

## Review on Mycotic Cause of Mastitis in Dairy Animals

Megersa Diriba and Bruk Abraha

Haramaya University College of Veterinary Medicine Ethiopia

**Abstract:** Mastitis is a common disease of dairy animals which has multiple and complex etiology. It is the most important factor that contributes for reduced milk production and increased losses in dairy farm in different expenses, which will be in charged for treatment cost, labor, veterinary and other costs that could affect the profitability of the dairy farm. It is on world wide spread in dairy herds and associated with a significant reduction in milk yield resulting in increased costs of production and deteriorated quality of milk and milk products. The most frequent isolated organisms among the mycotic cause of mastitis are the *Candida* species which are a group of unicellular opportunistic organisms, ever present in the natural surroundings of dairy cattle are normal inhabitants of the skin of the udder and teats, in which they exist in low numbers. According to some literature data, fungal infections in dairy animals account for 2–13% of all cases of mastitis in dairy animals and the frequently encountered fungi species in the pathology of mycotic mastitis in cows are *Aspergillus* spp., *Trichosporon* spp., *Cryptococcus* spp., *Penicillium* spp., *Cryptococcus neoformans*, *Rhodotorula* spp. and *Geotrichum candidum*. Mycotic mastitis has become an increasing problem in animals and humans due to the wide use indiscriminate antibiotic therapy contributory factor for fungal super infection of udders. Research aimed on investigation of mycotic mastitis is required in relation to the hygienic and management practices as well as pattern of antibiotic therapy adopted for the treatment of these cases and also effort should been encouraged to apply substitutes of antimicrobials such as probiotics and bioactive natural compounds for prophylactic and therapeutic use since mastitis has high public health hazard. Several authors in different country have reported the mycotic mastitis disease. But in Ethiopia still fungus is listed in etiology of mastitis however, there is on compiled information relating to its magnitude and risk factors. Good hygiene and sanitation practices of animal farm and judicious use of antibiotics will lower incidence of bovine mycotic mastitis. Further investigation regarding their pathogenicity and contribution to bovine mycotic cause of mastitis is needed.

**Key words:** Antibiotic Therapy • Mycotic Mastitis • Dairy Animals • Fungus and Treatment

### INTRODUCTION

Mastitis is a multi-etiological disease of dairy animals which is inflammation of the parenchyma of the mammary gland. The source of mastitis infection may be regarded as contagious or environmental. Contagious pathogens except some that invade the cow's udder after bacteremia are spread during milking. Most other species are opportunistic invaders from the cow's environment. The primary reservoir of infection is the mammary gland; transmission occurs at milking hand. Environmental pathogens are commonly known to cause clinical mastitis in most dairy herds. The bedding used for housing cattle is the primary source of environmental pathogens in

addition to other contaminated fomites as well as skin and teat lesions and vector parasite [1]. Fungi are on world wide spread in nature, being noted in bedding and gear from the stables on milking machines [2]. Yeasts and filamentous fungi infections are considered as the common cause of the bovine mycotic mastitis, which may infect small percentage of cows with possibility of being an outbreak in cattle based on the number and type of the infectious agent in mammary glands [3]. Fungal infections account for up to 2 to 13% of all cases of mastitis in cows [4]. Usually mycotic mastitis is unnoticed by clinician in the first attempt of treatment and administration of antibiotics may aggravate fungal mastitis as some of the antibiotics like penicillin and tetracycline act as a source

of nitrogen for various species of fungi [5]. Kitamura *et al.* [6] reported pseudohyphae, blastoconidia and hyphae in the mammary tissue and infiltration and granulation tissue in chronic mastitis due to *Candida maltosa* in a cow.

Mastitis is a serious problem that reduces milk production, affects the quality of milk and leads to public health hazards, results in the death of animals and consequently leads to economic losses [7]. The cost of clinical mastitis (CM) is direct cost discarded milk, cost of medicines and labor cost and indirect costs loss of future production and increased culling [8]. Prevalence of subclinical mastitis (SCM) in Ethiopia and other African regions may impose substantial costs due to indirect losses [9]. In Ethiopian highland crossbreed dairy cows have economic loss due to SCM. However, most dairy farmers in the country normally do not recognize subclinical mastitis, which incidentally occurs at a much higher frequency than clinical mastitis, while quite few ignore the disease [10]. Mastitis is a complex disease that interacts with microorganisms, host and the environmental factors. Methods commonly employed for diagnosis of mastitis are screening tests, bacteriological examination and physical examination [11]. In addition to the apparent clinical symptoms in animals, the diagnosis of mycotic mastitis can be conducted by examination directly in the preparation of disease-causing agents through a staining preparation. Examination of cultures which were inoculated on agar medium can also be done, however it requires quite a long time [12]. Fungus *Histoplasma farciminosus* can take the form of yeast fungus (spores) at temperatures of 37°C and the mycelium at 25-30°C (Dimorphic mold). In the media Sabouraud dextrose agar (SDA) the fungi produce short hyphae and irregular in shape, fungal hyphae surrounds the body of fungi, then eventually form a distinctive oval structure [13]. Diagnosis by using serological technique can save time. It includes blood agglutination test, fluorescent antibody test, ELISA test and hypersensitive skin test [12].

Treatment with antibiotic in case of bacterial mastitis can result in higher mycotic udder inflammations rate [14]. Mastitis treatment can be administered by inframammary infusing of antibiotics into the udder through the teat canal and parenteral treatment given by injection [15].

Mycotic mastitis have been reported in various parts of the world affected by mastitis, including Brazil, Poland, New Zealand and Tanzania [14, 16, 17, 18]. However, mycotic mastitis is poorly characterized given the few studies conducted to characterize fungi in the context of

this disease. In contrast, most studies have focused on prokaryotic etiologies, especially staphylococci. As a result, it remains unclear which fungal species is predominantly causing mycotic mastitis. Nonetheless, there is no compelling evidence to suggest that mycotic mastitis may not develop into a costly disease, as is the case with bacterial mastitis, in any part of the world, but may certainly become a farm specific problem. Since mycoses caused by fungi are common in mammals, such as humans and other warm-blooded animals, the possibility of mycotic mastitis being costly should not be ignored [19]. Several authors in different country have reported the mycotic mastitis disease. However, there is no compiled information relating to its magnitude, risk factors and economic importance. Therefore the objective of this review is to generate general information on the status of mycotic cause of mastitis and magnitude of bovine mycotic mastitis in dairy animals.

#### Literature Review

**Definition of Mastitis:** Mastitis is inflammation of the parenchyma of the mammary gland regardless of the cause. Mastitis is therefore characterized by a range of physical and chemical changes in the milk and pathological changes in the glandular tissue primarily result from invasion of pathogenic microorganisms through the teat canal. Characterization of mastitis depend on identification of the causative agent whether it be infectious or physical [11]. The classic meaning of the word mastitis is derived from the Greek word “Mastos” meaning breast or udder and the suffix “itis” meaning inflammation. It also defined as an inflammation of the mammary gland, almost invariably due to the effects of bacterial or mycotic pathogens. It is characterized by a physical, chemical and usually bacteriological change in the milk, as well as pathological changes in the glandular tissue [20]. Mastitis is one of the most complex diseases of dairy cows that generally involve the interplay between management practice and infectious agents, having different causes, degree of intensity and variation in duration and residual effect [21].

**Etiology of Mycotic Mastitis:** Among more than 72, 000 described species of fungi that are widespread in nature (soil, plants, water, air), over 300 are now recognized as real or potential pathogens responsible for mycoses in humans and animals [19]. The incidence of mastitis due to yeast sometimes occurred after inframammary infusion of antibiotics. The large doses of antibiotics may cause a reduction in the vitamin A, leading to injury to the udder’s

epithelium, thus facilitating the invasion of fungi [22]. Teat injuries may facilitate a yeast infection too [23]. Sometimes mixed infection of fungi and bacteria can occur in mastitis case [18, 24]. Fungi are opportunistic organisms that are considered as normal flora in the udder skin and soil but are able to establish disease when immune system is weak [25].

Mycotic mastitis had been documented to be caused by various genera of yeasts. In most cases, fungal infections of the mammary gland are mostly caused by yeast, the main genus of which is *Candida* [18]. However, the most frequently encountered species are *Candida* species, *Trichosporon* species, *Cryptococcus* species, *Saccharomyces* species, *Aspergillus* species, [4, 26]. Yeast infections of the mammary glands are responsible for 2–3% of clinical mastitis. *Aspergillus fumigatus* and *A. nidulans* have also been described as causal agents of bovine mastitis [27]. In over the quarters of cases, the cause of bovine mycotic mastitis is *Candida* species such as *C. albicans*, *C. glabrata*, *C. kefyr*, *C. tropicalis*, *C. krusei* and *C. parapsilosis*. Most of them are able to grow at 40°C [2]. *Cryptococcus neoformans* [28] and *Candida albicans* are by far the commonest causes but other *Candida* species have also been associated with bovine mastitis [29]. Usually mycotic mastitis is unnoticed by clinician in the first attempt of treatment and administration of antibiotics may aggravate fungal mastitis as some of the antibiotics like penicillin and tetracycline act as a source of nitrogen for various species of fungi [5].

#### **Modes of Transmission and Source of Mycotic Mastitis:**

Yeast is a microorganism, which may be found on a wide variety of substrates such as soil, plants and water. Most of these organisms are opportunists and sources of infection include the skin of the udder, udder secretion [27]. The most frequent isolated organisms among the mycotic mastitis are the *Candida* specie [5] which are a group of unicellular opportunistic organisms, ever present in the natural surroundings of dairy cattle environment and are normal inhabitants of the skin of the udder and teats, in which they exist in low numbers [25]. It is a direct pathway for causing mastitis by invading mammary glands cells. This disease has many symptomatic signs like strong pain in udder, continued fever, tenderness, deep inflammation in the mammary glands and lymph nodes and decrease of milk and bad quality [30]. Critically, animals may die due to intra-mammary cells infection by fungus such as *Aspergillus fumigatus* and *Candida* species [22]. Wrong antibiotic administration may lead to outbreaks of mycotic mastitis because of rising

subsequent infection during drying-off period [31]. Lack of hygienic conditions for the used milking and feeding equipment are the main reason of teats and milk ducts contamination [32].

#### **Epidemiology of Fungal Mastitis in Dairy Animals:**

Mastitis is a worldwide problem affecting mainly lactating animals but during their dry periods as well. It affects a wide range of hosts; different breeds, age, parity, lactation stage and milk yield categories [33]. Mastitis is one the factors contributing to reduce milk production [34]. The contributions of the dairy sector especially the smallholder system in Ethiopia to poverty alleviation and sustainable food production in the country is assumed considerable. However, among many factors the sector is constrained by mastitis, which incurs serious economic losses to the dairy industry [35]. Fungal infection of bovine mammary tissue is attributable to super infection by certain fungal species as consequence to strict mastitis control program that render natural udder immunity quiescent. Indiscriminate antibiotic therapy might also be a contributory factor for fungal super infection of udders. Moreover, factors like micronutrient inadequacy particularly that of vitamin A and zinc in cattle are precipitating. Contamination of teat dips, intermammary infusion of antibiotics and moldy surroundings play significant role. Fungi are opportunistic organisms that are considered as normal flora in the udder skin and soil but are able to establish disease when immune system is weak [25]. This might be due to unhygienic condition of the animal sheds and high humidity along with favorable environmental conditions supporting growth of fungal spores. Hence favorable conditions increase the chances of fungal spore to enter into the udder which provide suitable environment to these fungi [16]. Weakness of the cow immune system may result from several factors like; changeable weather, mineral-vitamin deficiencies and antioxidant deficiencies [14]. Relatively high isolation of fungi from mastitis cases suggested potential unhygienic conditions and poor management practices to be associated. Moreover, fungal association can be attributed to prolong treatment with antibiotics [12].

Widely used antibiotics increased incidence of mycotic bovine mastitis. Treatment with antibiotic in case of bacterial mastitis can results in higher mycotic udder inflammations rate [14]. Elimination of antagonistic bacterial flora during antibiotic therapy stimulate fungi to multiplication and also decreased amounts of vitamin A (antioxidant) in the glandular tissue and epithelium have positive impact on fungi growth [14].

Table 1: Fungi isolated from the mammary secretion of lactating cows and buffaloes

Genera	Species
<i>Candida</i>	<i>C. albicans</i> , <i>C. beigelii</i> , <i>C. brumptii</i> , <i>C. catenulata</i> , <i>C. ciferri</i> , <i>C. freyschusii</i> , <i>C. famata</i> , <i>C. glabrosa</i> , <i>C. globosa</i> , <i>C. guillermondii</i> , <i>C. hemuloni</i> , <i>C. humicola</i> , <i>C. inconspicua</i> , <i>C. ingens</i> , <i>C. intermedia</i> , <i>C. krusei</i> , <i>C. lusitaniae</i> , <i>C. lambica</i> , <i>C. membranaefaciens</i> , <i>C. mogii</i> , <i>C. norvegensis</i> , <i>C. parakrusei</i> , <i>C. parapsilosis</i> , <i>C. pelliculosa</i> , <i>C. rugosa</i> , <i>C. shehate</i> , <i>C. solani</i> , <i>C. sorbosa</i> , <i>C. stellatoidea</i> , <i>C. tenuis</i> , <i>C. kefyr</i> , <i>C. tropicalis</i> , <i>C. zeylanoides</i> , <i>C. variabilis</i> , <i>C. valida</i>
<i>Rhodotorula</i>	<i>R. glutinis</i> , <i>R. minuta</i> , <i>R. rubra</i>
<i>Trichosporon</i>	<i>T. cutaneum</i> , <i>T. asahii</i>
<i>Cryptococcus</i>	<i>C. albidus</i> , <i>C. curvatus</i> , <i>C. flavus</i> , <i>C. laurentii</i> , <i>C. luteolus</i> , <i>C. neoformans</i>
<i>Aureobasidium</i>	<i>A. pullulans</i>
<i>Pichia</i>	<i>P. ohmeri</i> , <i>P. membranaefaciens</i> , <i>P. farinosa</i> , <i>P. toletana</i> , <i>P. rhodanensis</i>
<i>Geotrichum</i>	<i>G. candidum</i> , <i>G. capitatum</i>
<i>Saccharomyces</i>	<i>S. fragilis</i> , <i>S. marxianus</i>
<i>Debaromyces</i>	<i>D. hansenii</i>
<i>Hansenula</i>	<i>H. fabianii</i> , <i>H. holstii</i> , <i>H. polymorpha</i> , <i>H. anomala</i>
<i>Aspergillus</i>	<i>A. fumigatus</i> , <i>A. flavus</i> , <i>Aspergillus</i> sp.
<i>Penicillium</i>	<i>P. chrysogenum</i> , <i>P. cyclopium</i> , <i>Penicillium</i> sp.
<i>Alternaria</i>	<i>Alternaria</i> species
<i>Epicoccum</i>	<i>Epicoccum</i> species
<i>Phoma</i>	<i>Phoma</i> species

Source: [36]

**Risk Factors for the Mycotic Mastitis in Dairy Animals:**

Mastitis is a complex disease and the difference in its prevalence and distribution might be due to differences in the breeds of cattle reared, lactation stage, parity, age of the animal, the geographical locations, weather conditions, the management system of the farm and previous episodes [1].

Mycotic mastitis is generally believed to result from an ascending infection subsequent to incorrect administration of antibiotic preparations during drying-off period [31]. The use of antimicrobials for a long period is pointed out as the main factor that propitiates the occurrence of mycotic mastitis because they affect the micro flora of mammary gland. Infections by yeasts have been known both in animals and humans for years. Although the majority of mycotic mastitis in cows is mild, some cases may result in death. Excessive and erratic use of antibiotics, corticosteroids, immuno-suppressive drugs and chronic diseases are the major contributing factors in increasing the incidence of diseases due to yeasts [37].

The other risk factor is contamination of the teat end or cannulas by environmental yeasts and fungi associated with lack of hygiene during the milking and poor equipment cleaning leads could favor further penetration into the mammary gland [34]. The large doses of antibiotics may cause a reduction in the vitamin A, leading to injury to the udder’s epithelium and affecting the microflora of the mammary glands, which acts as an animal natural defense, thus facilitating the invasion of

fungi and yeasts [30]. Relatively high isolation of fungi from mastitis cases suggested potential unhygienic conditions and poor management practices to be associated. Moreover, fungal association can be attributed to prolong treatment with antibiotics [12]. Fungi are opportunistic organisms that are considered as normal flora in the udder skin and soil but are able to establish disease when immune system is weak [25]. Weakness of the cow immune system may result from several factors like; changeable weather, mineral-vitamin deficiencies and antioxidant deficiencies [14]. In fact, large doses of antibiotic without bacteriological examination cause vitamin A deficiency that damage the udder’s epithelium and teat injuries can facilitate infection by yeast [38].

**Status and Significance of Mycotic Mastitis in Ethiopia:**

The contributions of dairy sector for smallholder poverty alleviation are considerable to be high. However, many factors are constrained by disease like mastitis especially subclinical one [39]. Usually Ethiopian farmer’s especially smallholders are not well informed about the invisible loss from sub clinical mastitis since dairying is mostly a sideline business among these farmers [34]. As mentioned in the above table (1), different species of fungi were isolated from cow milk. So that it indicates that mycotic mastitis is a serious problem. The percentages of the yeasts and molds, isolation from mastitis milk considerably in many countries are listed in below (Table 2).

Table 2: General epidemiology of bovine mycotic mastitis in different country and year

Country	Years	Prevalence (%)	Author
Brazil	1993	12.07	[26]
Sudan	2003	6.8	[40]
Saudi Arabian	2009	1.8	[41]
Tanzania	2009	16.7	[17]
Poland	2010	26.4	[42]
Egypt	2011	25.2	[43]
Iraq	2013	80	[44]
India	2013	64	[12]
Alexandria	2015	83.78	[45]
Ethiopia	2015	4	[46]
Algeria	2018	32.3	[47]
North Greece	2018	6.9	[48]
Ethiopia	2019	38.18	[49]
Iraq	2020	46.4	[24]

Table 3: Yeast species identified from milk of cows with clinical mastitis and sub-clinical mastitis in Sebeta town, Ethiopia

Identified yeasts (n = 220)	Number (%)
<i>Candida etchellsii</i>	16(7.3)
<i>Candida edax</i>	8 (3.6)
<i>Candida heamulonii</i>	2 (0.9)
<i>Yarrowia lipolytica</i>	24 (10.9)
<i>Rhodotorul agraminis</i>	5 (2.3)
<i>Rhodotorula glutinis</i>	2 (0.9)
<i>Rhodospiridium diobovatum</i>	5 (2.3)
<i>Galactomyces geotrichum</i>	8 (3.6)
<i>Geotrichum terrestre</i>	3 (1.4)
<i>Trichosporon</i> species	7 (3.2)
Saccharomyces species	4 (1.8)
No growth	136 (61.8)

Source: [49]

Table 4: Mold species identified from milk of cows with clinical mastitis and sub-clinical mastitis in Sebeta town, Ethiopia

Identified filamentous fungi Species (n=220)	Number (%)
Aspergillus species	15 (6.8)
Mucor species	13 (5.9)
Penicillium species	8 (3.6)
Fusarium species	8 (3.6)
No growth	176 (80)

Source: [49]

For the first time camel mastitis was reported by Pal [46] *Candida* species (4%) from the she camel in Ethiopia. The other one was from the current study as report by Tesfaye Bekele *et al.* [49] in Ethiopia around sebeta town over all prevalence was 38.18% which is listed in table (3). In other country yeasts and mycelial fungi (6.92%) were reported by Amel [40] in Sudan, 32.4% fungus prevalence from that yeast 29.54% and 3.4% filamentous fungi was reported by Akdouche *et al.* [47] in Algeria and Spanamberg *et al.* [31] isolated 68 species of yeast in Brazil and the most frequent genera were

*Candida* (37.9%), *Pichia* (19.1%), *Cryptococcus* (10.3%) and *Rhodotorula* (10.3%). The management practices adopted on dairy cows, like discarding first few strips of milk on ground while milking of animals as well as during treatment of mastitis animals and reluctance to disinfect hand between milking by milkers may contribute as potent source of lateral transmission of fungal and yeast infections [12]

**Pathogenicity of Fungi:** The ability of the fungus to attach to host tissue, the production of photolytic enzymes (secretary aspartyl proteases SAP), leucine aminopeptidase and immunomodulatory effects of fungal determinants are the virulence traits of fungi. These virulence factors have been attributed with possible roles in the pathogenesis of candidacies. Adhesion to tissues was found to be dependent on environmental conditions affecting the fungus [50]. Mastitis most commonly begins because of penetration by pathogenic microorganisms through the teat canal and establishment into the interior of the mammary gland and it can be explained using three stages, namely, invasion, infection and then inflammation [51]. In mycotic mastitis, the probable route of infection is ascending infection via the teat but mastitis may also arise via haematogenous spread following ingestion or inhalation of spores. Damage to the teat end caused by poor practice at milking predisposes to ascending infection. Antibiotic therapy could predispose to fungal infection due to introduction of spores when administering antibiotic therapies inframammary infusion. In addition, *candida* infections can arise due to the prolonged use of antibiotics creating an enhanced environment for their growth [11]. *Candida* species unicellular opportunistic organisms are found normally around dairy cattle environments like milking machines, treatment instruments, floors, hay, feeding equipment and they are also found naturally on skin (udder and teats) in low habitants [25]. It is a direct pathway for causing mastitis by invading mammary glands cells. This disease has many symptomatic signs like strong pain in udder, continued fever, tenderness, deep inflammation in the mammary glands and lymph nodes and decrease of milk produce and bad quality [30]. Critically, animals may die due to inframammary cells infection by fungus such as *A. fumigatus* and *Candida* species [22]. The ability of *C. albicans* to invade multiple organs of the host was attributed to wide range of virulence factor [52]. In particular adhesion and hydrolytic enzymes secretion appear to be critical for pathogenicity [52]. Additionally, using inappropriate

doses of antibiotics have a negative impact on animal health due to causing a vitamin A deficiency and killing the micro flora on udder epithelium cells that are important as an animal natural defense to prevent the fungal and yeast invasion [30].

**Economic Importance's:** Mastitis is a serious problem that reduces milk production, affects the quality of milk and leads to public health hazards, results in the death of animals and consequently leads to economic losses [7]. Costs due to mastitis include reduced milk production, condemnation of milk due to abnormal color, odor and test and due to antibiotic residues after treatment, reduced quality of milk in terms of some desired contents of milk responsible in determining quantity and quality of milk by-products, veterinary costs, culling of chronically infected cows and occasional deaths [53]. Economic loss to mastitis in the United States is estimated to be approximately \$ 185/cow annually. If we assume the same milk a total number of milking cows (9.5 million head) multiplies price and their value, the annual cost of loss per lactation for one infected quarter is about 1, 600 pounds [54]. Ethiopia produces approximately 3.2 billion liters from 10 million milking cows and average of 1.54 liters per cow per day over a lactation period of 180 days [55]. While investigating the total milk production losses in Ethiopia accounted for 78% caused by mastitis [56]. The economic losses from mastitis in the urban and per urban area of Addis Ababa are USD 58 and 78.65 per cow per lactation respectively [10, 49]. Generally, milk and milk products play a very important role in feeding the rural and urban population of Ethiopia and have a high nutrition value and is daily produced, sold for cash or readily processed. It is a cash crop in the milk-shed areas that enables families to buy other foodstuffs, contributing significantly to the household food security [57].

**Diagnosis of Mycotic Mastitis:** The udder, teat orifice and milker's hands are perfectly cleaned with water and soap and disinfected with 70% ethyl alcohol before collection of milk samples. The first streams of milk are discarded and about 20 ml of milk are collected in clean sterile capped bottle, each bottle was given a serial number. Samples are refrigerated 4°C during transportation to the laboratory and examined mycological and bacteriological as soon as possible [11]. Milk from cows with healthy udder should contain <200, 000 somatic cells count (SCC) in 1 ml of milk. SCC is an indicator of the udder health problems. Most somatic cells are white blood cells (e.g. macrophages and neutrophils) that influx mammary gland tissue from blood during inflammation [58].

**Mycological Examination and Fungal Identification:**

Samples from cows with a typical symptoms of clinical mastitis are centrifuged at 3000 rpm for 20 min for mycological examination then the cream, supernatant fluid were discarded, the sediment was streaked on Sabouraud dextrose agar, pure cultures are prepared from suspected colonies of yeast and molds. Shape, size and type of colony either *Candida albicans* or *Cryptococcus neoformans*. In case of mold based on the rate of growth, general topography, texture, surface pigmentation and reverse pigmentation. Gram stained film from the purified isolates of yeast were made on clean slides and examined microscopically for detecting their stain and morphological characters which appears distinctly round, oval or elliptical. Appearance of hyphae is distinct points of constriction simulating link sausages (pseudo-hyphae) with budding yeast forms (blastopores) often seen. In case of mold microscopic examination done by adhesive tape technique stained by lacto phenol blue and slide culture must always be carried out to confirm presumptive identification of colonies [59]. Yeast colonies are examined microscopically using Gram staining and species level identification is conducted by Omni log identification system using yeast microplates [60].

**Treatment:** Treatment of mycotic mastitis is a challenge, as many of these fungi do not respond to the antibiotics therapy rather they use some of the antibiotics like tetracycline as their source of energy [5]. *In vitro* antimycotic sensitivity of yeasts isolated from infected bovine mammary glands was studied in yeast cultures. These cultures were most sensitive to clotrimazole followed by ketoconazole, nystatin, miconazole and amphotericin B and least sensitive to 5 fluorocystine. The most sensitive yeast was *C. lusitaniae* (85.7%) and the least sensitive was *C. rugosa* (31.9%) [61]. Cure rates of 78-80% were obtained [62]. A case of bovine mycotic mastitis caused by *A. fumigatus* was successfully treated by combined intra-arterial and inframammary injection on three successive days of the antifungal drug miconazole. After evening milking 100 mg of miconazole (10 ml) was injected into the right external pudendal artery. Miconazole diluted with 50-ml saline was also infused into the affected udder [63]. As El-Razik *et al.* [43] reported *Lactobacillus rhamnosus* and *Bifidobacterium* isolated from cow milk showed antifungal activities against *C. albicans* and *A. fumigatus* with zone of inhibition equal (12, 10) and (10, 9) mm respectively. Also Feio *et al.* [64] proved that *Bacillus subtilis* inhibited the growth of several fungi. Magnusson *et al.* [65] found that *Lactobacillus salivarius*, *Lactobacillus plantarum* and

*Pediococcus pentosaceus* strains inhibited some fungi. *Lactobacillus acidophilus* indicated that the antifungal effect of these lactic acid bacteria could not simply be assigned to the low pH, but most probably to the formation and secretion of antifungal organic metabolites.

**Control and Prevention:** Control of mastitis requires understanding of its causes and management techniques which limit the spread of infection. The principle of mastitis control is that the disease is controlled by either decreasing the exposure of the teat to potential pathogens or by increasing resistance of dairy animals to infection [15]. Prevention of mastitis can be done easily and does not require expensive cost. Procedures of prevention should be followed: (1) Managing the environment to keep it enough ventilation and clean; (2) to ensure a clean feeding grass, it has to be washed; (3) Time for the treatment of mastitis cases must be suitable with milking period; (4) collecting milk has to go through the properly procedures aseptically way; (5) The treatment by drugs should be completely and treatment area must be clean; (6) Perform the method of dry enclosure (time when cattle is not milked); (7) Cattle with chronic mastitis must be culled; (8) nutrition should be given properly; (9) Consultation with a nutritionist to develop nutrition plans; (11) consultation with the veterinarian [66, 67]. Lactic acid bacteria (LAB) are among the most powerful prokaryotes when it comes to antimicrobial potential. A large number of LAB strains have effective antimycotic effect of the pathogens by depleting nutrients consumed by the pathogens and modulate the host immune response. Also, they release endogenous microbicides compounds including; lactic acid, bacitracin and hydrogen peroxide which have microbicide effect [65]. Probiotic bacteria present in the alimentary tract and vagina of man and animals prevent the overgrowth of *Candida* spp. and thereby decrease the occurrence of mucosal or systemic candidiasis [68, 69].

### CONCLUSIONS AND RECOMMENDATIONS

Mastitis is the most important factor that contributes for reduced milk production and increased losses in dairy farm in different expenses, which will be in charged for treatment cost, labor, veterinary and other costs that could affect the profitability of the dairy farm business and has public health importance. Fungal species were identified as causes of mastitis, hence further investigation regarding their pathogenicity and contribution to bovine mastitis is needed. The environmental contamination associated with lack of

hygiene during the milking and poor equipment cleaning leads to the development of mastitis. Mycotic mastitis has become an increasing problem due to the wide use of antibiotics in mastitis therapy. The effects of mastitis on dairy animals' health and milk production highlight an urgent need to develop effective strategy of prevention and control. The constantly changing predominance of etiological agents in different geographical locations must be considered while adopting and developing mastitis control strategies. Research aimed on investigation on the mycotic mastitis is required in relation to the hygienic and management practices as well as pattern of antibiotic therapy adopted for the treatment of these cases and also effort should be encouraged to apply substitutes of antimicrobials such as probiotics and bioactive natural compounds for prophylactic and therapeutic use since mastitis has high public health hazard.

Based on this concluding remark the following points can be recommended:

- Good hygiene and sanitation practices of animal farm and judicious use of antibiotics will lower incidence of bovine mycotic mastitis.
- Treating mastitis always with antibiotics without confirming cause means treating with antibiotics risk factor for mycotic mastitis.
- Further study should be conducted on the mycotic cause of bovine mastitis and its impact on dairy farm, pathogenicity and contribution to fungal causal agents' bovine mycotic of mastitis is needed.

### REFERENCES

1. Erskine, R.J., 2020. Mastitis in Cattle. MSD Veterinary Manual.
2. Lagneau, P.E., K. Lebtahi and D. Swinne, 1996. Isolation of yeast from bovine milk in Belgium. *Mycopathologia*, 135: 99-102.
3. Pengov, A., 2002. Prevalence of Mycotic mastitis in cows. *Acta Veterinaria Journal*, 52: 133-136.
4. Krukowski, H., A. Lisowski, P. Rozanski and A. Skorka, 2006. Yeasts and Algae isolated from cows with mastitis in the South Eastern part of Poland. *Poland Journal of Veterinary Science*, 9: 18-184.
5. Tarafarosh, M.A. and S.K. Purohit, 2008. Isolation of *Candida* species from mastitis Cows and milkers *Veterinary Scan.*, 3: 14-18.
6. Kimatara, H., A. Anri, K. Fuse, M. Seo and C. Itakura, 1990. Chronic mastitis caused by *Candida maltosa* in a cow. *Veterinary Pathology*, 27(6): 465-466.

7. FAO. (Food and Agriculture Organization), 2014. Impact of mastitis in small scale dairy production systems. Animal Production and Health Working Paper. No. 13. Rome Italy.
8. Huijps, K., T.J. Lam and H. Hogeveen, 2008. Costs of mastitis: Facts and perception. Journal of Dairy Research, 75(1): 113-120.
9. Petrovski, K.R., M. Trajcev and G. Buneski, 2006. A review of the factors affecting the costs of bovine mastitis. Journal of the South African Veterinary Association, 77(2): 52-60.
10. Mungube, E.O., B.A. Tenhagen, F. Regassa, M.N. Kyule, Y. Shiferaw, T. Kassa and M.P.O. Baumann, 2005. Reduced milk production in udder quarters with subclinical mastitis and associated economic losses in crossbred dairy cows in Ethiopia. Tropical Animal Health and Production, 37(6): 503-512.
11. Radostits, O.M., C. Gay, K.W. Hinchcliff and P.D. Constable, 2007. A text book of the diseases of Cattle, Sheep, Goats, Pigs and Horses. Veterinary Medicine 10<sup>th</sup> (ed.) Bailliere, Tindall, London, UK, pp: 1576-1580.
12. Pachauri, S., P. Varshney, S.K. Dash and M.K. Gupta, 2013. Involvement of fungal species in bovine mastitis in and around Mathura, India. Veterinary World, 6(7): 393-395.
13. Jungerman, P.F. and R.M. Schwartzman, 1972. Veterinary Medical Mycology, Lea and Fibiger, Philadelphia.
14. Wawron, W.Ł., M.A. Bochniarz and T. Piech, 2010. Yeast mastitis in dairy cows in the Middle Eastern part of Poland. Bull Veterinary institution Pula Way, 54: 201-204.
15. Blowey, R. and P. Edmondson, 2010. Mastitis control in dairy herds 2<sup>th</sup> (ed.) Treatment and dry cow therapy. International Cambridge United Kingdom, pp: 194.
16. Williamson, J.H. and M.E. Di Menna, 2007. Fungi isolated from bovine udders and their possible sources. NZ Veterinary Journal, 55: 188-190.
17. Mdegela, R.H., R. Ryoba, E.D. Karimuribo, E.J. Phiri, T. Løken, O. Reksen, E. Mtengeti and N.A. Urrio, 2009. Prevalence of clinical and subclinical mastitis and quality of milk in smallholder dairy farms in Tanzania. Journal of the South African Veterinary Association, 80: 163-168.
18. Dworecka-kaszak, B., A. Krutkiewicz, D. Szopa, M. Kleczkowski and M. Biegańska, 2012. High prevalence of candida yeast in milk samples from cows suffering from mastitis in Poland. Scientific World Journal, 1: 196-347.
19. Drouhet, E., 1998. Historical introduction: Evolution of the knowledge of fungi and mycoses from Hippocrates to twenty first century. Medical Mycology. Vol. 4. In Topley and Wilson's Microbiology and Microbial Infections 9<sup>th</sup> (edi.). Arnold London, UK, pp: 1-20.
20. Pretorius, C., 2008. The effect of Corynebacterium of Corynebacterium cutis lysate to control somatic cell counts in dairy cows, Dissertation submitted in accordance with the requirements for the degree Magister Scientiae Agriculture. Available at ed., pp: 77.
21. Harmon, R.J., 1994. Symposium: Mastitis and genetic evaluation from somatic cell count. Journal of Dairy Science, pp: 299-326.
22. Krukowski, H., M. Tietze, T. Majewski and P. Rózański, 2000. Survey of yeast mastitis in dairy herds of small-type farms in the Lublinregion, Poland. Myco-pathologia, 150: 5-7.
23. Gonzalez, R.N., 1996. Prototheca, yeast and bacillus mastitis. In The Proceedings of the National Mastitis Council (USA). Meeting. Nashville, Tennessee, USA, 35<sup>th</sup> pp: 82-92. Make references like this style.
24. Al-Haddadi, W., A. Elshafie, A. Al-Ansari, J. Al-Mawly, R. Al-Hatali, H. Al-Habsi, A. Al-Hshami, and A. Al-Ansari, 2020. Bovine mastitis in Oman is mainly associated with environmental bacteria that show high resistance to commonly use antibiotics. Archives of Microbiology and Immunology, 4: 38-50.
25. Santos, R.C. and J.M. Marin, 2005. Isolation of *Candida* spp. from mastitis bovine milk in Brazil. Revista Ibero AmericanMycopathologia, 59: 251-253.
26. Costa, E.O., C.R. Gandra, M.F. Pires, S.D. Coutinho, W. Castilho and C.M. Teixeira, 1993. Survey of bovine mycotic mastitis in dairy herds in the State of Silo Paulo, Brazil. Mycopathologia, 124: 13-17.
27. Richard, J.L., J.S. McDonald, R.E. Fitchner and A.J. Anderson, 1980. Identification of yeasts from infected bovine mammary glands and their experimental infectivity in cattle. American Journal of Veterinary, 48: 1991-1994.
28. Schalm, O.W., E.S. Carroll and N.C. Jain, 1971. Less common forms of mastitis. Page 275 in Bovine mastitis. Lea and Febiger, Philadelphia.
29. Yeh, S.G., K.Y. Chung and H.T. Cho, 1988. Prevalence of yeasts in bovine mammary gland infections and teat cups of milking machines. Korean Journal of Veterinary, 28: 361.
30. Seker, E., 2010. Identification of *Candida* species isolated from Bovine Mastitis milk and their in vitro hemolytic activity in Western Turkey. Revista Ibero American Mycopathologia, 169: 303-308.



31. Spanamberg, A., J.E.A. Wunder, D.I.B. Pereira, J. Argenta, E.M.C. Sanches, Valente and L. Ferreira, 2008. Diversity of yeasts from bovine mastitis in Southern Brazil. *Revista Iberoamericana de Micología*, 25: 154-156.
32. Gaudie, C.M., P.N. Wragg and A.M. Barbe, 2009. Outbreak of disease due to *Candida krusei* in a small dairy herd in the UK. *Veterinary Research*, 165: 535-537.
33. Rebeka, S., S. Beena, K. Ragini, M.R. Vineeth, V. Archana and I.D. Gupta, 2019. Effect of season, stage of lactation, parity and level of milk production on incidence of clinical mastitis in Karan Fries and Sahiwal cows. *Biological Rhythm Research*.
34. Fufa, A., F. Gemechis, M. Bekele and R. Alemayehu, 2013. Bovine Mastitis: Prevalence, risk factors and bacterial isolation in small holder dairy farms in Addis Ababa City, Ethiopia. *Global Veterinarian*, 10(6): 647- 652.
35. Getahun, K., B. Kelay, M. Bekana and F. Lobago, 2008. Bovine mastitis and antibiotic resistance patterns in Sellale smallholder dairy farms, central Ethiopia. *Tropical Animal Health and Production*, 40: 261-268.
36. Krukowski, H. and L. Saba, 2003. Bovine mycotic mastitis, Department of Animal and Environmental Hygiene University of Agriculture in Lublin, Str. Academic, 13: 20-950.
37. Watts, J.L., 1988. Etiological agents of bovine mastitis. *Veterinary Microbiology*, 16: 41-66.
38. Krukowski, H., M. Tietze and T. Majewski, 2001. Survey of yeast mastitis in dairy herds of small-type farms in the Lublin region, Poland. *Mycopathologia*, 150: 5-7.
39. Shirima, G.M., R.R. Kazwala and D.M. Kambarage, 2003. Prevalence of Bovine Tuberculosis in Cattle in different farming systems in the Eastern Zone of Tanzania. *Preventive Veterinary Medicine*, 57: 167-172.
40. Amel, M.A.M., 2003. Bacteria and fungi isolated from She-Camel mastitis in the red sea area of the Sudan, Master veterinary science thesis. University of Khartoum, Sudan.
41. Fadlilmula, A., A.M. Al-Dughaym, G.E. Mohamed, M.K. Al-Deib and A.J. Al-Zubaidy, 2009. Bovine mastitis: Epidemiological, clinical and etiological study in a Saudi Arabian large dairy farm. *Bulg. Journal of Veterinary Medicine*, 12(3): 199-206.
42. Przemysław, D., K. Krzysztof and K. Maciej, 2010. Adaptation of Microstix-andida Slide-test for Diagnosis of Bovine Mastitis Due to Anascogenic Yeasts. *Acta Veterinaria Journal*, 79: 113-120.
43. El-Razik K.A., K.A. Abdelrahman, S.I. El-Moez and E.N. Danial, 2011. New approach in diagnosis and treatment of Bovine Mycotic Mastitis in Egypt. *African Journal of Microbiology Research*, 5(31).
44. Al-Ameed, A.I., 2013. Isolation and identification of fungi from infected milk samples obtained from cattle with mastitis and studying the antifungal activity of Rosemary Ethanoic extract against the main strains. *Diyala Journal of Agricultural Science*, 5(2): 1-13.
45. Esraa M. Bakr, Abd El-kareem M. Abd El-Tawab, Tharwat M. Elshemey, H. Amir and Abd- Elrhman, 2015. Diagnostic and therapeutic studies on Mycotic Mastitis in Cattle. *Alexandria Journal of Veterinary Sciences*, 46: 138-145.
46. Pal, M., 2015. First Record of Camel Mastitis due to *Candida albicans* in Ethiopia. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, 36(1): 32-34.
47. Akdouche, L., M. Aissi and A. Saadi, 2018. Prevalence and identification of yeasts responsible for mastitis in dairy cattle farms in the SidiLahcene region in the Wilaya of SidiBel abbes- Algeria. *Journal of Advanced Dairy Research*, 6: 206.
48. Bourtzi-Hatzopoulou, E., A. Zdragas, E. Petridou and G. Filioussis, 2018. Yeasts as a causative agent of Bovine Mastitis in Greece. *Journal of the Hellenic Veterinary Medical Society*, 54(2): 105-110.
49. Tesfaye, B., L. Matios, T. Getachew, K. Tafesse, O. Abebe, Y. Letebrihan, T. Mekdes and D. Tilaye, 2019. Study on bovine mastitis with isolation of bacterial and fungal causal agents and assessing antimicrobial resistance patterns of isolated *Staphylococcus* species in and around Sebeta town, Ethiopia. *African Journal of Microbiology Research*, 13(1): 23-32.
50. Sheena, A. and L. Siegler, 1995. *Candida krusei* isolated from a sporadic case of bovine mastitis. *Canadian Veterinary Journal*, 36: 365-365.
51. Radostits, O.M., C.C. Gay, D.C. Blood and K.W. Hinchcliffe, 2000. *Veterinary Medicine, a text book of diseases of Cattle, Sheep, Pigs, Goats and Horses*. 9<sup>th</sup> (ed.) London: W.B. Saunders, pp: 603-660.
52. Mousa, W., W. Elmonir and E. Abdeen, 2016. Molecular typing, virulence genes and potential public health implications of *Candida albicans* isolated from Bovine milk. *Japanese Journal of Veterinary Research*, 64(2): 211-215.
53. Rahmeto, A., H. Hagere, A. Mesele, M. Bekele and A. Kassahun, 2016. Bovine mastitis: Prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Veterinary Research*, 12(1): 270.

54. Schroeder, J.W., 2010. Mastitis Control Program. Bovine Mastitis and Milking Management. North Dakota State University. USA, 701: 231-7708.
55. Tefera, T.L., 2010. Commercializing dairy and forage systems in Ethiopia: An Innovation systems perspective. Working Paper No. 17.
56. Schepers, J.A. and A.A. Dijkhuizen, 1991. The economics of mastitis and mastitis control in dairy cattle: A critical analysis of estimates published since 1970. *Preview on Veterinary Medicine*, 10: 213-24.
57. CSA (Central Statistical Authority), 2008. Agricultural Sample Survey. Report on Livestock and Livestock Characteristics, Vol. 2, Federal Democratic Republic of Ethiopia.
58. Akers, M.R. and C.S. Nickerson, 2011. Mastitis and its impact on Structure and Function in the Ruminant Mammary Gland. *Journal Mammary Gland Biology Neoplasia*, 16(4): 275-89.
59. Quinn, P.J., M.E. Carter, B. Markey and G.R. Carter, 2004. *Clinical Veterinary Microbiology*, Mosby. Elsevier limited, Philadelphia, USA.
60. Omnilog, 2010. OMNILOG data collection software, bacteria and fungi identification system, user guide part no.90311, Version 2.3.
61. McDonald, J.S., J.L. Richard, A.J. Anderson and R.E. Fichtner, 1980. *In vitro* anti mycotic sensitivity of yeasts isolated from infected bovine mammary glands. *American Journal of Veterinary Research* 41: 1987-1990.
62. VanDamme, D.M., 1983. Use of miconazole in treatment for bovine mastitis. *Veterinary medicine. Small Animal Clinician*, 78: 1425-1427.
63. Katamoto, H. and Y. Shimada, 1990. Intra-arterial and inframammary injection of miconazole for bovine mastitis caused by *Aspergillus fumigatus*. *British Veterinary Journal*, 146: 354-357.
64. Feio, S.S., A. Barbosa, M. Cabrita, L. Nunes, A. Esteves, J.C. Roseiro and M.J. Curto, 2004. Antifungal activity of *Bacillus subtilis* 355 against wood surface contaminant fungi. *Journal of Indian Microbiology and Biotechnology*, 31: 199-203.
65. Magnusson, J., K. Ström, S. Roos, J. Sjögren and J. Schnürer, 2003. Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. *FEMS Microbiology*. Mastitis: Causes and control. *Journal of Animal Science*, 86: 57-65.
66. Blomquist, N., 2008. Mastitis in Beef Cows frequently asked question. Alberta Agricultural and Rural Development.
67. McDonald, 2009. Mastitis in cow. Dairy cattle production 342-480. A McDonald campus of my gill university. Faculty of Agricultural and environmental sciences. Department of Animal Science.
68. Balish, E., 1986. Intestinal flora and natural immunity. *Microecol. Ther.*, 16: 157-167
69. Zwolinska-Wcislo, M., T. Brzozowski, T. Mach, A. Budak, D. Trojanowska, P.C. Konturek, R. Pajdo, D. Drozdowicz and S. Kwiecien, 2006. Are probiotics effective in the treatment of fungal colonization of the gastrointestinal tract? Experimental and clinical studies. *Journal Physiol Pharmacology*, 57(9): 35-49.