

# Essential Oils: An Effective Antimicrobial Therapy in Bovine Mastitis

Ameer Hamza<sup>1\*</sup>, Abdul Rauf<sup>1</sup>, Moaz Ghaffar<sup>1</sup>, Hafiz Muhammad Usman Siddiq<sup>1</sup>, Waleed Akarm<sup>1</sup>

1. University of Veterinary and Animal Sciences, Jhang Campus, Pakistan.

\*Corresponding author: [ameerr.hamzaa011@gmail.com](mailto:ameerr.hamzaa011@gmail.com)

## ABSTRACT

Mastitis is termed as the inflammation of the udder and is most common in dairy animals nowadays. Many pathogens are involved in causing mastitis but *Staphylococcus aureus* is the most common pathogen which is also the main etiological agent of many respiratory and skin infections in humans. The most commonly used treatment of mastitis is the use of antimicrobials against infectious microbes which is the main reason for antimicrobial residues (AMR) because pathogens may have developed resistance against them. Depending upon the recent literature, this conclusion can be withdrawn that use of essential oils is free of public health risks and may prove good alternative to antimicrobials in the control of bovine mastitis.

**Keywords:** Mastitis, Essential oils, AMR, Antimicrobials, *Staphylococcus aureus*, public health

### Introduction:

Bovine mastitis is the inflammatory response of udder tissues caused by various pathogens or trauma. The common etiological microorganisms include gram-negative (*Escherichia coli* and *Klebsiella spp.*), gram-positive (*Staphylococcus aureus* and *Streptococcus agalactiae*), and environmental pathogens (*Enterococcus spp.* and *Streptococcus uberis*). Recent studies showed that *S. aureus* is the most prevalent microorganism causing mastitis. Host factors like age, breeding and genetics, udder structure, host nutritional stress and immune system are also the main causes of bovine mastitis [1]. It is one of the most common diseases of dairy animals worldwide causing a huge economic loss to farmers. Moreover, it also results in low milk yield, treatment costs, medications, and culling of animals. The total failure cost due to mastitis is estimated to be about \$147 per cow in a year resulting in low milk production and culling [2].

Mastitis is characterized by clinical, subclinical, and chronic cases. Clinical mastitis is very common and is easily diagnosed because the abnormalities are visible like red and swollen udder and fever [1] and severe cases of clinical mastitis result in death threat dairy animals [3]. In contrast to clinical mastitis, subclinical mastitis does not show any abnormality to the udder or milk but there is a sharp decrease in milk production with the increase in the somatic cell count (SCC) [4] and the incidence of subclinical mastitis is also very high than clinical mastitis [5]. So, the economic losses due to subclinical mastitis are more due to its prevalence and reduction in milk yield.

### Diagnostic techniques in bovine mastitis:

As mastitis is causing huge losses to milk and dairy farmers, it is very necessary to diagnose the disease timely to overcome the damage. The earlier the disease is identified, the lower will be the losses. Also, mastitis-affected milk is unfit for human consumption. The easy way to diagnose the disease is a physical examination of the udder and to check for any abnormality, clinical signs, behavioral changes, or drop in milk yield. If there is any abnormality, we go for screening tests like, Somatic Cell Count, California Mastitis Test, or Surf Field Test. If any of these tests show positive results, then we can detect the pathogen through PCR-based tests, Microbial Culturing, protein-based tests, or Nano tech-based tests. After the detection of the pathogen, treatment can be initiated accordingly [6].

### Treatment:

#### Antimicrobial therapy:

As pathogens are mainly responsible for mastitis in dairy animals, it is also necessary to treat the animals against them. However, there are several ways to control and cure mastitis like the use of antimicrobials, vaccination, bacteriophage therapy, nanoparticle-based therapy, bacteriocins, and herbal therapy [7]. Among them, antimicrobial therapy is commonly used. The antimicrobials are administered through intramuscular, intravenous, or intramammary route [10]. The results of the use of vaccination in mastitis are not as good. Before antimicrobial therapy, the cause of infection should be known. Also, the selection of antimicrobials for treatment should be based on history, etiology, and antimicrobial sensitivity profile against the pathogen [8]. The common effective antimicrobials used against mastitis are florfenicol, cefoperazone, cephalixin, and ceftiofur, and resistance was found against norfloxacin, tetracycline, and trimethoprim-sulfamethoxazole [9]. The main disadvantage of using antimicrobials is antimicrobial resistance (AMR) which is harmful to public health.

Recent studies showed resistance against oxytetracycline, amoxicillin, and ciprofloxacin [8]. As *S. aureus* is a common pathogen, it has developed

resistance against several antimicrobials. *S. aureus* is a gram-positive bacterium and it is also associated with many infections in humans. Some common disorders caused by *S. aureus* in humans are endocarditis, urinary tract infections, sepsis, abscess of skin, food poisoning, mastitis, meningitis, and septicemia [23], [24], [25], [26].

Antimicrobials	Resistant gene(s)	Reference
Tetracycline	<i>tetK, tetM</i>	[11]
Aminoglycosides	<i>aacA-aphD</i>	[11]
Erythromycin	<i>ermA, ermB, ermC, ermT</i>	[12]
Penicillin	<i>blaZ, mecA</i>	[11]
Quinolones	<i>norB, norC</i>	[11]
Methicillin	<i>mecA</i>	[13]
Fluoroquinolone	<i>mepA</i>	[14]

Table 1: Antimicrobials resistance-related genes against *S. aureus*.

#### Essential oils (EOs) as an alternative therapy:

The treatment of mastitis is the use of antimicrobials through different routes but it has a risk of multidrug resistance and it is also associated with public health issues. So, there is a thriving need to use the latest treatment like products derived from natural sources [15], [16]. Milk is the most used product worldwide and for good quality milk, udder health is very important. Controlling the udder pathogens also reduces the foodborne illness. Essential oils are derived from various parts of plants containing different compounds that have therapeutic effects. EOs also have antifungal, antiviral, antimicrobial, and anti-inflammatory properties [31]. Recent studies showed the use of Essential Oils (EOs) are safe because there is no risk of residues reported yet. United States Food and Drug Authority also confirmed the use of Essential Oils as 'generally recognized as safe' (GRAS) [17]. There are many EOs used for the cure of mastitis but common are thyme (*Thymus vulgaris*) [18], lemongrass oil (*Cymbopogon flexuosus*) [19], Wild thyme (*Thymus serpyllum*) [18], mountain savory (*Satureja montana*) and oregano (*Origanum vulgare*) [20].

The use of plant-based products is also safe because they contain chemicals that are harmless to public health [21]. Silva in his study also reported that the use of lemongrass EO with MIC and MBC values ranging from 0.39 to 3.12 mg/mL and 0.39 to 6.35 mg/mL and thyme EO with MIC and MBC values ranging from 0.39 to 1.56 mg/mL and 0.39 to 3.12 mg/mL has very effective antimicrobial activity against *S. aureus* in bovine mastitis [22]. Latest data also showed that prolonged use of Essential Oils has little or no chance of AMR.

Name of EOs	Main component	MIC	MBC	Reference
<i>Thymus vulgaris</i>	Thymol (45.22%), p-Cymene (23.83%)	3.125 mg/mL	6.25 mg/mL	[18]
<i>Origanum majorana</i>	3-Cyclohexene-1-ol, 4-methyl-1-(1-methylethyl)-, (R)- (44.84%), α-Terpineol (6.83%), p-Cymene (6.75%)	0.62% v/v	1.25% v/v	[27]
<i>Cinnamomum aromaticum</i>	e-Cinnamaldehyde (94.67%)	0.625 μL/mL	1.25–10 μL/mL	[28]

<i>Salvia officinalis</i>	Carvacrol (61.01%), Thymol (20.41%), 1R- $\alpha$ -Pinene (7.88%)	0.625-1.25%	1.25-2.5%	[29]
<i>Melaleuca armillaris</i>	1,8-Cineole (72.3%), Limonene (7.8%)	6.25–25 $\mu$ L/mL	12.5–50 $\mu$ L/mL	[30]
<i>Lavandula stoechas</i>	17-Pentatriacontene (42.15%), Linalyl acetate (26.82%), Eucalyptol (18.87%)	4.37% v/v	8.75% v/v	[27]

Table 2: Main components of EOs with Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values used against *S.aureus* in bovine mastitis

**Conclusion:**

The irrational use of antimicrobials in mastitis results in antimicrobial residues that are also a threat to public health i.e., persistent chronic infections due to resistant microorganisms. It can be effectively controlled by accurate diagnosis of the causative agent. The accurate diagnosis helps in reduction of antimicrobial residues. The use of EOs as an alternative therapy is effective in mastitis because they are non-toxic, harmless to living tissues, can cross skin barriers, and have antimicrobial activity against pathogens.

**References**

[1] Cheng WN, Han SG. Bovine mastitis: Risk factors, therapeutic strategies, and alternative treatments—A review. *Asian-Australasian journal of animal sciences*. 2020 Nov;33(11):1699.

[2] Hogeveen H, Steeneveld W, Wolf CA. Production diseases reduce the efficiency of dairy production: A review of the results, methods, and approaches regarding the economics of mastitis. *Annual review of resource economics*. 2019 Oct 5; 11:289-312.

[3] Gruet P, Maincent P, Berthelot X, Kaltsatos V. Bovine mastitis and intramammary drug delivery: review and perspectives. *Advanced drug delivery reviews*. 2001 Sep 1;50(3):245-59.

[4] Abebe R, Hatiya H, Abera M, Megersa B, Asmare K. Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC veterinary research*. 2016 Dec;12(1):1-1.

[5] Martin P, Barkema HW, Brito LF, Narayana SG, Miglior F. Symposium review: Novel strategies to genetically improve mastitis resistance in dairy cattle. *Journal of dairy science*. 2018 Mar 1;101(3):2724-36.

[6] Ashraf A, Imran M. Diagnosis of bovine mastitis: from laboratory to farm. *Tropical animal health and production*. 2018 Aug; 50:1193-202.

[7] Gomes F, Henriques M. Control of bovine mastitis: old and recent therapeutic approaches. *Current microbiology*. 2016 Apr; 72:377-82.

[8] Sharun K, Dhama K, Tiwari R, Gugjoo MB, Iqbal Yato M, Patel SK, Pathak M, Karthik K, Khurana SK, Singh R, Puvvala B. Advances in therapeutic and management approaches of bovine mastitis: a comprehensive review. *Veterinary Quarterly*. 2021 Dec 15;41(1):107-36.

[9] Ribeiro MG, Riseti RM, Bolaños CA, Caffaro KA, De Moraes AC, Lara GH, Zamprogna TO, Paes AC, Listoni FJ, Franco MM. *Truiperella pyogenes* multispecies infections in domestic animals: a retrospective study of 144 cases (2002 to 2012). *Veterinary Quarterly*. 2015 Apr 3;35(2):82-7.

[10] Hossain MK, Paul S, Hossain MM, Islam MR, Alam MG. Bovine mastitis and its therapeutic strategy doing antibiotic sensitivity test. *Austin J Vet Sci Anim Husband*. 2017;4(1):1030.

[11] Zhang Z, Chen Y, Li X, Wang X, Li H. Detection of antibiotic resistance, virulence gene, and drug resistance gene of *Staphylococcus aureus* isolates from bovine Mastitis. *Microbiology Spectrum*. 2022 Aug 31;10(4):e00471-22.

[12] Abd El-Razik KA, Arafa AA, Fouad EA, Soror AH, Abdalhamed AM, Elgiously M. Phenotypic and genotypic characterization of erythromycin-resistant *Staphylococcus aureus* isolated from bovine subclinical mastitis in Egypt. *Veterinary World*. 2023;16(7):1562.

[13] Moawad AA, El-Adawy H, Linde J, Jost I, Tanja G, Katja H, Karsten D, Neubauer H, Monecke S, Tomaso H. Whole genome sequence-based analysis of *Staphylococcus aureus* isolated from bovine mastitis in Thuringia, Germany. *Frontiers in Microbiology*. 2023;14.

[14] Pérez VK, Custódio DA, Silva EM, de Oliveira J, Guimarães AS, Brito MA, Souza-Filho AF, Hejnemann MB, Lage AP, Dorneles EM. Virulence factors and antimicrobial resistance in *Staphylococcus aureus* isolated from bovine mastitis in Brazil. *Brazilian Journal of Microbiology*. 2020 Dec; 51:2111-22.

[15] Cheng WN, Han SG. Bovine mastitis: Risk factors, therapeutic strategies, and alternative treatments—A review. *Asian-Australasian journal of animal sciences*. 2020 Nov;33(11):1699.

[16] Yang WT, Ke CY, Wu WT, Lee RP, Tseng YH. Effective treatment of bovine mastitis with intramammary infusion of *Angelica dahurica* and *Rheum officinale* extracts. *Evidence-Based Complementary and Alternative Medicine*. 2019 Mar 3;2019.

[17] Leigh de Rapper S, van Vuuren SF. Odoriferous therapy: A review identifying essential oils against pathogens of the respiratory tract. *Chemistry & Biodiversity*. 2020 Jun;17(6):e2000062.

[18] Kovačević Z, Radinović M, Čabarkapa I, Kladar N, Božin B. Natural agents against bovine mastitis pathogens. *Antibiotics*. 2021 Feb 19;10(2):205.

[19] Gayathri P, ta Usha P. in vitro antimicrobial activity of lemongrass (*Cymbopogon flexuosus*) oil and citral against Methicillin resistant *Staphylococcus Aureus* (Mrsa) from bovine mastitis. *J. Vet. Anim. Sci*. 2020;51(2):184-8.

[20] Kovačević Z, Tomanić D, Čabarkapa I, Šarić L, Stanojević J, Bijelić K, Galić I, Ružić Z, Erdeljan M, Kladar N. Chemical Composition, Antimicrobial Activity, and Withdrawal Period of Essential Oil-Based Pharmaceutical Formulation in Bovine Mastitis Treatment. *International Journal of Environmental Research and Public Health*. 2022 Dec 11;19(24):16643.

[21] Paz JE, Contreras CR, Munguía AR, Aguilar CN, Inungaray ML. Phenolic content and antibacterial activity of extracts of *Hamelia patens* obtained by different extraction methods. *Brazilian journal of microbiology*. 2018 Jul; 49:656-61.

[22] Lopes TS, Fussieger C, Theodoro H, Silveira S, Pualetti GF, Ely MR, Lunge VR, Streck AF. Antimicrobial activity of essential oils against *Staphylococcus aureus* and *Staphylococcus chromogenes* isolated from bovine mastitis. *Brazilian Journal of Microbiology*. 2023 Jun 20:1-9.

[23] van Wamel WJ. *Staphylococcus aureus* infections, some second thoughts. *Current opinion in infectious diseases*. 2017 Jun 1;30(3):303-8.

[24] Singh SK. *Staphylococcus aureus* intracellular survival: A closer look in the process. *Virulence*. 2017 Nov 17;8(8):1506-7.

[25] Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler Jr VG. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical microbiology reviews*. 2015 Jul;28(3):603-61.

[26] Querques F, Cantilena B, Cozzolino C, Esposito MT, Passaro F, Parisi S, Lombardo B, Russo T, Pastore L. Angiotensin receptor 1 stimulates osteoprogenitor proliferation through TGF  $\beta$  - mediated signaling. *Journal of cellular physiology*. 2015 Jul;230(7):1466-74.

[27] Noori S, Rahchamani R, Kohsar JB, Binabaj FB. Antibacterial effect of *Lavandula stoechas* and *Origanum majorana* essential oils against *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli*.

[28] Benbelkacem I, Selles SM, Aissi M, Khaldi F, Ghazi K. In vitro assessment of antifungal and anti-staphylococcal activities of *Cinnamomum aromaticum* essential oil against subclinical mastitis pathogens. *Veterinaria*. 2019;68(1).

[29] Zarooni S, Rahchamani R, Ghanbari F, Khanahmadi A. Antibacterial effect of *Satureja hortensis* and *Salvia officinalis* essential oils against major bovine mastitis bacteria. *Iranian Journal of Veterinary Science and Technology*. 2021 Jul 1;13(1):75-81.

[30] Buldain D, Gortari Castillo L, Buchamer AV, Aliverti F, Bandoni A, Marchetti L, Mestorino N. *Melaleuca armillaris* essential oil in combination with rifaximin against *Staphylococcus aureus* isolated of dairy cows. *Frontiers in Veterinary Science*. 2020 Jul 15; 7:344.

[31] Neculai-Valeanu AS, Arion AM, Mădescu BM, Rîmbu CM, Creangă Ş. Nanomaterials and essential oils as candidates for developing novel treatment options for bovine mastitis. *Animals*. 2021 May 31;11(6):1625.