

Review

Potential target for exploring medicinal plants use of Bovine mastitis

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Mastitis is a disease of significant economical importance that affects farming and is a limiting factor in the world agribusiness of dairy cattle. It causes a considerable economic loss in milk production as a result of mammary gland inflammation. On that matter, the infections caused by *Staphylococcus aureus* also present public health implications, considering that thermostable toxins produced by this bacterium may remain stable in milk. This bacterium specie stands out as the cause of the higher incidence of mastitis due to its high resistance to antibiotics as a result of the indiscriminate use of these therapeutic agents. In this context, the process of discovering new antimicrobial agents is extremely important in a stage of rapid development of bacterial resistance. Thus, medicinal plants may be explored as alternative agents for treatment of this important animal disease. This minireview intends to enlighten briefly this disease, its economical and medical features using Brazil, one of the top-ten biggest dairy producers, as an example of the mastitis economical importance to point it out as a target for further research on medicinal plants, exploring their potential as new therapeutic options for treating mastitis.

Key words: Bovine mastitis, antimicrobial, public health, medicinal plants.

MASTITIS: AN INFECTION THAT AFFECTS BOVINE MILK PRODUCTION

Milk is one of the noblest animal products due to its high nutritional value. In addition, milk derivatives also lead to different food products and high income for several segments of the dairy production chain (Ribeiro, 2008). The milk composition includes proteins, fats, minerals, vitamins and water (Silva et al., 2012). Due to this chemical composition, several factors can alter milk such as animal age, race and feeding, stage of lactation, climatic variations and infections in the cow udder (Walstra et al., 2005).

Among the bovine infections, mastitis is one of the most frequent and important problem in dairy production. This disease is characterized by mammary gland inflammation and is caused mainly by bacteria. This infection directly affects not only the productivity in both quantity and quality aspects (for example, milk composition) but also alters milk physico-chemical characteristics (Silva et al., 2012). The changes caused by mastitis include not only the increase of cells and microorganisms in the milk but also compromise important nutritional components such as fat, protein and lactose (Muller, 2002).

According to the literature, 17-20% of the world dairy cows have mastitis at some stage of their productive lives, which may reduce milk production by 10 to 15%

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Figure 1. Diagnosis test of clinical and subclinical mastitis through Dark Background Screenhouse Mug (left) and California Mastitis Test methods (right) respectively (Source: UFLA, 2012).

(Silva et al., 2012). This production loss is more dramatic when a significant percentage of the cattle are infected (Rebhun, 2000).

Mastitis is the most common disease in adult dairy cows, accountable for 38% of all morbidity. Each year, three out of ten dairy cows have clinically apparent inflammation of the mammary gland. Of the affected cattle, 7% are disposable and 1% dies from this disease. The current data suggested that over 25% of the total economic losses in dairy cattle associated with diseases are directly related to mastitis (Peres Neto and Zappa, 2011).

Mastitis presents two forms: clinical and subclinical. The mastitis clinical form shows several signs and symptoms such as mammary gland swelling, pain and stiffening, increased temperature, and inflammatory secretion in the milk. Differently, mastitis subclinical form is characterized by not showing visible changes in the milk appearance and is the most prevalent form in dairy herds (Bandoch and Melo, 2011; Oliveira et al., 2011).

Due to the lack of symptoms or signs and therefore higher frequency as a persistent process, the subclinical form is more harmful than the clinical form (Langenegger et al., 1981). Currently, the California Mastitis Test (CMT) is used to determine subclinical mastitis detecting the increase in somatic cell count (SCC) in milk diagnosed (Oliveira et al., 2011). The CMT is a simple and low-cost test in which milk samples are taken from each teat and placed in a suitable tray. Then the somatic cells membranes present in the sample are disrupted by using a specific reagent, releasing the DNA that hydrates in contact with water and becomes viscous. The result of the test is evaluated according to the degree of gelling or

viscosity (UFLA, 2012).

The diagnosis of clinical mastitis is based on detecting signs of inflammation of the mammary gland and changes in milk (including blood presence) (Ribeiro et al., 2009).

On that purpose, the first milk jet from each teat is collected in a dark background screenhouse mug and analyzed for changes in color, consistency or presence of lumps or blood (UFLA, 2012) (Figure 1).

Depending on the diagnosis and/or the identification of the pathogenic microorganism, a fluid-based therapy may be employed in certain cases. Mastitis control aims at reducing new infections by adopting good hygiene practices during milking. Main control method consists of prevention through establishing good management practices on the farm, treating the infected animals and slaughtering the chronic cases (Schvarz and Santos, 2012).

Milking may promote the pathogen mastitis elimination and reduce new infections occurrence. The cow susceptibility of contracting infection during the first two weeks of the dry period is 15 to 20 times higher than the rest of the period. The intermediate stage of the dry period is one where the occurrence of mastitis is reduced. This explains why the mammary gland involution occurs at the peak of the absence of milk, which is an excellent culture medium (Teixeira et al., 2008).

Isolation of mastitis pathogens is a fundamental aspect of milk quality and udder health control programs. There is a current need to discuss public health and food safety issues associated with food borne pathogens found in or related to dairy products (Akram et al., 2013).

Table 1. Pathogens that cause bovine mastitis.

Pathogen Type	Species
Contagious or infectious	<i>Staphylococcus aureus</i> , <i>Streptococcus agalactiae</i> , <i>Corynebacterium bovis</i> , <i>Brucella abortus</i> , <i>Mycoplasma bovis</i> , Coagulase-negative <i>Staphylococcus</i> spp. <i>Listeria monocytogenes</i>
Environmental	<i>Streptococcus uberis</i> , <i>S. dysgalactiae</i> , <i>S. equinus</i> , <i>Escherichia coli</i> , <i>Klebsiella</i> spp., <i>Citrobacter</i> spp., <i>Enterobacter</i> spp., and <i>Pseudomonas aeruginosa</i>
Secondary or minor	Coagulase-negative <i>Staphylococcus</i> spp.
Uncommon	<i>Arcanobacterium pyogenes</i> , <i>Nocardia</i> spp., <i>Pasteurella</i> spp., <i>Mycobacterium bovis</i> , <i>Bacillus cereus</i> , <i>Serratia marcescens</i> , some species of anaerobic bacteria, fungi and yeast

MASTITIS: A DISEASE WITH A MAIN BACTERIAL PROFILE

More than 140 different types of organisms may cause mastitis, and these etiological agents are classified into: a) contagious pathogens (for example, *Staphylococcus aureus*), b) environmental pathogens (for example, *Streptococcus uberis*), c) secondary or minor pathogens (coagulase-negative *Staphylococcus* spp.), and d) uncommon pathogens (for example, *Arcanobacterium pyogenes*, some species of anaerobic bacteria, fungi and yeast) (Langoni et al., 2011; Radostits et al., 2002) (Table 1).

Currently, *S. aureus* is the main pathogen of bovine mastitis. This species contributes to mastitis pathogenesis due to the heat-stable toxin production that is active even in pasteurized milk (Contreras et al., 2007). *S. aureus* is highly contagious and can cause infection by more than 30 days by colonizing teats wounds, milkers hands of and/or mammary gland of infected cows. These bacteria cause huge losses to dairy farming by affecting milk production and quality (Bandoch and Melo, 2011).

Biofilm production by the *S. aureus* isolates is considered an important virulence factor responsible for the adhesion of these microorganisms on living or non-living surfaces. The intramammary infection caused by *S. aureus* strains that are biofilm producers is difficult to treat even with intra-mammary antibiotics. Thus the infections caused by biofilm producing bacteria should be properly considered, analyzed and treated (Raza et al., 2013).

The lack of resources for laboratory diagnosis or not using them when available often result to professionals making mistakes on mastitis treatment. This fact is associated with under dosing or inappropriate discontinuation of antibiotic therapy when detecting the animal clinical improvement, which contributes to the emergence of bacterial resistance (Mota et al., 2005). In fact, the widespread use of antibiotics on dairy farms

could lead to the selection and emergence of resistant strains (Walther et al., 2006). Interestingly, high rates of bacterial resistance to penicillin (53.5%), ampicillin (41.6%) and neomycin (38.6%) were found in the milk of cows infected with mastitis and created in organic dairy farms. Bacterial strains resistant to three or more antimicrobials were observed in 40 (39.6%) out of 101 isolates evaluated. According to the authors, since these organic dairy farms have only recently adopted organic farming, they are probably still affected by the previous inappropriate use of antimicrobials in mastitis therapy (Ribeiro et al., 2009).

Currently, mastitis is one of the major causes of antibiotic use in dairy cows. The use of antimicrobial agents is associated with the risk of inducing resistance to antimicrobial agents among bacteria, reduction of cure rates after treatment of clinical mastitis and transmission of resistance bacteria to humans via food chain (Suleiman et al., 2012).

The emergence of antibiotic resistance has been a major problem in human and animal medicine involving spontaneous mutation and/or recombination of genes (Mota et al., 2005). As a result, the main antibiotics are becoming ineffective, including against mastitis, due also to the transferring of the resistance bacteria-to-bacteria even among different species (Silva et al., 2012).

The coagulase-negative *Staphylococci* have become the most common bovine mastitis isolates in many countries. Therefore, they are considered now an important emerging mastitis pathogen (Laport et al., 2012).

Recently, a coagulase-negative *Staphylococcus* sp. resistant to five antibiotics (amoxicillin + clavulanic acid, enrofloxacin, gentamicin, vancomycin and penicillin G) was detected (Miguel et al., 2012). Thus, mastitis-causing coagulase negative *Staphylococci* can serve as a reservoir of resistance genes that can be transferred to other bacteria (for example, *S. aureus*). Importantly, this phenomenon increases the difficulties of controlling

and/or treating against mastitis (Leclercq, 2009).

S. agalactiae, *S. dysgalactiae* subsp. *dysgalactiae* and *S. uberis* are important mastitis environmental pathogens. According to the recent literature, the majority of the isolates were susceptible to all drugs except for aminoglycoside, macrolide, lincosamide and tetracycline (Rato et al., 2013).

Antibiotics are widely used in veterinary medicine and the indiscriminate use of these drugs contributes to the presence of antibiotic residues in milk that currently represents the main chemical contamination in dairy products. The milk can be contaminated with antibiotics when the producer does not respect the drug elimination period (Silva et al., 2012).

The milk contaminated with antibiotics is considered inappropriate for consumption and represents risk for public health. The risks of consuming dairy products containing antimicrobial residues include serious manifestations of hypersensitivity and other important human diseases (Andrade et al., 2001). Due to the serious public health problems arising from drinking milk contaminated with antibiotics, there is a need to analyze the most appropriate treatment to use in animals to ensure a product without risks to consumer health.

BRAZIL: A MILK PRODUCER STILL SUFFERING ECONOMICALLY WITH MASTITIS

According to the data of Gross Domestic Product (GDP) and trade balance, agribusiness is one of the main economic activities of Brazil. Lately, the agribusiness has favored the economy of Brazil, placing this country as one of the largest producers and exporters of the world, especially in the food production and exportation (Novaes et al., 2009).

On that perspective, milk production market is of great importance in Brazilian agribusiness. Activities related to the milk production, processing and marketing as well as its derivatives generate employment and income in rural and urban areas across the country, and provide food of high nutritional value for the population. According to Brazilian Institute of Geography and Statistics (IBGE), there are 1.2 million farms in Brazil producing milk and employing about three million people directly or indirectly (Guimarães and Lana, 2011). They contribute to more than six billion of the real value of national agricultural production (Langoni et al., 2011).

In the Brazilian food production, the dairy industry is the producer of the fourth most important product of Brazilian agriculture, after the meat industry, and coffee and sugar processing ones (Carvalho, 2010). The milk and its derivatives play an important role in the Brazilian agribusiness as there is an increase of about five dollars in GDP growth for every dollar increase in production in dairy agribusiness system. This places milk production ahead of important sectors such as the steel and textile industry (Barbosa et al., 2009).

Brazilian milk production has increased and currently Brazil has the world second largest dairy herd with 21,599,910 animals. According to the Strategic Management Advisory Board of the Ministry of Agriculture of Brazil that calculates the per capita consumption of dairy products, Brazil should account with the production of 33.12 billion liters of milk and consumption of 29.11 billion in 2012/2013 (AGE/MAPA, 2010; SEBRAE, 2010).

The Brazilian Southeastern is the largest regional producer of the country, with more than 10 billion liters or 36% of the national production. Secondly, this is followed by the South with more than eight billion liters or 29% of total production in the country (SEBRAE, 2010) (Figure 2).

Brazil is among the six largest milk producers in the world with the highest annual increase rate (Table 2). According to the literature, the Brazilian milk production will be soon the second largest in the world, only surpassed by the United States (Guimarães and Lana, 2011). Despite this milk production efficiency, currently the economic losses to the dairy farmer and the milk industry involving mastitis includes the production reduction, labor costs, professional fees, spending on drugs, animals death or disposal and low quality of the final product with decrease in industrial output (Langoni et al., 2011; Tozzetti et al., 2008). Importantly, the high quality control of the milk production is very important not only to provide a proper product but also for improving practices and procedures to reduce losses, waste and consequently costs of producing it (Scalco and Souza, 2006).

The identification of new treatments for mastitis for a country of the size of Brazil with this huge milk production and economic importance is of urgency. On that purpose, this new option should be of low-cost, safe and efficient to guarantee the use of the milk-derived products with quality and safety.

MASTITIS: ARE THE MEDICAL PLANTS FEASIBLE TREATMENT OPTIONS?

The use of plants with therapeutic purposes comes from long time ago and is described since the ancient ages. However, lately the formal use of these plants has increased due to the development of the scientific researches that have academically confirmed the medicinal activities. Due to the low-cost and easy access, medicinal plants have been used for treatment and prevention of several human diseases being considered as an alternative therapy (Degáspari and Dutra, 2011).

Essential oils derived from plants used as condiments represent a traditionally group of natural antimicrobials used in the food to enhance taste or aroma. They consist in complex mixtures including volatile substances whose components are related to different chemical groups: terpene hydrocarbons, simple alcohols, aldehydes,

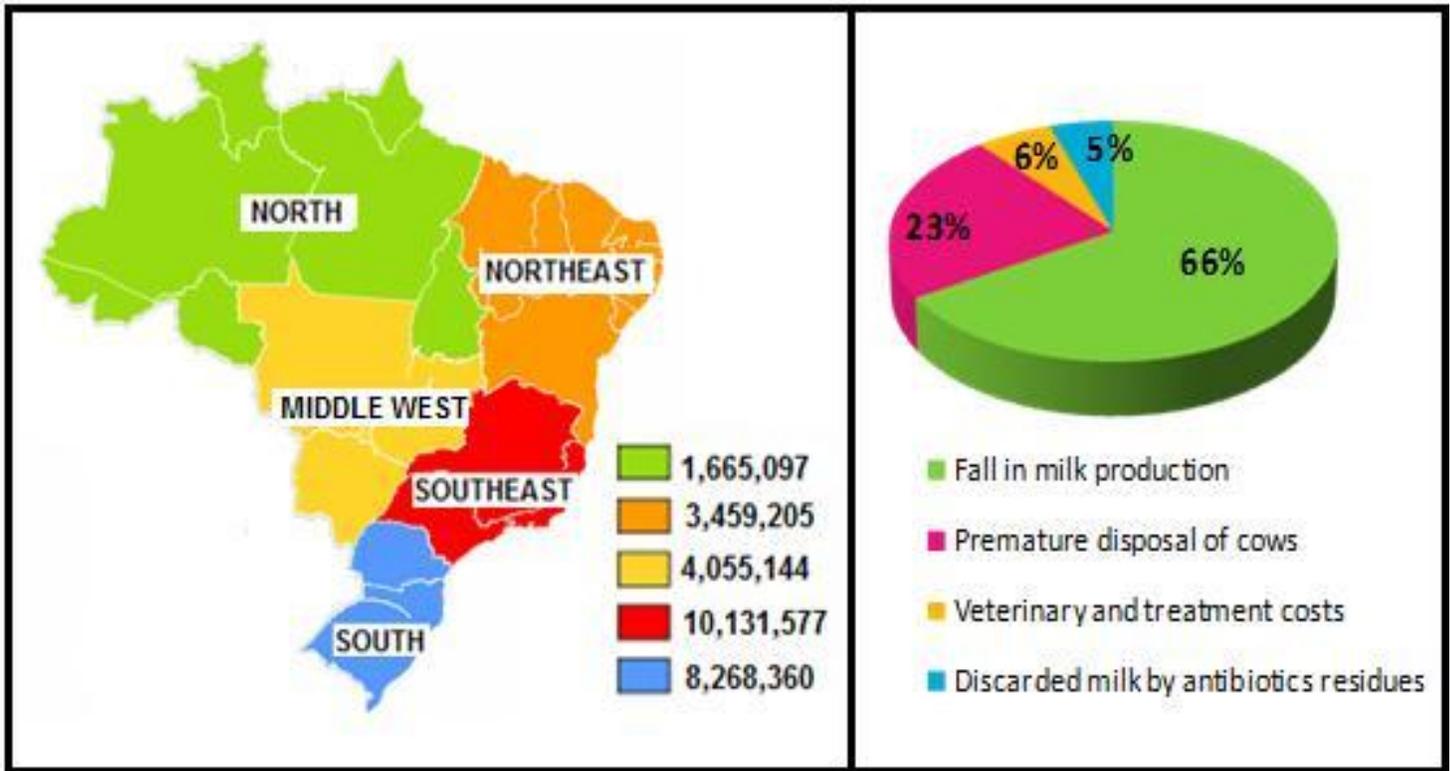


Figure 2. Geographic distribution of Brazilian milk production (billion liters) (left) and the economic losses caused by bovine mastitis (right) (adapted from SEBRAE, 2010 and Hand et al., 2012, respectively).

Table 2. Predictions of Strategic Management Advisory Board of the Ministry of Agriculture of Brazil for the per capita consumption of dairy products (billions of liters) in Brazil (adapted from AGE/MAPA, 2010).

Years	Production	Consume	Exportation
2009/10	31.12	27.33	1.10
2010/11	31.80	27.93	1.18
2011/12	32.46	28.52	1.27
2012/13	33.12	29.11	1.35
2013/14	33.78	29.71	1.44
2014/15	34.45	30.30	1.52
2015/16	35.11	30.90	1.60
2016/17	35.77	31.49	1.69
2017/18	36.43	32.08	1.77
2018/19	37.09	32.68	1.85
2019/20	37.75	33.27	1.94
Annual rate	1.98	1.98	5.78

ketones, phenols, esters, and organic acids in different concentrations (Simões and Spitzer, 2000).

The literature described few researches about mastitis and among them the antimicrobial activity observed on the essential oils of oregano (*Origanum vulgare*), mexican oregano (*Lippia graveolens*), thyme (*Thymus*

vulgaris) as well as on the most constituents of carvacrol, thymol and cinnamaldehyde is of importance. The biological activity was observed when testing these vegetal oils against *Staphylococcus* spp isolates from herds of dairy goats (Pozzo et al., 2011). Doss et al. (2012) recently evaluated the antibacterial activity of the

aqueous and alcoholic extracts of aerial parts of some selected medicinal plants against the mastitis causing bacteria. Interesting both extracts were active against *S. agalactiae*, *E. coli*, *S. aureus* and *K. pneumoniae* (MIC = 0.125-2.00 mg mL⁻¹).

The evaluation of the effect of medicinal plants and alternative medicine should be performed using the milk pathogens isolates, trying to detect an antimicrobial profile directly against them. However, the animal model should be used after that to verify the efficiency in a farm environment. This requirement affects the research in this area as it demands the proper research facilities, including the farm and animals to further exploration.

The consolidation of new research lines and of multidisciplinary groups is important if they have the goal of synthesizing and/or identifying new therapeutic options in medicinal plants and pharmacological evaluation of new active molecules against bovine diseases of economic importance. Researches through planning, modeling, and analyzing the biological studies should be encouraged in areas such as biotechnology, pharmacology, and applied microbiology. The identification of the antimicrobial activity and the use of molecular modeling with application of computational methods in the study of the theoretical toxicological profile (*in silico*) may contribute to a faster identification of these new plant molecules also identifying their specific bacterial target.

FINAL CONSIDERATIONS

Bovine mastitis causes milk quality loss, health risks to consumers due to antibiotic residues and pathogenic microbes, and serious economic losses to dairy industry. Currently, developing a better bovine mastitis control and prevention depend on:

- (a) the understanding of the processes related to antibiotics action and of the emergence of resistance,
- (b) the identification and pharmacological evaluation of new and more powerful antimicrobial agents,
- (c) the antibiotic therapeutic use in a rational way, and
- (d) the implementation of hygienic-sanitary standards suitable for the infections control.

Within this context, the search for new effective natural prototypes for the treatment of bovine mastitis does not compromise the milk quality which is important for a better quality of dairy farming and food production. On that perspective, medicinal plants may be a safe, efficient and a low-cost option for treating bovine mastitis that should also be further explored similar to the synthetic options.

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