarius*. 61% of them are found in Arab countries whereas the rest population is scattered in various countries. Camel meat constitutes almost 30% of the total meat consumption in Saudi Arabia and thus is considered a safe investment [13]. *Camelus dromedarius*

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has also served as a transportation source for supplying fibers for textile products and hides. Recently, these camels are used for recreational racing, which is an important tourist attracting sport in the Arabian Gulf [14]. Camel is a highly adaptable animal, particularly in marginal areas where it can reproduce and survive in harsh environments [15].

In contrast to other herbivores, milk production in camels continues even under severe environments [16]. Camels' average lactation period ranges from 14 to 16 months, which is longer than other cattle. The milk production in camels varies with the species, type of food and availability, stage of the reproductive cycle, and breed [17]. Generally, camels are resistant to several disease-causing agents but they are susceptible to mastitis-causing bacterial infections [18,19]. There is no regular veterinary care system for the camel herds leading to common mastitis in lactating females, which affects the milk quality and infants' health [19-21].

The copulation or presence of male dromedary induces female ovulation. Generally, the sexual cycle in camels starts at the age of two years in December (winter season). Depending upon the stress, food availability, and season, the average gestation period in camels could be up to 315 - 360 days or a maximum of 370 -375 days [22]. The first mating of camels is allowed at an age of 36-48 months and they can breed up to the age of 30 years [23]. The milk yield is comparatively higher in dromedary than other herbivores under the same conditions [24]. Their lactation period could range from 1 to 2 years and is directly correlated with stress, food availability, parturition, and climate conditions [25]. The first three lactation months are characterized by the highest production of milk followed by a reduction during the 4^{th} and 5^{th} months [26].

Camel milk is highly valuable for the people inhabiting semiarid and arid regions. Camel milk is rich in vitamin C and also contains fat and protein content [27]. Pastoralists earn handsome income from camel milk. Camel milk is preferred for raw consumption over other types of raw milk and is supposed to possess antimicrobial and therapeutic effects at certain lactation stages [28,29]. Camel milk is easily digestible, quenches thirst, and has more spoilage time than other types of milk [30]. The antimicrobial activity of camel milk could inhibit the growth of some specific human health-related bacteria [31]. Camel milk contains several enzymes such as Lactoferrin, peptidoglycan recognition protein (PGRP), Lacto peroxides, Lysozyme, N-acetyl-glucosaminidase (NAGase), and human immunity enhancing immunoglobulins [32,29]. These enzymes exhibit deleterious impacts against bacteria and viruses.

Camel milk also serves as an ideal medium for various bacteria, which can contaminate the milk through milking personnel or equipment at the start of milking, and during the exit from the teat canal. The milk can also be contaminated during storage or transportation at high ambient temperatures without refrigeration [33]. Local producers often consume raw camel milk, which could cause food-borne illness and affect human and animal health [34,35]. Camel milk is available at grocery stores in Saudi Arabia and some other countries [36]. The information about camel milk is limited because camels are not considered the main milk-yielding source globally. In comparison to cattle, only a few studies have investigated mastitis in Camelidae. Camel mastitis has been reported in Egypt, Saudi Arabia, Somalia, Kenya, Sudan, Ethiopia, and Israel [10,2,7,14]. The change in milk quality during mastitis depends upon the affected epithelial area and causative agent [37]. Mastitis signs in the udder include:

1. Enhanced permeability from blood to milk leading to an increase in enzymes, proteins, and ions.
2. Milk invasion by phagocytic cells.
3. Decreased synthetic capability of the gland leading to a decrease in certain milk components [38].
4. Some mastitis-causing pathogens generate toxins, which could cause human illness [39]. The affected quarter could produce acute phase proteins causing inflammatory reactions [40]. The microbial concentration in or near the teat orifice determines the rate of new mastitis infections [41].

What is Mastitis?

Mastitis is an injury or trauma-associated bacterial infection of the udder with serious economic impact [42]. Mastitis negatively affects mammary tissues, changes milk composition with an elevated somatic cell population, and causes pain to the animal [43]. Mastitis adversely affects farm profitability [44]. There have been continuous educational and extension efforts to tackle mastitis since the 1970s. However, subclinical and clinical mastitis remains the most influential health factor that affects milk quality and production [6]. Mastitis is a major concern of the milk industry because of the associated economic losses. Mastitis could be controlled with proper preventive programs during the early stages [45]. Mastitis-related economic losses include:

1. Permanent or temporary milk quality and yield reduction because of high SCC counts.
2. Loss of milk because of the antibiotic administrations, and increased expenses related to laboratory tests, treatment, veterinarian fees, and labor costs. Furthermore, the animal's reproductive cycle is compromised and the meat value of the mastitis-infected slaughtered animal is reduced [42,46].

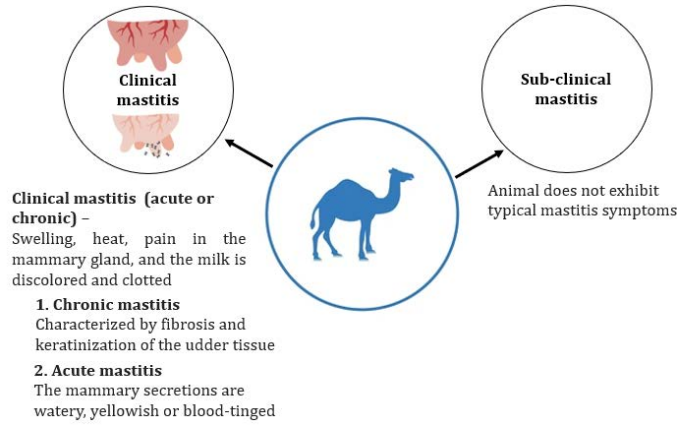
Types of *Camelus dromedarius* Mastitis

There are two types of mastitis known as clinical and sub-clinical mastitis (Figure 3). Clinical mastitis is visible from the appearance of milk and udder and does not require special diagnostic tests. Symptoms include hyperthermia, loss of appetite, and udder inflammation, which urges the mother to avoid neonates [47]. *Klebsiella pneumonia*, *Pasteurella haemolytica*, *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus agalactiae* are the major mastitis-associated bacteria [48]. Clinical mastitis could be mild, moderate, or severe as categorized by "International Dairy Federation (IDF)" in 1999. Mild mastitis causes clots and flakes in the milk. Contrarily, severe mastitis results in udder swelling and redness, dehydration, abnormal secretions in the milk, depression, fever, loss of appetite, and rapid pulse leading to animal death. The milk of animal suffering from severe mastitis is characterized by watery consistency [14].

Chronic or subclinical mastitis is characterized by undesirable changes in the udder and retarded growth of infant camel. The subclinical mastitis incidence rate is higher (1 in 15 diagnosed cases) than the clinical mastitis. Subclinical or chronic mastitis could degenerate the udder parts, and the udder surface becomes dotted with pus-containing lesions. In this case, the produced milk becomes contaminated with high cell count and pus [49]. The symptoms of sub-clinical mastitis are usually undetectable but it could decrease the milk yield and change the milk properties because of high SCC leading to the consumer's health concerns [50]. SCC is negatively associated with milk production. At an SCC level of above 300000, the milk is not normal whereas the

udder inflammation is also clearly visible [14]. Subclinical mastitis generally precedes clinical mastitis and it remains unnoticed for a longer period. However, it gradually starts affecting milk production and quality. Subclinical mastitis provides a suitable environment for the reproduction and growth of infectious organisms, particularly during the last ten days before giving birth and in dry weather. Chronic mastitis results in persistent inflammation of the mammary glands [51,19].

Figure 2. Schematic illustration of two types of camel mastitis with



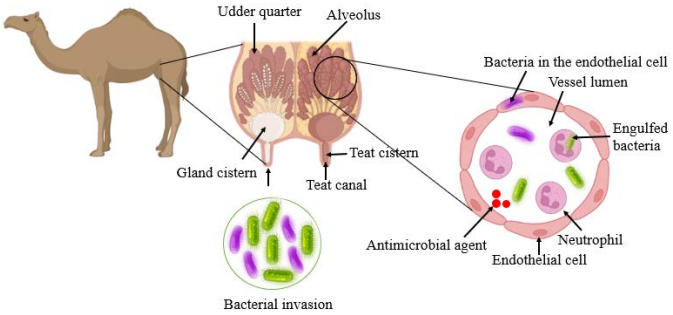
respective characteristics

Pathogenesis of *Camelus dromedarius* mastitis

Bacterial invasion of mammary glands and teat canal initiates mastitis. Then, bacteria multiply and release toxins to affect the milk-secreting tissues. This scenario leads to a rise in SCC, which reduces milk production. Teat serves as the first defense to protect the udder from infection through its sphincter that restricts bacterial entry and milk exit. A waxy material (keratin) protects the teat canal from the inside by binding mastitis-causing pathogens. The teat canal remains partially open for 1 to 2 hours after milking, which provides a chance for nearby bacteria to enter the canal and damage keratin. It ultimately affects the mucous membrane that protects the inner side of the canal. The mammary gland is the second defense mechanism after bacterial entry in the teat canal. The bacteria start multiplying and produce toxins after reaching the gland. However, the gland stimulates the release of inflammatory mediators, which attract phagocytes to counter pathogens. Pathogen and host factors determine the inflammatory response severity. Host age, lactation stage, SCC, immune status, and parity are crucial in determining disease severity [52].

Similarly, pathogen species, strain, inoculum size, and virulence also determine the disease severity. The population of leukocytes and somatic cells increases in the milk after the inflammatory response. Dead mammary epithelial cells and leukocytes are secreted in the milk where they form aggregations leading to clot formation. These clots block the duct and prevent milk excretion. Finally, the scars are formed in small pockets, which are difficult to treat with antibiotics. The practices such as improper animal preparation for milk stimulation, usage of mastitis-infected cannula and tubes, excessive milking, physical trauma, improper udder washes, handling wet teats without teat dips, and injuries of pathogens and their toxins increase the trauma to mammary glands. Mammary epithelium swells in response to persistent inflammation, which cannot be externally detected. Inflammation damages and deforms the alveoli gland. Extracellular fluid components (hydroxide, potassium, hydrogen, chloride, and sodium ions) enter the gland after the breakage of the blood-milk barrier. Then, they mix with milk and can also contain blood in case of severe damage [52] (Figure 3).

Figure 3. Schematic illustration of pathological insights into camel



mastitis

Bacteria Involved in *Camelus dromedarius* Mastitis

Gram-positive and Gram-negative bacteria, algae, mycoplasmas, and yeasts are the common source of camel mastitis [53]. Bacterial pathogens include Gram-negative bacteria (*Klebsiella pneumoniae* and *Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus, Streptococcus uberis*, and *Streptococcus agalactiae*) [54]. These microorganisms are also common human pathogens. Depending on the farm environment, multiple pathogens can be involved in mastitis [55]. Mastitis is also categorized as contagious or environmental mastitis. The contagious mastitis is transmitted by the pathogens (*Streptococcus uberis, Streptococcus agalactiae,* and *Staphylococcus aureus*) residing on/inside the skin/udder. The transmission occurs via sprays or splashes during milking and milk cross flow between teat cups with the operator's hands. Contrarily, soil bacteria transmit environmental mastitis through water, calving pads, manure, and bedding. These bacteria include *Streptococcus dysgalactiae*, *Streptococcus uberis,* and Coliforms (*K. pneumoniae* and *E. coli*) [54].

The same bacteria have been reported to infect camel's udder and cause mastitis in other animals [54]. Several bacteria are known to cause mastitis in bovine, goats, and sheep [54,56,57]. During a mastitis case, only a few pathogens were detected in the camel milk [58, 59,60]. *Micrococcus* spp., Streptococcus *agalactiae*, *Candida albicans,* Aerobacter*, Escherichia coli*, *Arcanobacterium* spp., *Staphylococcus aureus*, *Bacillus cereus, Corynebacterium* spp., and *Diplococcus pneumonia* are commonly involved in camel mastitis [61-65,59].

A survey in Eastern Saudi Arabia (1987 to 1985) depicted a decline in mastitis [66]. During that period mastitis was ranked at number six in comparison to other camel diseases. In 2011, a study was conducted in Al-Jouf Saudi Arabia to estimate subclinical udder infections. 120 milk samples were collected from 30 healthy camels and analyzed through SCC and culture techniques according to California Mastitis Test (CMT) [67]. Data revealed the involvement of Gram-positive cocci in udder infection. Average SCC in healthy camels was noted as 125,000 cells/mm^3 whereas the counts were much higher in infected camels. The study detected the presence of various organisms in milk samples including Gram-negative rods (4.3%), *Streptococcus spp.* (42.9%), *Staphylococcus aureus* (7.1%), *Escherichia coli* (12.9%), Micrococci (5.7%), and other Staphylococci (27.1%) [67].

The detection of subclinical mastitis is difficult and requires different laboratory tests. The detection of milk pathogens is the most accurate technique for detecting subclinical mastitis [68]. Serum Albumin, Adenosine Triphosphate (ATP), and SCC tests are also applied for its detection [62]. N-acetyl-ß-D Glucosaminidase and electrical

conductivity methods are not recommended for the diagnosis of camel mastitis [34]. A prompt detection followed by therapeutic measures can significantly restrict clinical mastitis. Subclinical mastitis often remains undetected, which could spread the pathogens to other lactating females [69,70]. Different genotyping and phenotyping techniques have been developed during the last two decades to investigate mastitis-causing bacteria in dairy cattle at subspecies and species levels. The genotyping methods include PCR, simple restriction digest, whole genome sequencing, and micro-arrays. These approaches can characterize mastitis-causing bacteria at the molecular level up to the subspecies level. They further facilitate in better understanding of pathogenic transmission routes, sources, virulence features, biological relationships, and their resistance against antimicrobial products [71].

The molecular techniques have helped in studying bovine mastitis-related pathogens. Molecular epidemiological investigations using online databases (multi-locus sequence typing and library typing) have revealed the host-adaptation mechanisms of major human and cattle pathogens [53]. Whole genome sequencing and virulence gene arrays have further enhanced the understanding of pathogenic evolution and adaptation to the host and its mammary glands. Electrophoretic banding patterns-based comparative typing methods are commonly followed in veterinary diagnostic laboratories. They help in applying molecular epidemiology methods for the farm and outbreak investigations. Data from these studies are also available for farm advisors and dairy veterinarians. However, the advancements in molecular epidemiology have not contributed much to controlling mastitis. Mastitis incidence can only be reduced through continuing efforts of herd manager, farmers, and their staff. However, molecular epidemiology has helped in better understanding pathogenic transmission routes and sources of mastitis. Molecular studies have also revealed the mechanisms of disease occurrence, host adaptation, infection-related pathogenic evolution, pathogenesis, and disease prognosis. The advances in molecular evolution studies have helped in controlling goat, sheep, camel, and bovine mastitis. Several molecular studies have contributed to developing mastitis diagnostic tools. Furthermore, they facilitate disease prognosis to assess the severity, duration, and cure. Recently, Whole genome sequencing of major mastitis pathogens has been performed. Molecular studies along with metabolomics, host genomics, proteomics, and transcriptomics can provide a comprehensive understanding of mastitis biology. This could further help in developing vaccines and understanding the potential risk of genetic material exchange between pathogen and host [72-76].

Treatment and control of *Camelus dromedarius* Mastitis

Daily intra-mammary infusion of antibiotic preparations has been recommended in various studies to treat camel mastitis. However, the anatomy of the Camelidae udder and the difficult administration procedure limits its applicability [67]. The use of anti-inflammatory drugs (flunixin meglumine) and systemic antibiotics (Aminoglycoside/ penicillin or trimethoprim–sulfamethoxazole) with regular stripping of mammary glands are the main mastitis-treating therapies. Hydrotherapy can also be effective in decreasing local edema. Camel udder teat contains three separate teat canals, which independently open into the teat sphincter. Separate gland complexes are drained by separate canals [77]. Therefore, for effective intra-mammary mastitis treatment, each gland complex of each quarter should be separately treated (one intra-mammary tube/ gland complex). The intra-mammary treatments should be carefully performed in camels because teat canal openings are comparatively smaller in camels than in cows that require a smaller cannula. The traumatic and unhygienic intra-mammary application could be more harmful than beneficial. Chronic mastitis is difficult to treat and could lead to the loss of an infected quarter [78]. *Streptococcus agalactiae* (Lancefield type B) based IMI in camels is commonly detected in the UAE, Somalia, Sudan, and Egypt [79-81,77]. *Streptococcus agalactiae* prevalence in camel dairy herds (50%) in Northern Kenya has emerged as a serious hazard for the owners. A successful parenteral camel mastitis treatment case has been reported [55]. However, published recommendations for camel mastitis treatments still require validation [82].

There are no specific recommendations for antibiotic administration in camels and the doses used are similar to the horses and cows [83]. This could generate undesirable effects leading to the camel's mortality . Therefore, establishing special diagnostic laboratories for camel diseases is important, which could better reveal camel-related bacterial resistance [84]. A survey in Oman demonstrated that oxytetracycline was the most commonly used antibiotic followed by tylosin, sulfonamide/trimethoprim, streptomycin-penicillin, and enrofloxacin [84]. In Saudi Arabia, the literature contains only a few published reports. The government reports depicted a rise in mastitis cases at camel farms because of hand milking leading to udder inflammation. Two Saudi Arabian government reports have revealed the detection of *Pasteurella* spp., *Streptococcus spp., Klebsiella spp. Diplococcus pneumonia,* Enterobacteriaceae, *Staphylococcus aureus*, *Corynebacterium bovis*, *Bacillus cereus*, and *Escherichia coli* from mastitis suffering camels (www.moa.gov.sa). The infection rate ranged between 10% – 50% where females were susceptible to chronic or acute mastitis. An acute form of mastitis has been noted in Saudi Arabian camels after a few days of parturition, caesarian section, or difficult birth. Similar to other regions, the mastitis symptoms in Saudi Arabia also include inflammation, fever, appetite loss, depression, and udder pain, which makes the mother avoid nursing her offspring. The camel milk becomes watery, reddish, or yellowish whereas the udder is reduced to a quarter with high SCC in milk during chronic mastitis. Chronic mastitis treatment is a complicated process that results in the loss of 25% of infected camels. Therefore, early diagnosis and treatment are important whereas injections into the udder are not recommended because of its special tissue structure [85]. Antibiotic injections (intravenous or intramuscular) are administered to treat acute mastitis including streptomycin, trimethoprim/sulphamethoxazole, flunixin meglumine, and penicillin. Sulphamethoxazole injection in the infected udder along with periodic milk removal can reduce the edema and inflammation [86].

In short, proactive measures should be adopted for the prevention of mastitis transmission. The dry or non-lactating phase is the ideal time for mastitis treatment when a success rate of 70% has been reported against Streptococci infections. It involves thorough washing of the farmer's hands with soap and water and sanitizing and washing of udder and teat before milking [87]. Curative and preventive herbal treatments have been developed in some countries, which are passed down from one generation to the next generation [88]. In this regard, nine plants were evaluated in Ethiopia for their anti-mastitis efficacy. Some of these plants exhibited fair potential against mastitis and other diseases. However, a comprehensive characterization of their active ingredients is required to determine a proper application dose [89]. The treatment of *Staphylococcus aureus* associated contagious mastitis is difficult during the lactation period. Therefore, the separation of infected animals from the herd is advisable. Contrarily, *Streptococcus dysagalctiae,* and *Streptococcus agalctiae* infections are easy to treat with antibiotics and good sanitation practices [90]. Mastitis transmission among herds can be prevented by ensuring a dry and clean farm environment, implementing effective farm sanitation procedures, and administrating antibiotics. Teat protection from pathogens is an important preventive measure as it can contract these agents through bedding, milking, and therapy administration process. Mud, manure,

or settled water serve as the pathogenic reservoir. Therefore, bedding should be dry and clean from food remains. Inorganic bedding could also reduce bacterial transmission. Teat dip in a sanitizing solution before and after milking is also an important preventive step to reduce mastitis-related pathogen transmission. The use of clean milking machines and washing of animal teats before milking also reduces pathogen transmission [91].

CONCLUSION

Camel mastitis has emerged as a global challenge during the last decade. Overall, the camel community experiences unhygienic conditions and a lack of health awareness and infrastructure, which are probably the key factors in mastitis emergence. The importance of camels as a multipurpose animal in these countries can be deduced from the previous data. Camels are important for their survival in harsh desert climates. Therefore, camel mastitis should be investigated in detail for developing effective antibiotic therapy and avoiding pathogenic resistance. Mastitis control should be a priority for all livestock owners and farm holders to avoid economic losses.

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