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Recent Developments on Bovine Mastitis
Treatment and Control

Edited by Kiro Petrovski

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Aims and Scope of the Series

Paralleling similar advances in the medical field, astounding advances occurred in Veterinary Medicine and Science in recent decades. These advances have helped foster better support for animal health, more humane animal production, and a better understanding of the physiology of endangered species to improve the assisted reproductive technologies or the pathogenesis of certain diseases, where animals can be used as models for human diseases (like cancer, degenerative diseases or fertility), and even as a guarantee of public health. Bridging Human, Animal, and Environmental health, the holistic and integrative "One Health" concept intimately associates the developments within those fields, projecting its advancements into practice. This book series aims to tackle various animal-related medicine and sciences fields, providing thematic volumes consisting of high-quality significant research directed to researchers and postgraduates. It aims to give us a glimpse into the new accomplishments in the Veterinary Medicine and Science field. By addressing hot topics in veterinary sciences, we aim to gather authoritative texts within each issue of this series, providing in-depth overviews and analysis for graduates, academics, and practitioners and foreseeing a deeper understanding of the subject. Forthcoming texts, written and edited by experienced researchers from both industry and academia, will also discuss scientific challenges faced today in Veterinary Medicine and Science. In brief, we hope that books in this series will provide accessible references for those interested or working in this field and encourage learning in a range of different topics.

Meet the Series Editor

Rita Payan Carreira earned her Veterinary Degree from the Faculty of Veterinary Medicine in Lisbon, Portugal, in 1985. She obtained her Ph.D. in Veterinary Sciences from the University of Trás-os-Montes e Alto Douro, Portugal. After almost 32 years of teaching at the University of Trás-os-Montes and Alto Douro, she recently moved to the University of Évora, Department of Veterinary Medicine, where she teaches in the field of Animal Reproduction and Clinics. Her primary research areas include the molecular markers of the endometrial cycle and the embryo–maternal interaction, including oxidative stress and the reproductive physiology and disorders of sexual development, besides the molecular determinants of male and female fertility. She often supervises students preparing their master's or doctoral theses. She is also a frequent referee for various journals.

Meet the Volume Editor

Kiro R. Petrovski is a production animal veterinarian with a special interest in bovine mastitis and dairy cattle. He has a master's degree and a Ph.D. He has published more than forty journal articles in prestigious journals. He has carried out consulting work on mastitis and milk quality problems in eleven countries. Dr. Petrovski has consulted with the farming, industry, and scientific communities in more than twenty countries about bovine mastitis and milk quality. Currently, he works as an academic veterinarian, teaching animal science, veterinary technician, and DVM students, in addition to working with farmers and veterinary professionals.

Contents

Preface

Bovine mastitis is the most frequent and economically important syndrome facing the dairy industry. The progress in its treatment and control is continuous, and thus, there is a need for an update. This book covers recent developments in the treatment and control of bovine mastitis through chapters that review the literature and present data from recent research. The book has three sections: "Basics of Bovine Mastitis", "Treatment of Bovine Mastitis", and "Bovine Mastitis Control".

Chapter 1 in Section 1 presents alternative approaches to the treatment of mastitis, such as herbal medication, and discusses the anti-inflammatory and antimicrobial activity of these alternative treatments. The chapter also discusses other aspects of ethno-veterinary practices related to bovine mastitis.

Chapters in Section 2 address recent findings in the antimicrobial resistance of mastitis-causing pathogens and the use of antimicrobials. Chapter 2 describes the current situation of antimicrobial resistance in various bovine mastitis pathogens and introduces alternatives to antimicrobial treatment. Chapter 3 reports on the attitudes towards the use of laboratory information and socio-political situation as they relate to the decision to use antimicrobials to treat bovine mastitis.

Section 3 addresses control of bovine mastitis, either clinical or subclinical. Chapters 4 and 5 describe current control measures for bovine mastitis. Chapter 6 discusses control using advanced pharmaceutical approaches, such as nanomaterials. Finally, Chapter 7 discusses the control of mastitis as it relates to biosecurity and the protection of food safety using appropriate milking and parlor strategies.

This book is a valuable contribution to the library on bovine mastitis. It is a useful summary of the recent concepts in the treatment and control of bovine mastitis, the most common disease in dairy cattle. The book is a useful resource for those who work in the dairy industry as well as students, professionals, and scientists interested in this subject.

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Section 1

Basics of Bovine Mastitis

Chapter 1

Management of Mastitis Using Trans-Disciplinarily Validated Ethno-Veterinary Practices

Balakrishnan M.N. Nair and Punniamurthy Natesan

Abstract

Mastitis is an inflammation of the mammary gland generally associated with intra-mammary infection (IMI) with certain microbes. Though common mastitis-causing bacteria are the main cause of mastitis, other organisms like viruses, fungi, yeast, certain microscopic algae (*Prototheca* spp.) and even physical injury might cause mastitis. The University of Transdisciplinary Health Science and Technology (TDU) conducted a participatory documentation of ethno-veterinary practice (EVP) and transdisciplinary assessment involving Siddha-Ayurveda, and Western pharmacology and established the safety and efficacy of EVP. *In-vitro* antimicrobial activity, clinical and molecular docking studies indicated anti-inflammatory and anti-microbial properties of EVP formulation for mastitis. The microbiome of milk from the cows with clinical mastitis indicates that after 6 days of treatment with herbal formulations, the average abundance of microbes causing mastitis was reduced substantially. Microbiome abundance in the milk of EVP-treated cows is almost similar to that of the control after 6 days. However, microbiome abundance in the milk from the antibiotic-treated cow varied substantially from the milk of the control. The clinical intervention with EVM preparations on 181,252 cows in a multicentric field study for a period of 5 years on the efficacy of EVP for subclinical, clinical and chronic mastitis with multifactorial aetiology resulted in a clinical recovery in 84.9% of cases.

Keywords: documentation, transdisciplinary validation, antibiotic resistance, herbal formulations, alternate to antimicrobials

1. Introduction

Mastitis is a perpetual problem of all milk-producing animals. Even though common bacteria are the main cause of mastitis, other organisms like *Mycoplasma* spp. [1, 2] yeast, [3] viruses and fungi might also be associated with the syndrome [4, 5]. Fungi such as *Aspergillus fumigatus*; *A. midulus*; *Candida* spp.; *Trichosporon* spp. and certain microscopic algae (*Prototheca* spp.) can cause mastitis. Physical or chemical injuries to the mammary region, poor hygiene and/or trauma also cause mastitis. Mycotoxins in cattle feed can suppress the immune system deepening negative energy balance and increasing the risk of metabolic disorders and infectious diseases [6, 7]. Indeed, other causes of immunosuppression also may be present.

1.1 Economic loss

The prevalence of subclinical mastitis in bovines in India ranges from 9.9 to 86.9%. The average drop in income due to mastitis is from Rs. 306–335 to 413–458 per cow per day [8]. Mastitis also causes permanent loss of productive ability of the cattle, reduces milk by 21% and butter and fat by 25%. The economic losses in mastitis are multifactorial and result a decrease in milk production, poor quality of milk, premature culling, higher veterinary service and labour cost [9, 10]. Subclinical mastitis accounts for 60–70% of the total economic losses by all mastitis types and thus causes three times more production losses than clinical mastitis [11–13]. In the last five decades, the loss due to mastitis in India has increased 115 times. The economic loss due to mastitis alone was 71,655 million Indian Rupees in the year 2012. The prevalence of the mastitis increased to more than 60% [14].

1.2 Conventional mastitis management and AMR

Non-infectious mastitis can usually be resolved without the use of antibiotics. Mastitis therapy protocols nearly always include antibiotics. The proportion of defined annual daily doses of antimicrobial per cow for mastitis alone administered on conventional dairy farms is 86.3% (**Figure 1**) [15]. The misuse of antimicrobials led to residue in animal products such as milk and meat, the development of the Antimicrobial Resistance (AMR) which is a threat to public health [16, 17].

2. Documentation and rapid assessment of EVP

The Trans-disciplinary University (TDU), more than a decade ago, developed a participatory documentation process of ethno-veterinary practice and a rapid assessment methodology to establish the safety and efficacy of EVP [18–20]. The veterinary dimension of Ayurveda or Siddha is used as a tool for studying folk/ethno-veterinary knowledge to assess the safety and efficacy of folk/EVP knowledge [21]. There is an inherent relation between the classical textual knowledge such as in Ayurveda or Siddha, which is classical Indian veterinary science and the folk knowledge

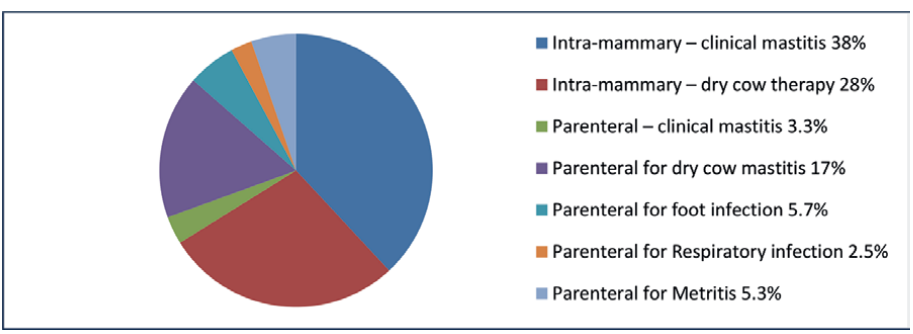

Figure 1.
Daily doses of antimicrobial per cow for mastitis alone per year administered on conventional dairy farms [15].

which forms the empirical foundation of theory and practice in the classical texts of Ayurveda and Siddha. This relationship forms the basis of the rapid method developed by TDU. The assessment is an essentially participatory method by cross-cultural triangulation of local experience with Ayurveda, Siddha as well as Western pharmacology. It involves the community, vaidyas (healers) and medical practitioners from various systems of medicine, pharmacologists, botanists and the facilitators like non-government organisations (NGOs) and People's Organisations [18]. Recording of the use of EVP from 24 locations in 10 states was done and rapidly assessed using Ayurveda/Siddha and established that 353 formulations out of 441 are safe and efficacious [20].

3. Standardisation of the ethno-veterinary formulation and the SOP for subclinical, clinical and chronic mastitis

3.1 EVP treatment protocol for subclinical and clinical mastitis

See (**Table 1**).
See (**Figures 2–5**).

3.2 Preparation and application

Method 1: A fine paste of *Aloe vera* leaves, turmeric and calcium hydroxide (**Figure 6**) is applied externally all over the udder with a circular massage after washing and removing the milk completely from the udder (**Figures 7–9**). Repeat

No.	Botanical name	Common name	Parts used	Figure	Amount
1	*Aloe vera*	Aloe	Leaves	2	250 g
2	*Curcuma longa*	Turmeric	Rhizome	3	50 g
3	—	Slaked lime	Powder	4	10 g
4	*Cissus quadrangularis**	Veldt grape, winged tree bine	Stem	5	Pieces 2

**In case of chronic mastitis.*

Table 1.
The botanical, local names and the ratio of ingredients in the formulation.

Figure 2.
Aloe vera.

Figure 3.
Curcuma longa–*rhizome.*

Figure 4.
Calcium hydroxide.

Figure 5.
Cissus quadrangularis.

the application after every 1 hour for ten times in a day for 5 days. Feed the affected animal with two lemons twice daily for 5 days besides the external application.

Method 2: A combination of *Aloe vera* 250 gr, *Curcuma longa* 50 gr. and calcium hydroxide 10 gr blended to form a reddish paste. Mix 75 grams of this paste with 150 ml sesame (Gingelly) or mustard oil. Clean the mastitis-affected udder with water and remove the milk completely from the udder. Dry the udder well and apply the mixture with your hand on all quarters (affected as well as non-affected) thoroughly

Figure 6.
Preparation of the herbal formulation for mastitis.

Figure 7.
Herbal formulation for mastitis.

Figure 8.
Application of the herbal formulation for mastitis on the udder.

with a circular massage. Apply this paste five times a day for 5 days. Cut two lemons into halves and feed the affected cattle twice daily for 5 days. Both these protocols can be used during dry periods to prevent mastitis [22].

3.3 Blood in milk

A paste of curry leaves (30 g) and jaggery (100 g) is also given to the animals having clinical mastitis with blood in milk till the condition is resolved.

Figure 9.
Application of the herbal formulation for mastitis on the udder.

Method 3: Chronic mastitis: A combination of *Aloe vera* 250 grams, *Curcuma longa* 50 grams, calcium hydroxide 10–15 grams and two pieces of *Cissus quadrangularis* blended to form a reddish paste. Mix 75 grams of this paste with 150 ml of water to dilute it. Clean the mastitis-affected udder with water and milk the animal completely. Apply the mixture with your hand on all quarters (affected as well as non-affected) thoroughly with a circular massage. Apply this paste 8–10 times a day for 15 days or till the condition is resolved. You can also use 150 ml sesame (Gingelly) or mustard oil instead of water and apply five times a day till the condition is cured. Cut two lemons into halves and feed the affected cattle twice daily for 7 days.

4. Transdisciplinary assessment of the EVP for mastitis

4.1 Ayurveda biology conceptual framework of the pathophysiology and management of mastitis in dairy animals

"*Sthanya* (breast) is considered as *upadhatu* of *Rasa dhatu* (lymphatic system) and mainly consists of *Twak* (skin), *Kandara* (connective tissues), *Mamsa* (muscle tissue), Rasavahini (lacticiferous ducts) and *Granthis* (glandular lobes or acini). The disease afflicting to all these components is *Sthanavidhradhi* (mastitis). *Sthana* is also a site for *Shotha* (inflammation), *Vrana* (ulcers), *Granthis* (benign tumours) and *Arbuda* (malignant tumours)" [23]. The *dosha* (*Vata, pitha and Kapha*) signifies the *dushanasvabhàva* (the nature of vitiation) and is the basic triggering factor in the disease causation. These, in turn, vitiate the tissue elements such as blood, muscle fat, etc., and manifest in the form of diseases. The same *doŝa* can produce several diseases [23].

Aloe vera L. (*Kumari*) has healing properties. It has the properties of *Deepana* (digestive), *Pachana* (carminative), *cold in potency* (pacifies pitta), *Pitta and Rakthashamaka* (pacifies Pitta and Raktha), *Krimighna* (reduce microbial load), *Vranashodaka* (cleanses wounds), *Vranaropaka* (promotes wound healing) and *Shothahara* (anti-inflammatory) [23].

Curcuma longa (*Haridra*) is Deepana (digestive), *Pachana* (carminative), *Uttejaka* (stimulant), *Rakthashodaka* (blood purifier), *Shothahara (anti-inflammatory), Krimighna (anti-microbial), Vranashodaka cleanses wounds* and *Vranaropaka* (promotes wound healing). Calcium hydroxide has the properties of *Srotoshodaka* (channel cleanser), *Shothahara* (anti-inflammatory), *Raktashodhaka* (blood purifier), *Vranashodaka* (Wound cleanser) and *Vranaropaka* (promotes wound healing) [23].

Mastitis can be compared with *sthanavidhradi* as described in Ayurveda which is a disease of *pitta* origin. The formulation consists of *Aloe vera*, *Curcuma longa* and *Calcium hydroxide* which are potent *pitta and Raktha shamaka* (Pacifies pitta humour). The formulation is *Agni deepana* (digestive), *Amapachana* (Carminative), *Krimihara* (reduce microbial load), *Puti rodhaka* (anti-infective) very good *Shothahara* (anti-inflammatory), *Srotoshodaka* (detoxifier), *Vranashodaka* (Wound cleanser) and *Vranaropaka* (promotes wound healing). Therefore, Mastitis (*Sthanavidradhi*) can be efficiently managed with this formulation [23].

4.2 Western science-based assessment

The *in-vitro* antimicrobial activity of aqueous, ethanol and ethyl acetate extracts of *Aloe vera* and *Curcuma longa* (turmeric) using agar well diffusion method exhibited antimicrobial activity against *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aurogenosa* [24]. A clinical study using the mastitis combination indicated that the selected parameters like pH, Somatic Cell Count (SCC) and Electric Conductivity (EC) of milk of the mastitis-affected animals became normal within 6–7 days of treatment. The milk production returned to near normal to the pre-mastitis level [25] (**Figure 10**).

Through an *in-silico* approach, bioactive compounds were tested for their effect against the target proteins of *S. aureus* using molecular docking studies [26]. Many bioactive components of *Aloe vera* and turmeric interact with the target protein. The pharmacodynamics study using the online server PASS reveals that the compound in the preparation possesses anti-inflammatory and anti-microbial properties. The anti-microbial activity of *Aloe vera* is attributed to the anthraquinones (aloin and emodin), flavonoids, tannins (active against MRSA), saponins, p-coumaric acid, ascorbic acid, pyrocatechol and cinnamic acid. Curcumin also possesses immunomodulatory and antioxidant activity [27, 28].

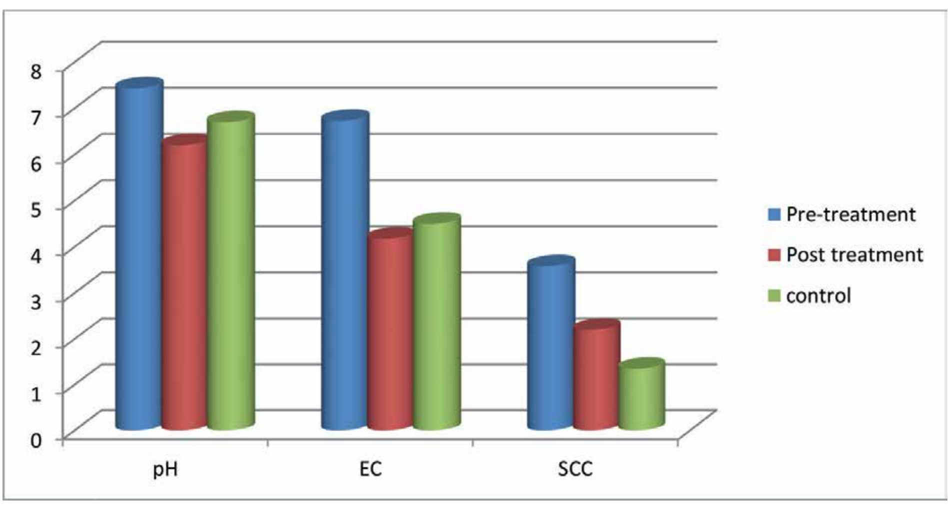

Figure 10.
The figure shows the reduction pH, electrical conductivity and somatic cell count of milk from mastitis-affected cows before and after treatment with an herbal formulation in comparison with values of normal cow: Pre-treatment – blue, post-treatment – red, normal cow – green bars.

Alkaloids, tannins, phenolics, terpenoids, phytosterols, saponins, flavonoids, glycosides, fatty acids such as palmitoleic acid and α-turmerone in fixed oils of *Curcuma longa* also possess antimicrobial activity against a wide range of bacteria. Curcuminoids have antioxidant, anti-inflammatory, antiviral and antifungal properties [29, 30]. Calcium hydroxide is known to possess anti-inflammatory action and reduces oedema formation. Thus all three ingredients in the formulation act at various steps in the inflammatory pathway and synergistically produce an anti-inflammatory effect [25, 29–32].

4.2.1 Changes in the microbiome of the milk from the cows with clinical mastitis before and after treatment with EVP

A preliminary study on the changes in the microbiome of milk from cows with clinical mastitis (The mastitis was confirmed with the California Mastitis Test) before treatment and after 6 days of treatment with ethno-veterinary herbal formulations. Abundance of *Streptococcus*, *Pseudomonas*, *Pseudomonaceae* family, *Klebsiella,* and *Enterobacteriaceae* family in the milk of mastitis-affected cows reduced to a minimum (**Figure 11**) [33].

Principal coordinates analysis (PCoA) indicates that the microbiome abundance in the milk of EVP-treated cows is almost similar to the control after 6 days. However, the microbiome in the milk from the antibiotic-treated cow varied substantially from the control animal (**Figure 12**).

5. Outcome of intervention of herbal formulation to prevent and cure mastitis as an alternative to antimicrobials

Eighty-eight per cent of subclinical mastitis cows turned CMT negative after application of the above oil-based ethno-veterinary herbal preparation (EVHP). The recovered animals produced 605 ml (7.3%) of additional milk per day. Ninety-two per

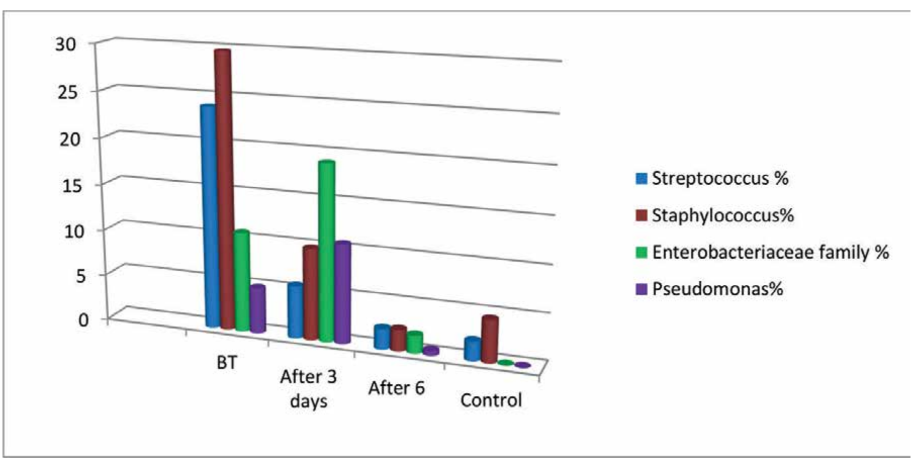

Figure 11.
The figure shows abundance of Streptococcus, Staphylococcus, Enterobacteriaceae family and Pseudomonas of control, mastitis-affected and EVP-treated cows. BT-before treatment, after 3 days of treatment, after 6 days of treatment and control.

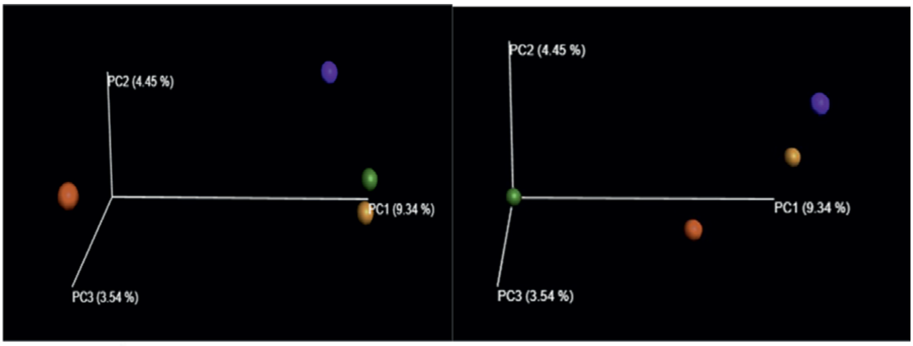

Figure 12.
(A) Principal coordinates analysis (PCoA) derived from one of the EVP-treated samples at different stages. Before EVP treatment, after 3 days, after 6 days of EVP treatment and control samples of F2 udder. Coloured dots are representative of the 4 samples. Red – F1 before EVP treatment; blue – After 3 days of EVP treatment; Orange – After 6 days of EVP treatment; green – Control. (B) Principal coordinates analysis (PCoA) derived from one of the antimicrobial-treated samples at different stages; before antimicrobial treatment, after 3 days of antimicrobial treatment, after 6 days of antimicrobial treatment and control samples. Coloured dots are representative of the 4 samples. Red – Control; blue – F1 before antimicrobial treatment; Orange – After 3 days of antimicrobial treatment; green – After 6 days of antimicrobial treatment.

cent of 3703 cattle and buffaloes treated with EVHP for clinical mastitis were clinically recovered after 5 days of treatment [34]. The application of EVHP shows a high clinical success rate of subclinical and clinical mastitis indicating the effectiveness of *Aloe vera*, turmeric and calcium hydroxide combination in mastitis management caused by a wide range of bacterial agents [35]. Ethno-veterinary herbal preparations comprising of Zambian grown *Aloe vera* and *Curcuma longa,* possess gram-positive antibacterial and antifungal spectrum of activity on bovine mastitis and other pathogenic microorganisms *in-vitro* [36].

The clinical intervention with EVM preparations on 181,252 cows in a multicentre field study for a period of 5 years from NDDB, Abbott and TDU on the efficacy of EVP in a stand-alone mode for subclinical, clinical and chronic mastitis with multifactorial aetiology and prolonged illness from 2017 to 2018 to 2021–2022, the recovery is 84.9.00% which is a reasonably high outcome by any standard from the clinical point of view (**Table 2**).

An intervention impact study indicated an 87.9% reduction of antibiotic residue in the milk and 12.1% of samples showed residues of beta-lactams or sulphonamides [37]. These 17 farmers used antibiotics along with EVP [37]. The incidence of mastitis was reduced to 83% in a selected area from 2016 to 2019 (**Table 3**).

The average expenditure for the treatment of mastitis with conventional medicine was Rs. 3324. EVP treatment has reduced expenditure for the management of mastitis from Rs.3324 to 120. The farmers saved an average of Rs 3204 for each episode of mastitis. The average milk production loss during 6 days during the treatment with Western medicine was 19.92 litres (Rs.518) and EVP was 3.6 litres (Rs. 93.6) (**Table 4**).

A little reflection on the data would suggest that the herbal formulations cannot possibly be arbitrary combinations because of their high effectiveness indicates that they have been obviously designed with some pharmacological logic. The logic is based on Ayurveda pharmacology called "*Dravya Guna Shastra*". Revalidating this formulation based on Western pharmacology is a cross-cultural or Trans Disciplinary

S. no	Mastitis	Total treated cases	Total clinical recovery	% Clinical recovery
1	Acute Mastitis	104,475	82,878	79.3
2	Chronic mastitis	52,791	41,502	78.6
3	Sub-clinical Mastitis	23,986	19,780	82.5
4	Mastitis (Abbott)	1692	1563	92.38
5	Mastitis (TDU)	1561	1432	91.7
	Total	184, 505	147, 155	84.9

Table 2.
Feedback from various milk societies from NDDB through INAPH, Abbott and TDU on the efficacy of EVP for mastitis in cattle from 2017 to 2018 to 2021–2022.

Year	2016	2018	2019
Average incidence of mastitis per union	65.63	36.5	10.6
Per cent reduction from 2016 to 2019		44.4	83.8

Table 3.
Reduction of the incidence of mastitis in cattle in the area selected for the studies when EVP was used from 2016 to 2019.

S. No	Mastitis	n	Average loss of milk/day (L)	Average loss for 6 days (L)	Financial loss in Rs.
1	Allopathic treatment	76	3.32	19.92	518
2	EVP treatment	76	0.6	3.6	93.6

Cost of Rs 26 per litre

Table 4.
Cost impact (production loss) when Western medicine and herbal formulations are used.

exercise. It would require extensive studies on advanced combinatorial chemistry as polyherbals are involved. It would further need systemic experimental pharmacology studies with innovative bioassays because conventional bioassays are far too fractured and inadequate to detect simultaneous changes on multiple targets and sensitively designed modes of action studies.

The current approach of creating objective and verifiable standards for traditional knowledge products and concepts is one-sided and therefore, an intercultural (subjective and objective) approach involving consultation between traditional and Western health sciences is necessary to promote mutual understanding which could create relevant quality standards [38–40].

In case of mastitis in over one hundred eighty thousand cases of dairy cattle, the recovery rate is 84.9% with EVM in a stand-alone mode (Field observational study and not double-blind controlled clinical study). The above data shows a reasonably high outcome by any standard from the clinical point of view, in a multicentre field study for a period of 5 years. Of course, we need further systematic studies. The field is wide open for exploitation of traditional herbal knowledge to benefit the farming community and save our precious animal wealth and a solution for the alarming misuse of antimicrobials and the related AMR issue.

6. Conclusion

Mastitis is a perpetual problem of all milk-producing animals. Mastitis therapy protocol includes a high percentage of antibiotics. The application of EVHP showed a high clinical success rate of subclinical and clinical mastitis indicating the effectiveness of *Aloe vera*, *Curcuma longa* and calcium hydroxide combination in mastitis management caused by a wide range of microorganisms. This formulation was validated using transdisciplinary understanding. Adopting ethno-veterinary science and practices to combat mastitis has been identified and tested as a key alternative in reducing the use of antibiotic(s). It is also indicated that the EVP formulations are cost-effective and could be prepared and used by the farmers themselves whilst being extremely helpful to prevent and manage mastitis of their cattle.

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Conflict of interest

The authors declare no conflict of interest.

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Section 2

Treatment of Bovine Mastitis

Chapter 2

Antimicrobial Resistance of Cattle Mastitis-Causing Bacteria: How to Treat?

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Abstract

Cattle mastitis continues to be a global burden for the dairy industry, and its control depends on preventive measures, rapid detection and identification of involved pathogens and accurate antimicrobial treatment. The (mis)use of antimicrobials initiated a rapid evolutionary process of bacterial resistance by natural selection and led to the increased frequency and spread of bacterial antimicrobial resistance (AMR). The global AMR emergency and the prudent use of antimicrobials in cows have raised questions about alternative treatment approaches; however, the use of antimicrobials remains the principal method for mastitis therapy. This chapter summarise the current knowledge on AMR in cattle mastitis as a multifactorial global problem, the trends in AMR patterns in the most common mastitis-causing bacterial pathogens and altering factors, the policies and actions restricting the use of antimicrobials in cows and related challenges in the treatment. The reasons influencing the outcome of treating an intramammary infection, such as the selection of appropriate antimicrobial agents, optimal drug regimens, the gaps in antimicrobial susceptibility testing (AST) of mastitis pathogens and interpretation criteria, and the paradoxical relationship between antimicrobial *in vitro* activity and *in vivo* efficacy are discussed. The importance of effective mastitis control programmes is emphasised by an overview of (accurate) diagnosis, the evaluation of the therapy, cow health control and farm management practices.

Keywords: cattle mastitis, bacteria, antimicrobial resistance, treatment, intramammary infection

1. Introduction

Mastitis, defined as the inflammation of the mammary gland, is the most frequent disease of dairy cattle and a significant economic burden for the dairy industry worldwide, affecting health, well-being, milk production and reproduction efficiency of cows [1, 2]. The most common cause of mastitis is an intramammary infection [3]. This disease can present in a clinical and subclinical form [4]. Clinical mastitis is characterised by visible abnormalities in the milk or in the udder, whereas subclinical mastitis is not visible and therefore is more difficult to detect. In addition, it occurs more frequently than clinical mastitis, and its duration is longer, which provides more opportunity for pathogens to spread between cows [3, 5, 6]. Depending on duration, mastitis occurs as peracute, acute, subacute and chronic [7]. Based on severity, it can be classified as mild (observable abnormalities in milk, generally clots or flakes, with little, or no signs of swelling of the mammary gland or systemic illness), moderate (visible abnormal milk accompanied by swelling in the affected mammary quarter with an absence of systemic signs of illness), and severe (sudden onset with grave systemic and local signs) [5, 8]. Severity and duration of mastitis mainly depend on the pathogen (s) involved, the host's health status/immune response and environmental factors [4]. Among various microorganisms associated with cattle mastitis, bacteria are the most frequently reported causative agents [9]. Traditionally and according to their primary source and transmission mode, mastitis causative agents have been classified as environmental pathogens (primary source is the habitat of the cow) and contagious pathogens (the main source is the mammary gland of infected cows) [7, 9, 10]. Furthermore, mastitis causative agents have been referred as major and minor pathogens, related to their prevalence, the severity of signs and the impact on cow health, milk quality and productivity [6, 11]. *Staphylococcus aureus*, *Streptococcus agalactiae* and *Mycoplasma bovis* are regarded as major contagious pathogens. Environmental pathogens are numerous: *Streptococcus uberis, S. equinus*, (*S. bovis*), (*S. dysgalactiae*), *Enterococcus* spp. (*Enterococcus faecalis*, *E. faecium, E. durans*), coliforms (*Escherichia coli, Klebsiella pneumoniae, K. oxytoca, Enterobacter aerogenes*), non-coliforms (*Proteus* spp., *Serratia* spp., *Yersinia* spp.), and others (*Pseudomonas aeruginosa*, *Trueperella pyogenes*) [6]. Minor contagious pathogens include coagulase negative staphylococci (CoNS) (*S. chromogenes, S. haemolyticus, Staphylococcus epidermidis, S. simulans, S. sciuri*), and *Corynebacterium bovis* [6]. The classification of pathogens as contagious or environmental is misleading, particularly for *S. aureus*, *S. uberis*, *S. agalactiae* and other streptococcal species because of potentially multiple transmission routes associated with the strains [12, 13]. The prevalence, dominance and distribution of mastitis pathogens vary temporally, within and between herds and countries [2, 14]. The changing trends of mastitis causative agents induces shifts in major and minor pathogens [11, 14]. Currently, *S. aureus* (25%), CoNS (20%), *E. coli* (11%), *S. agalactiae* and *S. uberis* (9%) are recognised as the major mastitis causative agents [15]. The control of the disease is challenging due to the difficulties in its prevention, diagnosis and treatment, largely depending on the effects of national mastitis control programmes. Regardless of the control strategy, antimicrobial treatment of mastitis in dairy cattle is an established component of mastitis control programmes [16]. However, the use of antimicrobials in dairy cows can contribute to increased antimicrobial resistance (AMR) [17], which is one of the major challenges for bovine mastitis therapy. The development of antimicrobial resistance is an adaptive response of bacteria to antimicrobials as environmental threats and their inappropriate use [18]. Reservoirs of antimicrobial-resistant pathogens, antimicrobial residues, zoonotic pathogens and antimicrobial-resistant bacteria in milk are risk factors of concern for public health [19]. Cow health and welfare on dairy farms are compromised not only by various (antimicrobial-resistant) pathogens and often untreatable infections, but also by the limitations of antimicrobial susceptibility testing (AST) and interpretative criteria, and the restrictions on use of antimicrobials in food-producing animals due to the AMR crisis.

2. Cattle mastitis in the era of antimicrobial resistance

The global crisis of AMR is induced by increasing globalisation, the needs of the large human population, intensive food production and changing climate associated with the increasing frequency of AMR among microorganisms and development of complex survival strategies as evolutionary response under the pressure of the widespread, inappropriate and extensive use of antimicrobials [18, 20, 21]. Thus, the AMR circle is linked to (pathogenic) microorganisms, the use of antimicrobials in animals and humans and the environment. The threat of globally increasing AMR to animal and human health, followed by the limited development of new antimicrobials and resolutions has led to national and international activities and rigorous measures [22–25] to reduce using of antimicrobial agents. The resulting limitations and increased need to combat the infections and AMR pathogens have prompted the search for alternative solutions and potential substitutes for antimicrobials in mastitis treatment for dairy cows [26, 27]. As the response to AMR, A Global Action Plan on AMR [23] addressing the challenge of AMR through a "One Health" approach was issued in 2015 by the World Health Organisation (WHO) in collaboration with the World Organisation for Animal Health (WOAH) and the Food and Agriculture Organisation of the United Nations (FAO) [28]. More recently, the European Commission (EC) has adopted a proposal for a Council Recommendation on stepping up the European Union (EU) actions to combat AMR in a One Health approach. The objectives are: strengthen One Health national action plans on AMR; reinforce surveillance and monitoring of AMR and antimicrobial consumption; strengthen infection prevention and control; strengthen antimicrobial stewardship and prudent use of antimicrobials; recommend targets for AMR and antimicrobial consumption in human health; improve awareness, education and training; foster research and development, and incentives for innovation and access to antimicrobials and other AMR medical countermeasures; increase cooperation; and enhance global actions [29]. Mastitis (42%) and respiratory disease (20%) are the main indications for antimicrobial use in cattle in Europe [30, 31]. Considering an estimated 60–70% of all antimicrobials used on dairy farms are for preventing and treating mastitis [6], further efforts to improve mastitis control may significantly contribute to reduction in the use of antimicrobials [32]. The rules laid down in the Veterinary Medicines Regulation in the EU [33], which provide a wide range of measures to fight AMR, including the prudent and responsible use of antimicrobials in (food-producing) animals, the restrictions on prophylactic and metaphylactic use in animals, and reserving certain antimicrobials for the treatment of infections in people, have been applied from 2022. In response, pragmatic national and farm-level recommendations in support of improved mastitis control and intramammary antimicrobial stewardship in the Irish dairy industry have developed [32]. The measures applied in Denmark and the Netherlands showed substantial reducing on-farm antimicrobial usage over the last decade [32]. The shared actions included a ban on the prophylactic use of antimicrobials, a national database of antimicrobial usage allowing objective measurement and benchmarking and transparent reporting, clarity on the level of veterinary oversight required, detailed treatment guidelines, national reduction targets in antimicrobial usage, and restrictions on the usage of specific antimicrobials. Several antimicrobial agents used for mastitis therapy, categorised in Veterinary Critically Important Antimicrobial Agents (VCIA) or Veterinary Highly Important Antimicrobial Agents (VHIA) [25] are critically important for human health and should not be as used a first-line treatment. Strict control and reducing inappropriate antimicrobial use in animals and humans are among the high priorities for addressing the AMR crisis; however reserving antimicrobials for human use only and limiting their use in veterinary medicine raise the question of the inability to treat infections in cows and consequences for cow health and welfare. Strategies that are more comprehensive should be promptly adopted in order to contain spread of infectious diseases among and between cows and humans, as policies to manage reservoirs of the pathogens related to cows, humans and the environment. A recent study [34] found a bidirectional association of antimicrobial consumption and AMR between humans and animals: animal antimicrobial consumption is positively linked with resistance in the human bacterial pathogens, while increased antimicrobial use in humans is associated to increased animal AMR. Moreover, socioeconomic factors play a significant role in the spread of AMR, implying antimicrobial consumption as a secondary risk factor, which reduction alone will not be sufficient to combat the worldwide AMR crisis [34]. This observation was supported by a more recent study [35], which showed that decrease in usage only slowly decreases resistance with no evidence of a reversal of resistance, and thus reducing usage is not a complete solution to alleviating high levels of resistance. Although antimicrobial consumption is considered as the most important factor contributing to AMR, resistance transmission appears to be the main driver for AMR levels [36].

Surveillance and monitoring of AMR is essential for assessing the trends related to the prevalence, source, spread and geographical distribution of AMR bacterial pathogens, for the early detection of emerged resistance and to provide information for evaluating antimicrobial usage and effects of actions to combat AMR [37]. National monitoring systems for AMR in bacterial pathogens of animals have been implemented by numerous countries [38–40]. Since the monitoring of AMR in bacterial pathogens of animals is not currently coordinated at European level, the European Union Joint Action on Antimicrobial Resistance and Healthcare Associated Infections (EU-JAMRAI) recently has recommended building the European Antimicrobial Resistance Surveillance network in Veterinary medicine (EARS-Vet) [39]. Despite numerous AMR surveillance systems on national and international level, such systems are still lacking in many countries. An online platform for surveys and maps of AMR in animals (resistancebank.org) that centralises information from low- and middle-income countries was recently introduced [40]. VetPath is an ongoing pan-European antimicrobial susceptibility monitoring programme collecting pathogens from diseased cattle, pigs and poultry not recently treated with antimicrobials [41]. The results of third VetPath monitoring period (2015–2016) for bacteria isolated pre-treatment from cows with acute clinical mastitis across European countries showed that mastitis pathogens were susceptible to most antimicrobials with exceptions of *S. aureus* (25.5%) and CoNS (29.1%) against penicillin, *S. uberis* against erythromycin (24%) and tetracycline (37.5%) and *S. dysgalactiae* against tetracycline (43.2%). High ampicillin and tetracycline resistance of 24% and 23.6%, respectively was observed in *E. coli*. The percentage resistance and the MIC values (the minimum inhibitory concentration-the lowest concentration of the antimicrobial required to prevent the replication of the bacteria) [42] of most antimicrobials for the major pathogens remained stationary when compared to those of the preceding VetPath surveys [43].

European Food Safety Authority (EFSA) [44] reported *S. aureus* and *E. coli* as being the most relevant antimicrobial-resistant bacteria in cattle in the EU. *Staphylococcus* spp. isolates have been reported to be resistant to β-lactams, tetracyclines, aminoglycosides, amphenicols, macrolides, trimethoprim, lipopeptides, and lincosamides in Europe [45]. On the global level the highest mean levels of resistance in *S. aureus* were observed for penicillin, according to the data collected in the period from 2010 to 2021. The mean proportions of resistance in Oceania (23.9%) and Europe (32.1%) were substantially lower than in Africa (57.7%), South America (59.9%) and Asia (64.2%). In European countries, the highest penicillin resistance was reported in Italy (63.1%), whereas the low levels of resistance were observed in Sweden (4%), Austria (10%), (14%) and Denmark (17.5%). Methicillin resistance was less common in Oceania and South America (< 3%), when compared to Africa (8.8%), Europe (9.9%) and Asia (19.1%). Resistance to the third generation cephalosporins (3GCs) was less apparent, ranging from 0% for ceftiofur in Africa to 13.7% for cefoperazone in Europe. Although resistance to the lincosamide pirlimycin was generally low (< 5%), 47% of 100 isolates exhibited resistance in Austria. Mean fluoroquinolone resistance levels were higher in Asia (20.5%) than in other continents, including Europe (7.9%), except in Italy where 36.9% of 122 isolates were resistant to enrofloxacin. Resistance to macrolide erythromycin was highest in Asia (30.9%) and Oceania (28.8%), while in South America and Europe was estimated at 4.9 and 5.5%, respectively. Resistance to neomycin was generally low, with exceptions of Canada (18.3%; reported as the resistant and intermediate) and South Africa (16.7%; reported as intermediate). Very low levels of resistance were observed for sulfonamide–trimethoprim in most continents, including Europe (0.6%). The highest mean resistance proportion (37.9%) was detected in Asia. Data obtained for penicillin–novobiocin showed no or very little resistance suggesting this antimicrobial to be effective for the treatment of *S. aureus* mastitis [44]. Similar findings have been recently reported; the highest overall prevalence of resistant *S. aureus* was against penicillin followed by clindamycin, erythromycin and gentamycin, while ceftiofur and cephalotin had the lowest overall prevalence. However, the AMR to almost all the antimicrobials showed an increasing pattern over time, among which clindamycin, gentamycin, and oxacillin had a higher increase in their AMR prevalence [46]. Contrary to *S. aureus* mastitis that respond poorly to antimicrobial therapy [46], intramammary infections caused by CoNS are usually self-limiting, although some clinical mastitis cases require antimicrobial treatment [47].

A weighted mean proportion of 10.9% resistance in *E. coli* was reported for 3GCs [44]. Despite the detection of 43.3% of 102 isolates resistant to ceftiofur in Ukraine, less than 8% of *E. coli* isolates were found to be resistant to 3GCs in Europe. Only one study tested cefoperazone and reported low resistance of 0.8% among 135 isolates in France. Resistance levels for aminopenicillins were similar in Africa (44.9%) and Asia (40.1%) and higher than in Europe (31.1%). In addition, in France 34% of *E. coli* isolates were resistant to amoxicillin, while the highest resistance percentage of 77.45 was recorded in Ukraine. Ampicillin resistance ranged from 11.3% in Denmark and 12% in Germany to 39.4% in the UK. Mean resistance levels were lower for amoxicillin–clavulanic acid compared with ampicillin, with the highest levels detected in Chine (81% of 100 isolates). In Europe the resistance was estimated at 13.3%. Mean proportions of fluoroquinolone resistance were low, particularly in Europe (3%). Contrary, the mean resistance proportions of 22% were detected in Asia. Higher mean resistance percentages were observed for gentamicin (35.4%) and neomycin among isolates in Asia (11.8%) compared with Europe (20.6% and 9%), where the highest resistance was observed for gentamicin in Ukraine (26.5%). Lower average levels of resistance were observed for sulfonamide–trimethoprim (12.6%) and tetracyclines in Europe (22.4%) when compared to other continents [44]. Resistance to β-lactams, tetracyclines, and amynoglycosides appears to be widespread [45]. In comparison, higher levels of resistance were observed in *E. coli* isolates from gastrointestinal cases than from mastitis cases for clinically important antimicrobials. Treatment of mild or moderate *E. coli* and other Gram-negative mastitis cases with antimicrobials is not warranted, while the use of antimicrobials to treat acute cases may be considered [30, 44].

Resistance levels were found to be similar for *S. uberis* and *S. dysgalactiae*. Overall mean levels of resistance for 3GC and penicillin were low and less than 7% in Europe. For the macrolides, most studies reported less than 25% resistance [44]. For lincosamide pirlimycin the resistance in *S. uberis* and *S. dysgalactiae* from Europe was 15.9% and 7.6%, respectively [43]. The mean proportion of sulfonamide–trimethoprim resistance for *S. uberis* ranged from 4.9% in North America and 12.7% in Oceania to 15.2% in Europe, while for *S. dysgalactiae* was 0.3% in North America, 7.4% in Europe, 14.3% in Asia, and 17.2% in Oceania. For fluoroquinolones, the mean proportion of resistance was 27.4% for *S. uberis* and 22.4% for *S. dysgalactiae* in Europe [44]. A recent review on AMR in bovine mastitis pathogens in European countries reported resistance of streptococci to macrolide, lincosamide, and streptogramin and the differences between *S. uberis* and *S. dysgalactiae* in the sense of a higher resistance prevalence in *S. dysgalactiae.* Generally low resistance to β-lactam antimicrobials was observed [45].

A few groups of antimicrobials are considered to be effective against mycoplasmas [48, 49]. The resistance in *M. bovis* to tetracyclines, macrolides, lincosamides, aminoglycosides, chloramphenicols, and fluoroquinolones appears to be rising [50]. High MIC values for spectinomycin, gentamycin and kanamycin were reported for isolates from milk [50]. Significant differences on the MIC values were found among Belgium, Germany and Italy for lincomycin, spiramycin, tylosin, oxytetracycline, florfenicol, enrofloxacin; however, a high level of resistance for macrolides and a low level of resistance for tiamulin and doxycycline were observed in all countries [51]. In China, the isolates had low MIC values to enrofloxacin and tiamulin [52]. Valnemulin was found to be effective against Spanish isolates [53]. Sensitivity of *M. bovis* to pirlimycin, danofloxacin and enrofloxacin, but not kanamycin, oxytetracycline, tilmicosin or tylosin was reported in Japan [54]. However, *M. bovis* mastitis is considered to be untreatable, and culling is the most common recommendation for its control [55].

Despite observed low resistance of major bovine mastitis pathogens to several cephalosporines and fluoroquinolones and the significance of these antimicrobials in veterinary medicine (categorised as VCIA or VHIA) [25], they meet the criteria related to human health: "A": High importance of the antimicrobial to human health to treat serious, life-threatening infections that have no or limited availability of alternative treatments and B: Risk of transmission of resistance to the antimicrobial from animals to humans, including cross-resistance or co-selection of resistance to other crucial antimicrobials [56]; "Critically Important" antimicrobials for human medicine, and thus should not be used as the first-line treatment in animals [57].

The AMR rates and patterns may vary by country or in one region over time, mainly depending on the (non)use of specific antimicrobials, bacterial species/strains and variable level of resistance among them, and the development and transmission of antimicrobial resistance. However, the data on AMR should be taken with precaution due to the limiting factors, such as the lack of information from many countries, the geographical and temporal variations, variable number of tested isolates being collected prior to antimicrobial treatment or after, the variety of available antimicrobials, methodologies (disk diffusion, broth microdilution, agar dilution), interpretative criteria (clinical breakpoints/epidemiological cut-off values) and the differences related to the Clinical Laboratory Standard Institute (CLSI) [58] and the European

Committee on Antimicrobial Susceptibility Testing (EUCAST) [59] guidelines [44, 46]. Because of the differences between CLSI and EUCAST recommended disk contents and commercial availability in the countries, these organisations initiated common criteria for development of optimal disk contents (potencies) in 2017 [60]. CLSI [58] is the only organisation providing internationally available methods and breakpoints specifically for many bacteria from animals [41, 61]. The results of AST provide guidance of potentially suitable antimicrobials; however, harmonised AST methods, veterinary-specific interpretive criteria are not available for all antimicrobials, bacterial pathogens, animal species and sites of infection, including those for bovine mastitis pathogens [41, 61]. The correct evaluation of AST results requires veterinary-specific clinical breakpoints (VSCBs) and quality control ranges [61]. Thus, the accurate status of AMR among mastitis-causing bacteria is largely unknown and the data so far reported are uncertain. These drawbacks underscore the urgent need for standardised guidelines for the AST and interpretation criteria, as prerequisites for adequate therapy, AMR monitoring and reporting at national and regional levels, and the harmonisation of a global AMR surveillance system.

3. Treatment of bovine mastitis: success or failure?

The outcome of mastitis treatment depends on many factors, such as the resistance of the causative pathogen against the chosen antimicrobial agent [61] and the lack of correlation between antimicrobial *in vitro* activity and *in vivo* efficacy [16]. Therapy with antimicrobials to which bacterial isolates showed susceptibility *in vitro* results in a low proportion of cure *in vivo*, and conversely bacterial isolates that are resistant to antimicrobials *in vitro* may cure following treatment *in vivo* [16]. This antimicrobial *in vitro*/*in vivo* paradox is difficult to explain, mainly because of unexplored host-pathogen–antimicrobial interactions and resulting responses/effects. However, special consideration should be given to several factors. Insufficient improvement of clinical signs might be related to specific physicochemical conditions at the site of infection (e.g., pH value, oxygen partial pressure and perfusion rate) [61]. Appropriate choice of antimicrobials, the pharmacodynamic and pharmacokinetics properties of antimicrobial agents, drug interactions, the selection of optimal antimicrobial drug regimens: dosing, duration of therapy, routes of administration and optimal therapeutic concentrations should be carefully addressed [30, 61].

The most common *in vitro* AST methods are disk diffusion, broth (micro) dilution and agar dilution [59, 62]. Based on the breakpoints the bacterial isolates are categorised as "S" "Susceptible, standard dosing regimen", when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent; "I" "Susceptible, Increased exposure" when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection; "R" "Resistant" when there is a high likelihood of therapeutic failure even when there is increased exposure [59]. According to CLSI "I" still stands for intermediate and "SDD" is a separate category "Susceptible Dose-Dependent". Clinical breakpoints are defined according to *in vitro* and in *vivo* data to predict the likelihood of clinical cure [30]. Thus, the determination relies on the distribution of MICs within the target bacteria species, combined with pharmacokinetic-pharmacodynamic parameters and data from clinical efficacy studies [30, 61]. These data are still unavailable for various antimicrobials for bovine mastitis pathogens [41]. Analysis for many antimicrobials specific to animals, including cattle and disease depends on breakpoints based on data specific for humans (MIC data, pharmacokinetics, particularly the serum concentrations, and clinical outcome of human patients) [41]. Currently, several antimicrobials have interpretive guidelines for bovine mastitis pathogens, and categorisation of susceptibility and resistance still relies on clinical breakpoints developed for humans [41]. In view of clinical efficacy, incorrect data on AST can be misleading for the choice of antimicrobial drugs resulting with inadequate therapy and AMR [61]. In addition, standardised procedures for MIC testing of antimicrobials against veterinary mycoplasmas (including *M. bovis*, one of the major bovine mastitis pathogens) and criteria for interpretation are lacking, while standard procedures such as the disk diffusion method are not recommended for mycoplasmas due their fastidious nature [49, 63]. There is the lack of ECOFFs/ECVs (i.e., the highest MIC for organisms devoid of phenotypically detectable, acquired resistance mechanisms, which defines the upper end of the wild-type MIC distribution), a necessary step when setting clinical breakpoints to guide therapy. This also prevents the separation of isolates with (non-wild-type) and without (wild-type) phenotypically detectable resistance and affects AMR surveillance and early warning of developing resistance [62, 64]. Nevertheless, ECOFFs are not adequate for classification of isolates as clinically resistant or to calculate the percentage of isolates that are multidrug-resistant (MDR) (defined as an isolate that is not susceptible to at least one agent in at least three antimicrobial classes) or extensively drug resistant (XDR) (defined as an isolate that is not susceptible to at least one agent in all but one or two antimicrobial classes) [65, 66] due to the lack of relevant pharmacological data [30].

Antimicrobial resistance occurs when bacteria have or develop to avoid the mechanisms of the drugs against them, the ability to replicate and not just survive in the presence of a drug [42, 67]. The most common measure of the level of resistance is MIC and a higher MIC corresponds with a higher level of resistance [42]. Natural resistance may be intrinsic, which is always expressed in the species, and induced, when naturally occurring genes are only expressed to resistance levels after exposure to an antimicrobial [68]. Acquired resistance is exhibited when a previously sensitive bacterium acquires a resistance mechanism [67] by mutations in chromosomal genes or acquisition of the genetic material from an exogenous source by horizontal gene transfer (HGT) that can occur through transformation, transduction, and conjugation [18, 67, 68]. Sub-MIC antimicrobial concentrations can positively select for resistance mutations, increase HGT of antimicrobial resistance genes (ARGs) and mutation rates [69]. The inoculum effect (higher initial density of cells resulting in lower susceptibility to some antimicrobials) may lead to the failure for treating infections because the actual MICs of bacterial populations are higher than those determined *in vitro* (e.g., bacteria producing antimicrobial-inactivating enzymes, higher rates of degradation correlate with higher number of bacterial cells) [69, 70]. Switch from resistance to susceptibility is not common [70]; bacteria are able to survive antimicrobials without encoding specific resistance mechanisms [69].

The contribution of non-inherited, phenotypic resistance to antimicrobial treatment failure appears to be significant [71]. Drug indifference occurs when the antimicrobial is effective only in a specific bacterial physiological condition (e.g., non-dividing cells are resistant to some antimicrobials, whereas other antimicrobials are active against stationary cells, but their level of activity is lower than when cells are actively growing). The antimicrobial concentrations required for curing an infection are directly related to the duration of the infection [70, 71]. In addition, the phenomenon known as “bacterial persistence”, “adaptive resistance” and “phenotypic tolerance" [71] may be responsible for the differences between the *in vitro* and *in vivo* effectiveness of an antimicrobial and involved in the clinical failure of antimicrobial treatments. It describes transient resistance to one or more antimicrobials, induced by a specific environmental signal (e.g., stress, subinhibitory levels of antimicrobials) or due to epigenetic phenomena like persistence that allows bacteria to respond more rapidly to antimicrobials [67, 72]. The increase in resistance as a response to environmental changes may not completely revert upon removal of the stimulus. This can lead to a gradual increase in MIC over time [67]. Both persistence and tolerance describe increased survival in the presence of an antimicrobial without an increase in the MIC [42] and allows bacteria to resume normal growth once the antimicrobial is removed [70]. While resistance and tolerance are considered properties of a population, persistence refers to the ability of a subset of the population to enter a state of dormancy and survive exposure to high concentrations of antimicrobial, whereas the rest of the population is rapidly killed [42, 70]. Therefore, persisters (persistent cells or a subpopulation of tolerant bacteria) [42] are predominantly dormant and can survive courses of antimicrobials, since antimicrobials are most effective against actively-metabolising cells. Moreover, they are also relevant in biofilms [73]. The level of persistence (the size of the persister subpopulation) will only weakly depend on the concentration of the drug if it is far above the MIC. The survival advantage of persisters is often observed for antimicrobial treatments belonging to different classes of antimicrobials [42]. Poor therapy response can also be explained by the lack of microbiological testing, inappropriate diagnosis [61] and polymicrobial infections [70]. Undetected mixed/polybacterial or polymicrobial infections and mastitis pathogens missed by standard culture pose a high-risk for treatment failure, the occurrence of recurrent infections, reservoirs of infection and dissemination of pathogen (s) among the cows. Culture-negative milk samples have been frequently observed from cases of clinical mastitis (40% of samples) [16], whereas, for example mycoplasmas have been rarely investigated in undiagnosed cases of mastitis (over a quarter of clinical and nearly 40% of subclinical cases) [55, 74]. Considering that multiple bacterial and/or other pathogens may be involved, such as fungi and algae, or mycoplasmas undetectable by conventional methods, antimicrobial therapy most likely was ineffective if microorganisms isolated from a mastitis sample are not primary pathogens [30]. Moreover, clinical susceptibility may not provide the probability of treatment success of polymicrobial infections where pathogens are embedded in complex multispecies microbial communities due to intra- and interspecies interactions that alter species responses under antimicrobial exposure [70]. Because resistance is determined by the interactions within that specific community AST should be conducted upon communities in addition to single-cell cultures [70]. Survival strategies of bacterial communities in the presence of antimicrobials are: (1) Collective resistance, interactions within a community that elevate the ability of its members to resist the action of an antimicrobial and continue to grow in the presence of antimicrobials thus increasing the MIC of the community; (2) Collective tolerance, interactions within a community that alter cell state, such as slowing down metabolism, and thus slow down the rate of cell death during transient exposure to antimicrobials without an increase in MIC; (3) Exposure protection, interactions within a community that protect its sensitive members during antimicrobial treatment by reducing the effective concentration of antimicrobial. These three main modes can additionally be enhanced by biofilm formation [70].

A biofilm is often defined as "an aggregate of microbial cells adherent to a living or non-living surface, embedded within a matrix of extracellular polymeric substances (EPS) of microbial origin". EPS is combined of extracellular macromolecules including nucleic acids, proteins, polysaccharides and lipids. Clinical biofilm-associated infections should be distinguished from microbial colonisation with non-pathogenic organisms [75]. Microorganisms (single or multiple microbial species) initiate biofilm formation under environmental pressure, such as antimicrobial treatment and subinhibitory concentrations of antimicrobials [70, 76]. Biofilms serve as barriers against host immune responses and drugs and protect their members through limiting the diffusion of antimicrobials into the population and increasing the protection provided by antimicrobial inactivation [69, 76]. This leads to resistance to antimicrobial treatment and reduction the possibility of eradicating infections [76]. Biofilms can also increase the proportion of persister cells within the population and levels of resistance by altering the expression of pre-existing ARGs [70]. One of the reasons for difficulties in resolving chronic mastitis cases is biofilm formation. However, most of the studies on biofilm associated with bovine mastitis are *in vitro*, living a gap on the composition, mechanisms of biofilm development, interactions between the host and biofilms, and the factors that can affect outcomes (increased or decreased biofilm formation) not necessarily linked to the use of antimicrobials [77]. Many bovine mastitis pathogens are able to produce biofilm, including *S. aureus,* CoNS, *E. coli*, *S. agalactiae*, *S. dysgalactiae*. *S. uberis, E. faecalis* [78], mycoplasmas [79], *Candida* spp. [80], and *Prototheca* algae [81]. The high resistance of biofilms to current antimicrobials makes its eradication very difficult; nevertheless, there are new promising strategies like antimicrobial peptides, nanotechnology, ozone, bacteriophage therapy, apitherapy and phytotherapy [27, 77].

Other factors involved in treatment success or failure include the lack of microbiological testing, AST and the evaluation of antimicrobial therapy. Microbiological testing of milk samples and AST of the isolates should be performed prior to therapy, in a prompt and timely manner. Repeated microbiological testing, approximately 7 days (depending on the used antimicrobial) following course of antimicrobial therapy is necessary to ensure clearance of infection and to exclude carriage. The postponed clinical responses should be avoided, as the delayed onset of improvement should not be interpreted as treatment failure [61]. Spontaneous cure (in the absence of antimicrobial treatment) of intramammary infections is recognised in dairy cattle, and thus antimicrobial treatment is not always required for resolution of clinical signs or bacteriological cure of intramammary infections [16].

4. Mastitis control programmes

Preventive measures based on cow health control, biosecurity and farm management are essential for effective mastitis control [82, 83]. Improving udder health at farm level is based on the reduction in duration of existing intramammary infection and reducing the incidence of new intramammary infection [4]. The "five-point plan" in the UK (routine maintenance of milking machines, post-milking teat disinfection, identification and antimicrobial treatment of clinical cases, whole herd antimicrobial dry-cow therapy and the culling of chronically infected cows) has been very effective in managing contagious pathogens until the rise in environmental pathogens and, therefore a need for some adaptations [32]. Following the ten-point mastitis control programme by National Mastitis Council (NMC) of USA was based on ten steps: 1. establishment of goals for udder health, 2. maintenance of a clean, dry, comfortable environment, 3. proper milking procedures, 4. proper maintenance and use of milking equipment, 5. good record keeping, 6. appropriate management of clinical mastitis during lactation, 7. effective dry-cow management, 8. maintenance of biosecurity for contagious pathogens and marketing of chronically infected cows, 9. regular monitoring of udder health status, 10. periodic review of mastitis control programme [84]. Mastitis control programmes and their effectiveness vary by country, and in some states, such as Norway and the Netherlands [85, 86] appear to be very successful. Unfortunately, such programmes lack in many parts of the world. Regardless of the control strategy, mastitis control programmes include antimicrobial therapy [16] and knowing the mastitis pathogens is critical to the rational use of antimicrobials [30]. Culturing of mastitis cases can dramatically reduce the number of cows that are treated with an antimicrobial [30]. For detection in a timely manner and to avoid false-negative results, milk samples from individual cowsand pooled with the number of milk samples lower than those in bulk tank milk should be examined for pathogens on a regular basis and sequentially, using culture-based methods combined with real time PCR or other highly sensitive molecular technique. The preventive and control measures should also include enhanced biosecurity on farms, regular controls of animal/human movement, quarantine and testing of purchased cows prior to introduction to farms, separation of suspected and removal of infected cows, proper milking and environmental/housing hygiene, correct dry-cow management, nutrition and vaccination and other actions contributing to improvement of cow health, immunity and welfare. Increasing farmer awareness of mastitis control strategies and AMR and communication with veterinarians are also crucial in combating and preventing cattle mastitis and reducing of overuse and misuse of antimicrobials. Efforts toward effective control and prevention of mastitis and prudent use of antimicrobials reflect in research on the development of new vaccines and alternatives to antimicrobials such as the use of bacteriophages, nanoparticles, cytokines, animal- and plant-derived antimicrobialcompounds, antimicrobial proteins, probiotics and prebiotics and homoeopathy [26, 27, 30].

5. Conventional therapy/prevention and alternatives to antimicrobial therapy

The emergence of resistant bacteria related to the treatment effectiveness, public health risks and the environment have raised the need for the novel therapeutic approaches [27]. However, the main course of treatment of bovine mastitis still relies on antimicrobial use. Antimicrobials are most often administered either by intramammary route or the systemic route [27].

After the advents of the antimicrobial era that produced an effective intramammary treatments, antimicrobial usage in dairy cows usually occurs as [87, 88]:

Primarily, clinical mastitis is mostly treated by intramammary administration of antimicrobial formulations (local treatment). Severe mastitis requires additional antimicrobials administered parenterally. Secondly, local antimicrobial treatment is performed at the day of drying-off, 45–60 days before the next calving. Drying-off treatment has shown significant effect in the reduction of mastitis and has enabled many dairy farms to reduce or even eliminate specific pathogens from their herds (dry-cow treatment – DCT). Usually, this procedure has been recommended for all cows at dry-off worldwide. Quite often when antimicrobial treatment is done (intramammary or parenteral) during any time of lactation period, milk is not suitable to be used and has to be discarded because of drug residues. After antimicrobial treatment is finished, withdrawal period has to elapse whose duration depends on pharmacological properties of used drug and during that time farmers experience economic losses. Beside main antimicrobial therapy, in more severe cases additional symptomatic and supportive therapy are of crucial importance to reduce local inflammatory process in the affected quarters, to enable a better perfusion of antimicrobial through tissues, as well as more rapid mammary tissue healing and restoring milk production. Regarding the current discussion about AMR, the described blanket antimicrobial DCT seems obsolete, although no data confirm that DCT bears relation to the emergence of AMR of mastitis or human pathogens [88]. Unfortunately, common use of antimicrobial therapy has made it undesirable in many aspects considering public health. Common utilisation of antimicrobials intentional or not may leave the significant residues in ecosystems and food chain and lead to development and spreading of resistant microorganisms. In the industry of fermented milk products these residues may cause a serious and even disastrous problem affecting all lines of production and health of final consumers.

Currently, according to reports approximately 30,000 humans in EU and 700,000 humans globally die every year from infections caused with multiresistant bacteria. Without solutions leading to a reduction in AMR, since 2050 approximately 10 million people annually are in great death risks from bacterial infections caused by bacteria with AMR [88]. Moreover, through cows and their products possibility of creating new resistant strains always exist and that fact have affected public concern, emphasising importance for the reduction of antimicrobial usage in food-producing animals.

Despite the use of antimicrobial dry-cow therapy, influence of pathogens from the environment to the appearance of intramammary infections or clinical mastitis is still quite common [88]. Formation of keratin plug in teat canal during dry period could be delayed or insufficient, which is a great risk factor for development of new intramammary infections. Mammary quarters with open and/or damaged teat canal have almost double risk to develop new intramammary infection during the dry period, compared to closed or undamaged teat canal. Using combination of antimicrobial dry-cow therapy and internal teat sealant to mimic the protective effects of the keratin plug and provide protection during the entire dry period, provides benefits over antimicrobials alone through improved prevention of new intramammary infections, subclinical mastitis, reduced somatic cell count and reduced use of intramammary antimicrobials in next lactation [89, 90].

The discovery and development of new treatment agents as alternatives in bovine mastitis therapy, comes together with consumers demand for antimicrobial-free products, which has led to several new options in therapy and prevention.

6. Vaccination

Considering historic and modern importance of vaccination in almost all areas of animal breeding, it is logical choice to formulate and implement certain vaccinal programmes in bovine mastitis prevention. In veterinary medicine vaccination programme is important and effective method in prevention and control of many infective diseases. However, unfortunately just several vaccines proved to be effective in routine practice. True success for any vaccinal regime depends on quality of vaccine, route of administration as well as coverage of vaccination among cows. Reports indicate that results in vaccine efficacy are quite different and to obtain satisfactory results many control measures have to be implemented as part of mastitis control strategy, because vaccination alone will not be solution on its own [27].

6.1 Nanotechnology

This relatively new technology often called nanotech has become a growing methodology in the 21st century with a great potential to be used as comprehensive tool in various industries. Nanoparticles offers many new and different types of materials to be used in veterinary medicine (nanotherapy), as well as reducing the problem of AMR and drug residues. Nanotherapy is making a significant economic influence in dairy industry, reducing the quantities of discarded milk and culled cows from herds. New delivery systems created by nanoparticles enables antimicrobial drugs to be used efficiently in low dosages directly into the target cells with shorter withdrawal period, leading to the reduction of side effects and financial losses. Nanoparticles are able to perform higher intracellular drug uptake compared to other typical ways of drug delivery systems. In this manner accumulation, antimicrobial activity and the retention time of the drug is increased, AMR is decreased and finally biofilm formation is inhibited [91]. To establish better control and overcome therapeutic difficulties against *S. aureus* related mastitis, inorganic nanoparticles like nanogels and antimicrobials have proven to act synergically and highly effective [91, 92].

6.2 Probiotics

According to numerous studies, probiotics have great potential for improving health and well-being. Classification of probiotics as probiotic drug mean that probiotic is associated with a certain medical condition and can be used as therapy or to prevent disorders. Lactic acid bacteria originating from the teat canal microbiome could be used in mastitis prevention. Live culture of *Lactococcus lactis* after intramammary administration proved in some cases to be effective like antimicrobial treatment, but without any withdrawal period. The infusion of *L. lactis* into the bovine mammary gland promoted recruitment of neutrophils, and increased concentrations of milk acute-phase proteins and expression of genes encoding cytokines IL-8 and IL-1 β. Isolates of the *Lactobacillus* and *Lactococcus* genera showed inhibitory activity toward some major mastitis pathogens like *S. aureus*, *S. uberis* and *E. coli* [93].

6.3 Phytotherapy

Utilisation of plants is part of traditional medicine worldwide and is one of the most promising alternative options in the prevention and treatment of health disorders. Many traditionally used medical plants possess antimicrobial, anti-inflammatory, antioxidant and immunomodulatory potential. Biological diversity of herbs from numerous world regions provides an almost endless choice of raw materials with huge potential in medicine. Possibility of being used synergically with antimicrobials, highlights the importance of medical plant–antimicrobial combinations against common pathogens, as well as against resistance-modifying agents. Some essential oils extracted from plants express even antibiofilm properties. Plant extracts may be utilised in different ways like infusion, gel, spray or ointment. Some researchers reported that the effect of phytotherapeutical remedies used in mastitis treatment was similar to conventional antimicrobial therapy but without an irritating effect on the udder and had minimal residues in milk. One of the promising alternative phytotherapeutics against mastitis pathogens is Cinnamon essential oil. It shows powerful bactericidal characteristics with beneficial anti-inflammatory effects and a reduction in tissue damage in mammary gland. Essential oils have also shown strong antimicrobial activity against causative agents of protothecal mastitis, as well as against some other typical mastitis pathogens like *Staphylococcus sp*., *Streptococcus* sp., *Bacillus cereus* and *E. coli*. Unfortunately, undesirable properties like instability, biodegradability and low solubility of essential oils in certain solutions exist but could be improved in combinations with nanomaterials to improve their transport and efficiency. Phytotherapy utilisation may highlight significant economic benefits, especially with a focus on subclinical mastitis, because it is responsible for most of the financial losses [27, 92, 94] and could have a great potential to be used not just in conventional but especially in organic farms.

6.4 Bacteriophages

They are defined as viruses with ability to infect bacteria and to continue replication inside of them, suppressing their proliferation. Their important ability is to be able to target specifically only the pathogens of interest, while microbiome of the host is not affected. The main limitation of the phages is their specificity. Single bacteriophage can affect only a certain number of bacterial strains, and treatment of infection caused by several possible bacteria requires different phages. To increase potency, administration of phages can be in the form of cocktails or together with some antimicrobials. This makes them desirable for treatment against multidrug-resistant bacteria, possessing low probability of resistance development. Moreover, phages are degraded in nature after solving infection, while antimicrobials can persist for a long time. Phages are a powerful option for the post-antimicrobial era, especially against drug-resistant bacteria, where they also reduce the number of somatic cells, contribute to inflammatory factors, relieve the signs of mastitis in cattle and even potentially be used in the development of vaccines [95].

6.5 Low intensity laser radiation

In recent years this methodology represents an alternative and non-pharmacological therapeutical way with many previous positive uses in humane medicine. To perform treatment, every udder quarter have to be irradiated, divided in daily treatments and cycles. Beneficial response from the cows is expressed in the form of decreased number of microorganisms, more receptive microorganisms to antimicrobials treatment or blood vessel regeneration. Laser irradiation stimulates the phagocytic activity of milk granulocytes, becoming more active in destroying the etiological agents of mastitis. Irradiation treatment increases healing rate from mastitis treated intramammarily or intramuscularly with antimicrobials. Compared to antimicrobial treatment alone, irradiation enables faster regression of clinical signs such as redness, pain, hardening, inflammation, and oedema, promotes healing of wounds, deeper tissues and nerves and prevents cell death and tissue damage, as well as faster disappearance of macroscopic changes in milk and better elimination of intramammary infections. The supportive effect in treated mammary glands is probably due to the regulatory effect on pro- and anti-inflammatory cytokines *in vivo* and *in vitro* and with stimulation of the immunological system in vivo. Temporarily, the higher number of somatic cells in treated cows may be due to a bio-stimulating effect because, at the tissue level, laser therapy stimulates the immune system by accelerating phagocytosis, blood and lymph circulation and intracellular generation of active oxygen forms. Effectiveness of treatment increases when repeated for several days [96–98].

6.6 Ozone

Ozone as a gas represents polimerised oxygen (O_3), created by ozone generator or under the influence of ultraviolet light. Application of ozone in desinfection and reduction of microbial population and decontamination is well documented. The bactericidal, fungicidal and virucidal properties of ozone, through a strong oxidation effect, seem to have significant potential in the treatment of mastitis in bovines [99]. Local and systemic signs tend to improve after ozone administration to clinically inflamed quarters. Compared to antimicrobial administration, milk is not discarded during treatment or after. Chronical mastitis proved to be more difficult to cure totally by ozone administration alone, and certain microbials like *S. uberis* shows good resistance against ozone treatment. In these situations, ozone therapy should be combined with antimicrobial treatment. In general, ozone treatment is cheap and with similar effectiveness as antimicrobial treatment for clinical or chronical mastitis, but also reduction in somatic cell number seems to be somehow faster [99, 100]. Other reports coincide with statement that ozone administration in cases of clinical and subclinical mastitis in dairy cattle may lead to elimination of causative agents and detoxification of the inflamed quarter [101, 102]. Even ozone water seems more effective when compared to antimicrobial administration, especially in cases of coliform mastitis when irrigation of a quarter is needed. Irrigation with ozone water may cause lower endotoxin release from *E. coli* to the milk other than the treatment with local or systemic antimicrobials [101]. Minor problems could appear in routine practice with ozone treatment because it usually requires several cycles of treatments [100–102].

6.7 Apitherapy

Apitherapy has been used as a traditional remedy since ancient times for possessing various therapeutic activities (antimicrobial, anti-inflammatory, antioxidant, antiproliferative, immunomodulator). This method is very safe, highly effective, easily applicable and extremely economic. Apitherapeutic management is gaining more importance in the modern medicine and could be used for many varieties of different health disorders. This is especially important in food-producing animals as it is highly safe for cow products and very effective. Apiproducts like honey, pollen, propolis and venom, proved to have wide effectiveness, which depends on botanical, geographical and seasonal conditions, leading to differences in their potency. Multiple compounds contained in bee products act synergistically and are very effective in different concentrations against even multi-drug-resistant bacteria, besides boosting immunity and antioxidative effect. Apiproducts act as natural compounds with none or minimal irritation to tissue, even to sensitive mammary tissue, which is very susceptible to irritation. Administration of honey intramammary has beneficial effects against bacteria, but it also led to an increased number and activity of total leucocytes, helping to resolve mastitis and eliminate causative agents [103]. Diluted or even undiluted honey may be used as intramammary treatment against bacteria like *S. aureus*, *P. aeruginosa* and *E. coli*, while it is harmless to mammary tissues and without undesirable residues in milk. Because repeated administration does not produce any microbial resistance, it could be a good choice for mastitis treatment in conventional and organic farms [104–106]. Besides honey, propolis is also one of the well-known and used honeybee products. This substance has a complex chemical composition with many expressed biological activities. Intramammary administration of propolis showed significant antimicrobial, antioxidant, immunostimulatory and anti-inflammatory abilities.

These activities in mammary gland makes propolis great alternative to conventional therapy, and some reports proved it is even more effective against Gram-positive bacteria than Gram-negative bacteria [107–109]. Propolis have ability to even reduce the growth of typical mastitis pathogen like *S. aureus* to an average of zero [107], but to reduce reaction of mammary tissue concentration must be lower [108]. Surprisingly, even honeybee venom usually utilised as pain reliver and in treatment of inflammatory diseases is highly effective against typical mastitis pathogens including methicillin-resistant *S. aureus* (MRSA) without side effects even in its lowest concentrations [110, 111].

6.8 Homoeopathy

In the last decade, this method is gaining popularity for food-producing animals, especially in organic farms. Methodology is based on a holistic approach with the goal of stimulating the cows' immune system and fighting against AMR. In India, therapies with homoeopathic remedies and their combinations have proved effective against mastitis. Even their combination with certain antimicrobials could be part of the solution in the successful control of bovine mastitis. Side effects or particular allergy reactions are not common, and there are no residue problems or withdrawal period in milk or the product, non-environmental pollution and these remedies are for many farmers. However, references to homoeopathy are still relatively limited and more research in the area of holistic remedies and treatments is required to prove real medical efficiency of this approach [112–114].

6.9 Bacteria-derived antimicrobials

This group of antimicrobial peptides is active against many Gram-positive and Gram-negative bacteria. Compared to antimicrobials, these peptides like bacteriocins have a very narrow spectrum of antimicrobial activity, which allows them to target only specific pathogens and work efficiently even against antimicrobial-resistant strains. Bacteriocin nisin produced by lactic acid bacteria (*L. lactis*) proved to have an inhibitory effect against mastitis-related pathogens like *S. uberis* and *S. agalactiae*. These peptides are becoming desirable therapeutic options in food-producing animals for activities against mastitis-related and foodborne pathogens. There are some new reports about successful mastitis treatment with bactofencin, nisin and reuterin. All of them were highly active against multidrug-resistant mastitis isolates, while nisin could even express antimicrobial activity on biofilm-producing *S. aureus* cultures. Certain bacteriocins can act synergistically with conventional antimicrobials, leading to reduced drug concentrations, decreased side effects, and the appearance of new resistant strains [115–118].

7. Animal-derived antimicrobials

Milk contains peptide substances like lactoferrin and other similar proteins with antimicrobial properties such as immunoglobulins, lysozyme, β-defensin and lactoperoxidase. These peptides have a broad spectrum of activities, which control many biochemical processes. They all can potentially be used in treating various infectious diseases caused by bacteria, fungi and protozoa. Their activity can neutralise toxins, inactivate bacteria, and limit or prevent bacterial adherence to the mammary tissues.

The property of lysozyme to hydrolyse the essential bacterial cell component peptidoglycan, was used successfully in increasing antimicrobial efficacy against *S. uberis* and *S. dysgalactiae*. Spectrum of antimicrobial activity may even increase in combinations like lactoferrin and β-lactoglobulin, because of their different activities on different bacteria. More than 60 antimicrobial peptide drugs have already reached the market, while more therapeutical peptides are yet to come, waiting to finish preclinical and clinical development [119]. All above-mentioned peptides could be considered as possible non-antimicrobial agents against bovine mastitis-related pathogens with further potential to be used with antimicrobials [119–122].

8. Conclusions

The use of antimicrobials remains the major approach for mastitis treatment. The standardisation of AST, determination of clinical breakpoints and interpretive guidelines for bovine mastitis pathogens are crucial for the appropriate selection and use of antimicrobials, AMR monitoring at national and regional levels, and the harmonisation of a global AMR surveillance system. The lack of routine microbiological testing of milk samples and AST of the isolates may lead to improper therapy, the persistence of mastitis, increased transmission of pathogens and AMR rise. Multiple mastitis pathogens missed by standard culture and biofilms are high-risk factors for treatment failure. The success of mastitis treatment highly depends on appropriate choice of antimicrobials, the pharmacodynamic and pharmacokinetics properties of antimicrobial agents, drug interactions and the selection of optimal antimicrobial drug regimens. In addition, AST of the isolates prior to therapy and the assessment of antimicrobial therapy are among the main steps for AMR monitoring and prevention of its occurrence. Implementing national mastitis control programmes and evaluation their effectiveness are imperative. Some countries are ahead of others in terms of improved approaches to mastitis management and control of antimicrobial consumption on dairy farms; their experience can guide the development of further strategies. Cow health control, udder health monitoring, improving farm management practices, identification and reducing the risk factors of mastitis, pathogen introduction and spreading, monitoring and restricting antimicrobial usage (and reserving the antimicrobial agents for the therapy), should be regularly applied until the development and implementation of more effective control measures, alternative farming systems and/or the decrease in consumption of cattle products.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 3

Attitudes, Social Influences and Decision-Making in the Choice of Antimicrobials for the Treatment of Bovine Mastitis

Richard Zapata-Salas, José F. Guarín and Leonardo A. Ríos-Osorio

Abstract

The excessive and irrational use of antimicrobials for the control of bovine mastitis has been the subject of study throughout the world for many decades. Currently, the use of antimicrobials in livestock is of great interest from the "One Health" approach. Scientific research has revealed that the reasons for the inappropriate use of antimicrobials in production are related to human behavior, political, commercial, and economic factors. The objective of this study was to understand the attitudes toward the use of the laboratory and the social influences for decision-making in the choice of antimicrobials in the treatment of bovine mastitis in the North of Antioquia, Colombia. The study was developed through mixed methods (cross-sectional and grounded theory) with a convergent triangulation design. Two hundred and sixteen dairy farmers and 9 veterinarians participated. The results allow us to conclude that the problem of antimicrobial resistance related to mastitis and udder health planning depends on both technical aspects and access to knowledge and to laboratory services and, perhaps, even more importantly, on the culture and social relations that are established between the dairy farmer and the other actors of the dairy chain in the north of Antioquia.

Keywords: social influences, decision-making, antimicrobials use, laboratory use, bovine mastitis, antimicrobials resistance

1. Introduction

Bovine mastitis is the most important bacterial disease in primary milk production [1]. In the dairy sector, antimicrobials are widely used as a strategy to control this disease, once an inflammatory process has been detected in the mammary gland [2, 3]. In several countries, such as Ireland, Canada, and the Netherlands, approximately one-fifth of the antimicrobials used in dairy farming are used for the treatment of clinical mastitis and about half is used in the treatment of dry cows [4–6].

It is estimated that more antimicrobials are used in animals than in humans. In addition, many of the classes of antimicrobials used in humans are also marketed for use in animals, including broad-spectrum beta-lactams and quinolones. In addition, other antimicrobials such as tetracycline, triazoles, and streptomycin are used both in humans and in animals, and even therapeutically in plants. This has raised concerns in terms of public health due to its impact on human, animal, and environmental health [7].

Antimicrobial resistance has been shown to be easily transferred between different ecosystems and bacterial populations; even resistant zoonotic bacteria can be found in soil, which can infect plants, animals, and humans. For example, Methicillin-resistant *Staphylococcus aureus* (MRSA) is the cause of serious infections in hospital settings, and it is also the main mastitis-causing bacteria in dairy systems. In many cases, these resistant strains have arisen in food-producing animals, due to the irrational use of antimicrobials, and are transmitted to humans through interaction during production [8, 9].

The use of antimicrobials in the dairy sector is influenced by social factors, where dairy farmers are sensitive to social norms set by other producers and rely on the expertise of other actors, such as veterinarians. In addition, public policies, education, communication, time, finances, and adequate language are considered alternatives to transform inappropriate practices in the use of antimicrobials among dairy farmers and to promote appropriate decision-making [10, 11].

In Colombia, there are no specific policies on the restricted use of antimicrobials in the dairy sector, and no studies have been carried out on the use of the laboratory and social influences to promote an appropriate use of antimicrobials in the prevention and control of bovine mastitis. Therefore, the objective of this study was to understand attitudes toward laboratory use and social influences on decision-making on the choice of antimicrobials in the treatment of bovine mastitis in the north of Antioquia, Colombia.

2. Materials and methods

Study design: Mixed methods (Cross-sectional and Grounded Theory) with a convergent triangulation design [12].

Study subjects: In the quantitative phase of the study, non-probabilistic sampling stratified by municipality and size of the production system was used to select 216 dairy farmers in the north of Antioquia. From each farm, the owner, administrator, or leading milker in charge of production was chosen. This person had complete knowledge of the management of the production system, use of the laboratory, and choice of antimicrobials.

Dairy farmers and veterinarians who attend the farms of the participating farmers took part in the qualitative phase. The number of participants was defined through a theoretical sampling by category saturation [12], the saturation of pre-established and emerging categories was achieved with 17 dairy farmers. The selection of participants was carried out through a sampling of maximum variation, with the aim of capturing the maximum plurality of discourses that characterize human reality in relation to the objective of the study: gender, age, farm size, municipality, educational level, and functions on the farm. Likewise, these participants were considered as key actors according to the results of the survey. For the population of veterinarians, the saturation of pre-established and emerging categories was reached with 9 veterinarians. Sampling by maximum variation included: gender, age, work experience as a dairy farm veterinarian, municipality, and employment relationship.

Information Collection: A 6-question survey was applied to characterize age groups, gender, educational level, socioeconomic stratum, functions, and size of the production system. Additionally, we worked with an instrument of 7 questions on the use of antimicrobials and the use of the laboratory and training and its association with the biological indicators of udder health (BTSCC and CFU). Subsequently, an initial appearance validation and a content validation of the selected items were performed to determine the relevance of the item structure, as well as the completeness, exclusivity, and precision of the variables. Subsequently, 40 subjects from the study population evaluated the preliminary instrument, determining its acceptability and applicability.

A semi-structured interview was developed from the categories identified in two systematic reviews on udder health [13, 14]. The instrument underwent an initial appearance validation and a content validation of the selected themes. The interview inquired the meanings, representations, and attitudes about the self-consumption of milk, the use of milk with antimicrobial residues, and the formal and informal milk trade that dairy farmers and veterinarians experience in their daily lives. The interview starts from the following scheme: (a) Contextualization of the study and informed consent, (b) self-consumption of milk, (c) use of antimicrobials, and (d) formal and informal milk trade, without it following a pre-established order. Two to three face-to-face interviews were conducted with each participant according to the open format, axial coding, and selective grounded theory [15].

Essential methodological criteria of the qualitative component: In the study, the criteria of credibility, auditability, and transferability were applied [16].

The data on the udder health indicators (BTSCC and CFU) were supplied by the dairy company to which the farmer sells his milk, with his prior endorsement. The laboratories are accredited under the NTC-ISO/IEC 17025 standard: 2005. The averages for these variables were calculated by taking biweekly data during the period between September 2019 and August 2020. The CFU and BTSCC variables are presented according to ranges based on Colombian regulations and adapted by Múnera-Bedoya et al. (2017) [17].

Analysis of information: Absolute and relative frequencies are described for the categorical variables. The BTSCC and the CFU were defined as dependent variables. The association between the survey variables and the dependent variables was evaluated using the Mann Whitney U and Kruskal Wallis H tests, after verifying noncompliance with the assumption of normality evaluated using the Kolmogorov-Smirnov test with Lilliefors correction. Data were analyzed using SPSS-IBM version 25® software. In all the analyses, a statistical significance of $p \leq 0.05$ was taken.

All interviews were recorded. The recordings were transcribed with the Transcribe version 4.13.0 software, reviewed, and corrected manually, guaranteeing their total accuracy. Later, they were imported into the Atlas.ti version 22 software. The interviews were analyzed following the open, axial, and selective coding stages. Open coding allowed the conceptualization from the abstract representation of the phenomena described by the participants. In this sense, a code was assigned to each fragment of the text. These codes were compared according to their common characteristics and meanings. This coding is born from theoretical categories pre-established by the authors and from the words of the participants. Axial coding arises from the codes created in open coding. Here, the categories and subcategories were established, and their relationship according to their properties and dimensions. The central category (Use of milk with antimicrobial residues) was determined by selective coding, and all categories were integrated to propose a theoretical construct. The central category was defined based on the following criteria proposed by Strauss and Corbin: I. That all the main categories are related to the central category, II. Where each one of them, or the majority, contributes indicators to the concept, III. That the relationship between the categories allows a solid explanation, IV. That it explains the contradictory cases, or alternatives, to the central idea of the category, V. That the concept is refined when it is integrated with other concepts. The theoretical scheme made it possible to eliminate excess data and complete the underdeveloped categories, through additional theoretical sampling. The constructed theory was validated by comparing it with the raw data and by the recognition of the theoretical proposal by the participants as an approximate conceptualization of their realities [15].

Based on the methodological proposal for mixed methods with a convergent triangulation design, an analysis of the integration of results was carried out through the comparison of qualitative and quantitative results in a matrix and theoretical discussion [12].

Ethical aspects: This study was approved by the bioethics committee of the University Research Headquarters- SIU of Universidad de Antioquia, approval document 19–101-876, governed by Resolution 8430 of 1993 from the Ministry of Health of Colombia, the principles of the Declaration of Helsinki, the code of federal regulations, title 45, part 46 for the protection of human subjects of the department of health and human services from the national institutes of Health of the United States (1991), and resolution 2378 of 2008 from the Ministry of Social Protection of Colombia. Signed informed consent was obtained from each participant, and endorsement to request data about udder health indicators from the dairy company to which they sell their milk.

3. Results

The analysis by age groups shows that the majority of dairy farmers participating in the study are located in the adult category (77.8%), followed by older adults (17.1%) and by young people (5.1%). Only 6% of the participants were women. 80.6% of the surveyed farmers only studied up to secondary school, while only 7.9% reached technical-technological training (7.4%), and 11.5% achieved professional training with or without specialization. The highest proportion of farmers was classified in socioeconomic stratum 2 (49.5%), followed by socioeconomic stratum 3 (27.8%). Half of the surveyed people perform all functions in the production system and are associated with a cooperative. 40.2% represent small farmers (less than 1529 liters/week), 32.9% medium-sized farmers (between 1530 and 3822 liters of milk/week), and 26.9% large farmers (more than 3822 liters/week).

The sanitary quality of the milk (BTSCC) was deficient in 67.6% of the farms, while 31% of them had an acceptable or good sanitary quality, and only 1.4% had an excellent sanitary quality. Regarding the hygienic quality of milk (CFU), 54% of the farms obtained an excellent rating, 24% obtained an acceptable or good rating, and 22% presented deficiencies in this aspect (see **Table 1**).

A relation was found between those who intend to sell their milk in the town, when the dairy industry does not buy it because it has a high BTSCC, and the BTSCC variable. Which is more prevalent in those who plan to sell their milk in the informal trade. Likewise, a relation was identified between those who intend to sell their milk in the town when the dairy industry does not buy it due to a high BTSCC and the intention to choose antimicrobial treatment, based on laboratory results. 91.1% of those who plan to sell their milk in the informal trade disagree with

BTSCC			CFU		
Classification (range: cells/mL)	**n**	**%**	**Classification (range: Units/mL)**	**n**	**%**
Excellent (< 150,000)	3	1.4	Excellent (< 75,000)	117	54
Good (150,001 - 250,000)	19	8.8	Good (75,001 - 150,000)	39	18
Acceptable (250,001 - 400,000)	48	22.2	Acceptable (150,001 - 250,000)	12	6
Poor (> 400,000)	146	67.6	Poor (> 250,000)	48	22

Table 1.
Tank milk quality according to somatic cell count (BTSCC) and Colony forming units (CFU).

making use of the laboratory to select an adequate treatment (p < 0.001). CFUs are higher for those who perform less than one antimicrobial treatment per month; those who do not agree to perform cultures and antibiograms in milk samples from cows with mastitis, to select a suitable antimicrobial according to the susceptibility of the isolated bacteria; those who do not train their workers in mastitis prevention; or those who do not have workers. The BTSCC and the CFU are higher for those who do not receive an offer of laboratory services for their cows from the collecting company (see **Table 2**).

			BTSCC average	CFU average
		%	**Mean (IQR)**	**Mean (IQR)**
Antimicrobial treatments for mastitis per year per farm (treatments per month)	0 to 6 in a year (less than 1 treatment per month)	31.5	514,533 (380945–670,480)	113,609 (32656–303,134)*
	12 to 24 in a year (from 1 to 2 treatments per month)	43.1	543,467 (369875–690,933)	42,950 (22222–131,453)*
	26 to 416 in a year (more than 3 treatments per month)	25.5	439,650 (363973–654,786)	53,099 (20556–139,133)*
The antimicrobials that I use are solving the mastitis problem on my farm	In disagreement	11.6	644,000 (398444–670,480)	68,022 (34422–303,134)
	In agreement	88.4	495,089 (357127–670,480)	55,600 (22166–169,444)
I choose antimicrobial treatment based on culture results and antibiogram	In disagreement	73.6	522,911 (378933–670,480)	79,000 (26267–274,750)*
	In agreement	26.4	439,888 (327550–670,480)	37,333 (15044–112,468)*
Perception of cost of inputs and animal health vs. price of milk	Fair	0.5	188,400 (188400–188,400)	13,555 (13555–13,555)
	Unfair	99.5	495,156 (368067–670,480)	63,622 (22682–206,622)
The company to which you sell your milk offers laboratory services for your cows	Yes	52.3	465,822 (332600–670,480)*	48,220 (25267–110,267)*
	No	47.7	634,350 (398235–687,727)*	110,000 (18640–303,134)*

			BTSCC average	CFU average
		%	Mean (IQR)	Mean (IQR)
Mastitis prevention training	Yes	63.9	487,557 (345356–670,480)	51,827 (18822–154,456)
	No	36.1	511,828 (398444–670,480)	85,245 (31867–220,467)
Training of workers in mastitis prevention	Yes	25	439,769 (365800–670,480)	40,820 (15555–124,529)*
	No	43.5	480,622 (350042–670,480)	53,429 (20866–206,622)*
	Has no workers	31.5	590,605 (408814–671,174)	93,356 (30267–303,134)*

** indicates the association between each of the independent variables and the dependent variables BTSCC or CFU when the p value is less than 0.05 ($p < 0.05$).*

Table 2.
Use of antimicrobials, use of laboratory, and training.

4. Categories and subcategories built on the analysis of actions, related to decision-making regarding the choice of antimicrobials, and the risk of promoting bacterial resistance to antimicrobials

The qualitative analysis of the interviews with dairy farmers (DF) and veterinarians (V) allowed us to recognize 4 categories: use of the laboratory (culture and antibiogram), social influences on the use of antimicrobials, resistance to antimicrobials, education, and technical assistance.

4.1 Use of the laboratory (culture and antibiogram)

Dairy farmers are reluctant to perform cultures and antibiograms, which leads to indiscriminate use of antimicrobials, and this hinders medical work to treat mastitis.

V4. People use antimicrobials indiscriminately. It is very difficult that we have to get used to working without tools. People do not want to do cultures.

Due to the culture of intervention in cases of mastitis, in which a negative attitude toward the use of the laboratory and self-medication is encouraged, veterinarians experience pressures that lead them to recommend the use of antimicrobials without performing antimicrobial susceptibility tests. In some cases, this results in an unsatisfactory cure for mastitis.

V5. Normally, you recommend an antimicrobial, and the person simply says, "It did not work for me, because I have been using this one and this one, and none of them work for me. And the one you prescribed did not work for me either." So, it also became a very complex issue because you no longer know what to recommend for one of those types of mastitis. Ideally, a culture plus an antibiogram should always be done and thus know what is going to work, but this does not happen.

The use of culture and antibiogram is the last option and is performed when the cow has already received several antimicrobials and mastitis continues, suspecting bacterial resistance to antimicrobials.

V1. They have a problem with mastitis, and they have already used penicillin, cephalosporin, and you are like, what else do I prescribe? What do I do? You really feel handcuffed.

Then, you have to resort to other options that are not the most viable, a culture and an antibiogram.

For those who sell their milk to the only collection company that provides a laboratory service to evaluate mastitis in their cows, it is easier to reach an appropriate diagnosis and the description of susceptibility to antimicrobials.

V3. The most recommendable thing is that the laboratories and the company can do the culture and antibiogram to the farmer.

The most committed farmers, working together with the veterinarian, send milk samples for culture and antibiogram; however, they report resistance to all antimicrobials at times.

V9. We make use of the veterinary diagnostic laboratory. Some of the most diligent dairy farmers send the samples. However, cultures and antibiograms are performed, and the animals do not respond.

The long distances between the farms and the veterinary diagnostic laboratories generate a lack of interest in the use of the culture and antibiogram for the diagnosis of mastitis. In turn, the time in the delivery of test results affects adherence to this service, which has direct effects on decision-making in the appropriate use of antimicrobials and bacterial resistance.

DF17. I have land in San José de la Montaña, and taking a moment to go to San Pedro is not viable. You waste time. You arrive in the afternoon.

DF17. They even call me often, because in the surveys, I tell them the good and bad things. I am always very fair, and the bad thing is that they are very slow. What good is it to me if I send a milk sample for CFU today, Monday, and I get the answer the other Monday? When the payment stub arrives on Thursday, then I did nothing. If I was going to correct a problem, then why should I send that?

4.2 Social influences on the use of antimicrobials

The veterinarian plays a crucial role in the appropriate choice of antimicrobials and in the implementation of udder health interventions. However, farmers are often more influenced by the recommendations of other farmers, rather than following the professional's instructions. This social behavior, together with the lack of interest in using the laboratory, forces veterinarians to design strategies to analyze each case of mastitis and make decisions based on the disposition of the farmer. Often, the irrational use of antimicrobials is resorted to, before sending a milk sample for culture and antibiogram.

V4. You have to consider several things: First, the farm and what works there, because I do not know its trajectory; second, the clinical review; and third, the treatment that has been given, if it works or if it does not, and with it, you, more or less, have a spectrum of options, and if things are complicated, you make a culture. But that is like one of the last options. There are clients to whom I can tell, "Let's do a culture now," to see how it is, and they accept, but there are others for whom it is more complicated. The other thing is that they always say "My neighbor put aguapanela with veterflucin in the udder and that worked."

DF13. Here, more than anything, you rely on farmers who have more experience. They say, "What does one do with those antimicrobials? Use this drug," and the first thing they read is the withdrawal time.

Farmers are often receptive to recommendations from agricultural store staff. These untrained personnel promote irrational use and resistance to antimicrobials during mastitis treatment.

V3. As soon as a salesperson who often does not have technical training recommends antimicrobials at a store counter, the problem begins.

4.3 Antimicrobial resistance

Farmers and veterinarians are immersed in a culture of inappropriate use of antimicrobials, where the decisions to choose these are based on availability, previous experience, or empirical recommendations. Veterinarians are aware that many farmers are reluctant to send milk samples to the laboratory for culture and antibiogram but feel it is their duty to ask about this as a measure to promote udder health and public health.

V2. The antimicrobial that used to work for me no longer works. Then, what happens? They do it and we sometimes fail at it too. Why? Because you have a cow with mastitis. Before saying, "Give it this penicillin, give it this cephalosporin," whatever, ask the farmer if they have the economic possibility of sending a milk sample to the laboratory and find out with an antibiogram which one is needed. We do not do that. So, we already know that depending on the case and the analysis we do in the field, we send a certain antimicrobial, but sometimes it does not work. Sometimes when we arrive, we find that the farmer has already used a very strong antimicrobial, so to speak, with a very broad spectrum or already used, well, so many that you do not know what to do.

Self-medication, indiscriminate mixing of antimicrobials, underdosing, and errors in the application route are behaviors that decrease the probability of success in the treatment of mastitis and promote resistance to antimicrobials, which represents a significant risk to public health. This situation is further aggravated when antimicrobials for human use are used in animals.

V6. Yes, the majority of cases, I think it may be more than 90% of the cows that have come to the consultation for mastitis treatment have had resistance to antimicrobials and not just once, but at least twice. Dairy farmers like to mix tetracyclines with beta-lactams, even if it does not work. They combine and even overdose, and besides that, they apply medications through the non-recommended route; medications that are intramuscular, they apply them as intramammary. This is very common, added to the issue of medicines for human use.

DF5. There are people who do not have enough knowledge. A cow gets mastitis, and if the person, the boss, or the worker does not have much knowledge, they treat it for a day or two, when it should be five or six days. So, in 1 or 2 days, if the cow improves, they leave it like that, but after eight days, it returns to the same thing, and why? To avoid many times the cost overrun for investment.

Farmers, in their desire to avoid culling cows that produce large amounts of milk but have mastitis caused by multiresistant bacteria such as *Staphylococcus aureus*, often combine this with poor milking practices. This promotes the spread of the bacteria among the cows in the herd and increases the incidence of complex mastitis in the productive system, which in turn results in high economic losses.

V6. So, when you send a culture and antibiogram, and it comes out resistant, and unfortunately if it is sensitive to an antimicrobial, or it is not available, or you cannot find it in the indicated presentation, then the appropriate treatment is greatly limited. Almost always, these animals end up being culled, or the agent that caused the mastitis is a resistant agent such as S. aureus, where there is not much to do. So, they start fighting with an agent who is very difficult to handle within a milking routine and where the recommendation is to cull the cow, and they do not do it because the cow is very good, and they end up with the entire herd harmed.

According to veterinarians, another behavior that may be contributing to antimicrobial resistance in the udder is the administration of antimicrobials without the concomitant use of anti-inflammatories and analgesics, because this can limit the penetration of the drug into the tissue, in the concentrations necessary to fight the infection.

V6. The other thing is that they treat cows without giving them an anti-inflammatory and analgesic, but they only use the antimicrobial. The drug does not reach an udder that is inflamed so easily; the concentrations are lower, so the issue of resistance is very high, too high.

The indiscriminate use of antimicrobials in drying therapy has been a common practice in the dairy industry. However, its nonselective use and without antimicrobial susceptibility testing may favor the development of mastitis in early lactation and increase antimicrobial resistance in cows.

V4. Antimicrobial resistance is rampant, and this is not even due to mastitis; it is due to drying. After drying, cultures must be carried out to see what antimicrobial to dry with, because there are already resistant bacteria and when the cows calve, mastitis begins.

Choosing antimicrobials based on milk pH is another nonassertive veterinary practice that represents unnecessary use of antimicrobials and promotes bacterial resistance.

V3. There is another methodology based on a pH marker in milk. Depending on the pH of that milk affected by mastitis, you make the decision. If it is acid milk, some antimicrobials are prescribed, if it is basic milk, other antimicrobials, in order to change the pH to those bacteria that are accustomed to that udder.

Bacterial resistance to antimicrobials is promoted in calves, with the widespread use of milk from cows undergoing antimicrobial treatment for mastitis.

V9. They are raising the calves of those cows, with cows with mastitis. In my opinion, the resistance is transmitted to these calves. Probably in the future, the bacteria of these calves will have resistance to antimicrobials. There are going to be no antimicrobials that work on those calves.

DF8. What did we start doing here? We pour all that withdrawal milk into the little calves, and when the calf gets sick, I tell them to bury it because there is nothing left to do. The antimicrobial no longer works.

Farmers before consulting with a veterinarian have tested all the antimicrobials they have. Sometimes, the veterinarian sees the drug options exhausted and opts for alternative medicine.

V1. I have used a thousand things on this cow and nothing works. So, it's up to you how to resort to new molecules, an antimicrobial that you know is super strong or one that has already worked, or resort to other alternative therapies, such as homeopathic ones.

Lack of commitment and lack of training of workers in the antimicrobial application can promote antimicrobial resistance in mastitis-causing bacteria.

DF17. When you do not have a trained staff doing a good milking routine, I can almost guarantee that antimicrobial will not work for you either.

4.4 Education and technical assistance

Knowledge transfer to dairy farmers has been deficient. Farmers often find it exhausting to attend multiple trainings where the same basic mastitis topics are repeated. This has led to a lack of motivation on the part of farmers to attend educational events, which could be different if they were better planned.

DF11. People say: I went there and they only talked about mastitis. Next time no, why am I going there again? To hear them talk to me about mastitis, I already know what that is. People say why to go there if they are going to repeat the same thing again.

It is common to find training offered to dairy farmers by suppliers of inputs and medicines, but often these talks have a commercial focus and promote their own products. This situation creates a conflict of interest and may result in inappropriate decisions in the management of mastitis. It is necessary to look for more objective training alternatives free of commercial interests to improve the knowledge and decision-making of farmers.

DF11. They tell us: Well, we are going to do some training, four, five training sessions, but they also promote their product.

Training on the proper use of medicines to treat mastitis and other diseases can be more successful if they are carried out in areas close to the production systems, and scientific issues are explained in a language appropriate to the needs of dairy farmers. Local dairy continuous education programs are successful. These initiatives are based on a collective interest and therefore can involve different actors, such as collecting companies, input suppliers, and educational institutions. Veterinarians and farmers have highlighted positive experiences, where the location of the event was close to farmers, continuity was maintained in the meetings, current issues were addressed, and field practices were included to apply what was learned.

V8. You can see that here, at least during the time I was director and veterinarian in the administration, we had a program to strengthen good livestock practices. Within this compendium of good livestock practices, the proper use and management of veterinary drugs enters as a fundamental issue, to which we also, in the company of other entities such as SENA, emphasized training issues. First, it was Salazar rural settlement. We would go there, and we would give the course right there, and what did that prevent? Displacements; we avoided loss of time and encouraged more people to participate, because it was in the community center. Here, I had a group of 30 farmers, precisely in the Salazar settlement, where they were given a weekly class of two hours making the difference and explaining the use of each group of drugs, what was a dose, what was a dosage, what was a drug, what was pharmacodynamics, what was pharmacokinetics, what were withdrawal times, and what were withdrawal times for the use of agrochemicals, antimicrobials, antimicrobial resistance, anti-inflammatories, NSAIDs, corticosteroids, and hormonal.

DF11. We were in a little dairy school here with a veterinarian from a formal X collector, and we set up a little dairy school for the farmers on the last Friday of every month. The veterinarian from X company came and as such invited some people from different laboratories, and there was even one from SENA accompanying us. Through the collector and some loans from farms, we had resources to do some practices. That is why I really liked that dairy school because we had not only a theory but also practices. They took us there to a neighbor who lent three or four cows that had unhealthy udder problems, mastitis as such, in short, a series of things that they were going to talk to us about, and we were going to learn to differentiate them. So, in that little dairy school, I think we did pay good attention; we learned something that was going to be useful not only for that day but also for the future.

The availability of continuous education for dairy farmers is crucial for public health. There is a lack of knowledge on the part of some farmers about the use of antimicrobials and their impact on public health. However, other farmers are aware of the implications of their actions. Therefore, educational programs must not only address technical knowledge but also work on the transformation of inappropriate attitudes and behaviors, taking into account the cultural dimension.

V2. I believe that training is important because some people are unaware, but for me, it is a very small percentage. Most of them know what they are doing; most of them know where their milk is going and how clean they do their milking routine. There are others who do not care about anything; they just produce that milk no matter what; if they sell it for a thousand pesos, it does not matter; what they need is the money. But they do not see that this milk is going to a final consumer, which can be even their children, or even themselves. I think it is something so cultural.

When the importance of mastitis is brought to economic terms in personal advice or training, there is greater receptivity from dairy farmers.

V6. Within the farms that I attend to, they are given a report on how their herd is doing in somatic cell count. Most people do not care about this. What have I tried to tell them? That mastitis is a disease and that beyond knowing the number of somatic cells, it is the money they stop receiving for having a cow with mastitis. When you change that concept or that chip, or transform that information into money, which is what really matters to a farmer, they understand the magnitude of the problem.

The low knowledge that dairy farmers have about the importance of udder health for the production of quality and quantity milk, added to the culture of milk production in large volumes, has generated a negative attitude and lack of interest toward their participation in educational events on mastitis and udder health. On many occasions, the lack of time and the distance of the meeting are argued as excuses for not attending. However, there is a greater interest and attendance in training related to reproduction.

V2. In this company, we have done several training sessions on various topics. If you want to gather all the farmers, let us say here in los llanos, in Santa Rosa, people do not go because not everyone has the means to go or the time, so we have sent the events to the settlements, but you arrange the meeting. For example, we are going to hold this meeting on milk quality in the community center, and only 8 or 10 people attend. But if you tell them: we are going to do an artificial insemination course, 30 to 35 people attend. They think that to have milk, the cow has to give birth. They have it so in their head that they just give more importance to the reproduction part.

Training constitutes a collective action event and is essential for effective udder health interventions that also do not put public health at risk. Veterinarian-led interventions can only be successful if the dairy farmer understands the process and the importance of their role in mastitis control.

V7. Training for farmers is necessary because as a veterinarian, you cannot be the nurse and the owner of the herd. You are the person who is going to direct a process; they are going to help you organize the process, but you have to train people so that they understand what you are doing.

The socio-cultural and economic differences between farmers have an impact on the ability to achieve the objectives established in the training. Illiteracy is an obstacle to learning and the adoption of a critical attitude to identify problems in management and milking practices that affect udder health and the use of antimicrobials.

V8. Training issues for the most vulnerable populations are more complex, especially the issue of understanding, there is a higher level of illiteracy than on the farms or in the villages where there is a higher economic level or a much better production. This leads the farmers themselves to make an analysis and a difference on the issue of why they have a problem related to udder health. In other words, they often know how to differentiate if it is environmental mastitis, if it is mastitis due to a problem in the milking equipment, a pressure issue, or in the pulsators. If they suddenly have subclinical mastitis, they do constant CMTs (California Mastitis Test). While in the most vulnerable populations, it is much more complex for them to assume good milking routines, decide what treatment to do, and a good monitoring of udder health.

The extension is essential to improve udder health. This type of accompaniment promotes a critical attitude for decision-making in dairy farmers.

V7. Bovine mastitis is due to inappropriate decision-making and the lack of comprehensive technical assistance. You have to go 3, 4, or 5 times to observe, help, and educate. I have been very extensionist with farmers. I always tell them, "if I leave tomorrow, or something happens to me tomorrow, what will happen with the interventions?" The idea is that they know how to solve the problem we have here.

5. Discussion

The results of the quantitative component indicate some attributes that in their integration and comparison with the theory built through the grounded theory allow a greater understanding of the phenomena around decision-making, in the choice of antimicrobials for the treatment of bovine mastitis, and the risks of bacterial resistance (see **Table 3**).

Variable	Characteristic	Theory (based on qualitative analysis)
Antimicrobial treatments for mastitis per month per farm.	31.5% less than 1 treatment/month Higher CFU for those who do less than 1 treatment per month. Bab BTSCC, regardless of the number of treatments.	Social influences on the use of antimicrobials The veterinarian is the main social influencer of dairy farmers for making assertive decisions in the choice of antimicrobials. Farmers are receptive to antimicrobial recommendations by untrained agricultural store staff or other dairy farmers. Antimicrobial resistance Farmers choose antimicrobials based on availability. Self-medication, mixing antimicrobials without knowledge, underdosing of antimicrobials, and errors in the application route have decreased the probability of success. Conservation of cows with mastitis caused by multi-resistant bacteria without measuring risk to the herd. Supplying antimicrobials without applying anti-inflammatory, limits the entry into tissue. The use of antimicrobials in non-selective drying therapy and without susceptibility testing promotes bacterial resistance. Choosing antimicrobials based on milk pH is another non-assertive practice. Feeding calves with milk from cows with mastitis, and under treatment, promotes resistance in their bacteria. Lack of commitment and lack of training of workers in the application of antimicrobials can promote resistance.
Choose antimicrobial treatment based on culture results and antibiogram.	73.6% disagree, that is, they do not do it. Higher CFU and BTSCC for those who do not perform culture and antibiogram to choose the antimicrobial.	Use of the laboratory (culture and antibiogram) Dairy farmers do not want to do cultures. Veterinarians are pressured to recommend antimicrobials without susceptibility testing. The culture and antibiogram are done when several treatments have been done without success. The remoteness of the laboratories and the delivery times of results affect adherence to the use of culture and antibiogram.

Variable	Characteristic	Theory (based on qualitative analysis)
The company to which you sell your milk offers laboratory services for your cows	47.7% do not receive a laboratory service offer for the diagnosis of mastitis in their cows. Higher CFU and BTSCC for those who do not receive an offer of laboratory services for the diagnosis of mastitis in their cows.	Use of the laboratory (culture and antibiogram) Only one collection company offers a service for collecting milk samples, diagnosis for mastitis, and antibiogram. Farmers who do not sell to this collector hardly have access to diagnostic services for mastitis.
Training of workers in mastitis prevention	25% do it. Lower CFU and BTSCC for those who train their workers.	Education and technical assistance The transfer of knowledge to the dairy farmer has failed. Most of the training are offered by suppliers of inputs and medicines and are for commercial purposes. The training are successful when they take place close to the farms, in a language according to their knowledge, with continuity of meetings, current issues, and practices. The offer of continuous education for dairy farmers is a fundamental action for public health. There is greater receptivity in advice or training when the health problem together with the economic impact is explained to the farmer. There is disinterest in participating in mastitis talks. Culturally, the interests of farmers are focused on reproduction. Illiteracy has made it difficult to learn and build a critical attitude.

Table 3.
Similarity comparison matrix and integration of qualitative and quantitative results.

6. Use of the laboratory (culture and antibiogram)

The reasons why farmers in other countries are reluctant to carry out culture and antibiogram tests are similar to the case of the north of Antioquia. A third of Swiss and Scandinavian farmers consider that results take longer than they can expect to treat mastitis; however, more than 60% state that they decide which antimicrobial to treat with, based on culture and antibiogram results [18, 19]. In the Netherlands, most farmers do not treat all clinical mastitis cases with antimicrobials; they prefer to select cows for treatment based on the severity of the mastitis case, that is, with low SCCs, or treat them with antimicrobials only after a non-antimicrobial treatment has failed. Most farmers perform a bacteriological culture only occasionally, mainly to better understand the mastitis problem on the farm or to choose the appropriate antimicrobial [4]. In the case of farmers in the north of Antioquia, the use of the laboratory is occasional and generally when the producers have already used several antimicrobials indiscriminately without success. This behavior depends on many factors, among them are the offer of services and the delivery times of results. Concerns of this same nature prompted New Zealand researchers to evaluate new technologies for the identification and antimicrobial susceptibility testing of the main mastitis-causing bacteria in the country, with results within 24 hours that were similar to those obtained by conventional tests [6]. If in Colombia and in other countries policies that favor the use of the laboratory are not designed, in order to choose appropriate antimicrobials to control bovine mastitis, and additionally, if technological developments are not implemented, which allow obtaining results of susceptibility to antimicrobials in a shorter time, it will be difficult to transform the culture of dairy farmers, and a series of events in the use of antimicrobials will continue, which will favor the development of resistance to antimicrobials.

7. Social influences on the use of antimicrobials

Dairy farmers recognize other producers, veterinarians, and staff in pharmacies and agricultural stores as influential players in the choice of antimicrobials to treat mastitis. Dairy farmers' decision-making has been found to be a complex process influenced by many factors, including external controls such as financial rewards or sanctions [20], internal controls such as motivations [21], individual values and beliefs [22], and analysis of recommendations in the context of experience and characteristics of the production system [21]. The social referents for decision-making are related to internal controls, since behaviors, attitudes, and beliefs are built in relationships with the actors whom the dairy farmer trusts [23].

Consistent with the findings of this study, dairy farmers in the UK are commonly an influential source of information to their peers on antimicrobial use [24]. Veterinarians continue to be considered as reliable and influential reference actors in decision-making on the use of antimicrobials and biosafety issues [21]. In contrast to our study population, the study by Swinkels et al. (2015) found that nutritionists are also reference actors in decision-making on the use of antimicrobials [10], and dairy cooperatives are determining factors in mastitis problems [25].

In any of the cases, trust in the recommendations on the choice of antimicrobials and their use by actors with or without training in health is a problem, unless the recommendation is to resort to the clinical laboratory to perform culture and antibiogram tests on milk samples from cows with mastitis. This is recommended by veterinarians when, at the time of consultation, it is discovered that the farmer has applied various antimicrobials without success. However, the adoption of the veterinarian's recommendations depends on the length of the relationship with the dairy farmer, given that long relationships are considered trustworthy and valuable in decision-making [26]. This has been a problem in the north of Antioquia, where the working and salary conditions of veterinarians do not promote their permanence in the region and, on the contrary, promote their rapid migration.

8. Antimicrobial resistance

Self-medication, antimicrobial choice based on farm availability, underdosing, errors in the application route, lack of commitment and education of dairy farmers and workers, nonselective antimicrobial drying therapies, lack of antimicrobial susceptibility testing, keeping cows with mastitis caused by multiresistant bacteria, and feeding calves with milk with antimicrobials are actions related to the use of antimicrobials and the control of bovine mastitis that have been identified in research and that can promote antimicrobial resistance in bacteria from humans and animals. These actions are common among farmers from different countries, which is why professionals in microbiology and organizations focused on public health, such as the World Health Organization (WHO), recommend, above all, using antimicrobials only based on the results of culture and antibiogram with guidance from a veterinarian [27] and only when this accompaniment is not possible to follow the indications on the label [28]. Other recommendations are: The restricted use of parenteral antimicrobials only for severe cases of mastitis [29], avoidance of priority antimicrobials for use in humans [27], and when an antibiogram is not possible, use narrow-spectrum antimicrobials and always performing selective dry cow therapy [30].

In order to transform practices related to the use of antimicrobials in dairy farmers and workers, educational programs, training, and specific campaigns are a priority. In addition, the support to the farmer should focus on the implementation of effective measures to prevent mastitis [31]. In short, there are many errors made by dairy farmers included in the study in relation to the use of antimicrobials. Based on the experiences of other studies and of our study population, the best way to reduce the use of antimicrobials and the likelihood of promoting antimicrobial resistance is to implement measures that reduce the occurrence of mastitis in cattle, through strategic udder health planning, according to the characteristics of each farm.

9. Education and technical assistance

Regarding education on the use of antimicrobials in the treatment of mastitis in dairy farmers, failed processes have been described by some farmers and veterinarians due to commercial interests and the lack of consideration of the producers' needs. That is why, in order to guarantee an efficient and decentralized education, it is necessary to focus on the priority problems of particular groups of dairy farmers [21].

The prudent use of antimicrobials in the treatment of mastitis is complex and requires support in decision-making, for both farmers and veterinarians. To achieve assertive decision-making in the use of antimicrobials, it is essential to transform practices through continuing education and the application of skills [32]. In this sense, theory must be put into practice to achieve successful results, as has been demonstrated in experiences reported in the north of Antioquia, where theoretical-practical interventions have been developed [33].

Veterinary monitoring is essential in choosing the appropriate molecules to treat mastitis because it is one of the main sources of information for farmers. However, to change ingrained practices in the dairy farmer, it is important to have a reliable and stable advisor in the area since building trust depends on time and permanence [34]. Otherwise, the technical assistance could be a failure in terms of reaching the objectives, which depend to a large extent on adherence to the professional's recommendations.

10. Conclusion

This chapter refers to the excessive and irrational use of antimicrobials for the control of mastitis in the dairy sector of Northern Antioquia, as an example of a region with a great agricultural vocation but that presents typical dairy problems like those of many countries in the world. This problem is influenced by various cultural and social factors, such as the lack of state control and support, limited attention to mastitis, self-medication, and lack of commitment and education. In addition, bacterial resistance to antimicrobials may be related to the inappropriate choice of antimicrobial based on availability, the non-use of cultures and antibiograms, underdosing, errors in the application route, and the conservation of cows with multi-resistant mastitis.

In this sense, the veterinarian is an important social reference for dairy farmers, but more technical and educational support is required from these professionals to improve the choice and proper use of antimicrobials. The training offered by suppliers of inputs and medicines can be useful, but it is important that they are offered close to the farms, in an appropriate language and with continuity of meetings, always maintaining the health of the udder as the common objective, so that a transparent commercial relationship with farmers prevails.

In conclusion, the fight against antimicrobial resistance and udder health planning in the dairy sector depends on technical, cultural, and social aspects. It is important to promote adequate access to knowledge and laboratory services, as well as to improve social relations between dairy farmers and other actors in the dairy chain in northern Antioquia.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Section 3

Bovine Mastitis Control

Chapter 4

Bovine Mastitis Control Strategies with Emphasis on Developing Countries

Blaise Iraguha

Abstract

Mastitis is a major problem that reduces milk production and quality in the whole world. Because mastitis is an endemic disease, farmers in most countries have mastitis control programs to keep mastitis prevalence at a low level of less than 5%. However; a mastitis control program cannot be implemented unless the prevalence and the risk factors of mastitis are known. Therefore; a systematic review was conducted to provide broad information on mastitis prevalence and associated risk factors and propose appropriate control strategies with emphasis on developing countries. This book chapter recommends dairy farmers to monitor milking cow's udder health, improve hygiene, regular use of teat dips, practice dry cow therapy, appropriate and effective treatment of affected animals.

Keywords: milk, mastitis, risk factors, mastitis detection, mastitis control

1. Introduction

Mastitis prevalence rates from the developing countries tend to be much higher than those from developed countries. Plozza et al. [1], working in New South Wales in Australia and using the California Mastitis Test (CMT), reported a mastitis prevalence rate of 29% while Fadlelmoula et al. [2] reported a prevalence rate of 27.6% from Germany. Elbers et al. [3] reported the lowest prevalence of 12.7% from The Netherlands.

In Bangladesh Rahman et al. [4] used CMT and estimated that the prevalence of mastitis was 19% in the dry and 44% in the wet season. In Uruguay, Gianneechini [5] used CMT and reported a mastitis prevalence of 52.3%. Figures from Ethiopia vary from between 10–23% for clinical mastitis and 22–71% for subclinical mastitis. Abera et al. [6] from Ethiopia using the CMT found an average prevalence of 46%. Of this 10% was due to clinical mastitis and 36% from subclinical mastitis. Using the same method (CMT), Almaw et al. [7] reported a subclinical mastitis prevalence of 25.22% from Gondar, Ethiopia. Mekibib et al. [8] from a different location also in Ethiopia reported a cow level mastitis prevalence of up to 71%. Of this prevalence, 22.4% was due to clinical mastitis and 48.6% was due to subclinical mastitis. Girma [9] conducted a study on prevalence of bovine mastitis on crossbreed dairy cows around Holeta, Ethiopia, and found that the prevalence of mastitis in general was 44.1%.

About 10.3% was due to clinical mastitis and 33% was due to subclinical mastitis. According to Biffa et al. [10] the general prevalence of mastitis in lactating dry cows in southern Ethiopia was 34.9%.

Karimuribo et al. [11], reporting from Tanzania and using the CMT and culture, estimated the prevalence of mastitis at 75.9% and 43.8%, respectively. Kivaria et al. [12] found the prevalence of mastitis was 43.3% in smallholder dairy cows in Dar es Salaam region, Tanzania.

In Rwanda official publications indicate that the prevalence of subclinical mastitis was more than 67% by Mpatswenumugabo et al. [13] and 52% by Iraguha et al. [14]. Based on sampling from bulk chilling tanks in Nyagatare District, Chatikobo [15] reported a prevalence rate of 58.6%. However, this figure does not indicate mastitis prevalence at farm and cow levels which are the focus of any mastitis control measures. Moreover, the report of Shem [16] broadly states that mastitis is a problem in dairy herds in Rwanda but does not have adequate figures.

2. Mastitis risk factors

The risk factors associated with mastitis are many and no single article has ever attempted to deal with all of them. Individual factors that are of particular importance in the individual mastitis cases have been extensively reviewed by Cunningham [17].

According to Abera et al. [6], Almaw et al. [7], Mekibib et al. [8], and Biffa et al. [10], in Ethiopia the main risk factors identified to be associated with mastitis are age (over 6 years), housing systems (muddy houses), lactation stage (over 6 months), wearing gloves, using paper towels, feeding after milking, injured teat, udder conformation, udder condition (unwashed udder), season (long rain season), and system of production. Benhamed et al. [18] from West Algeria also added breed as an important factor. Karimuribo et al. [11] and Kivaria et al. [12] from Tanzania identified body condition score, parity stage, and udder consistency, housing condition and milking practices as important factors.

According to Iraguha et al. [14], the prevalence of infected quarters increases with age, peaking at seven years and teat end conditions (damaged teats). Most new infections occur during the early part of the dry period and in the first two months of lactation, especially with the environmental pathogens.

The greater the prevalence of the disease in the herd, the greater the risk of new infections, Blood and Anderson [19] assert that the incidence of mastitis is greater in Holstein Friesian than in other breeds.

High milking rate and large teat canal diameter have been associated with increased somatic cell count (SCC) or risk of intra-mammary infection. Normal teat ends with a slight amount of callosity do not appear to increase the risk of mastitis and may be a beneficial response of the teat during machine milking. However; abnormal teat ends that are extremely rough and showing evidence of hyperkeratosis are associated with an increase of new mastitis infections as [14] and Vitamin E, vitamin A and selenium may be involved in resistance to certain types of mastitis [20].

Poor housing and bedding quality management increase infection rate and incidence of clinical mastitis due to environmental pathogens [21]. According to Rahman et al. [4] a clean environment and udder were mandatory for reducing mastitis in Bangladesh. This would apply across the whole world. Although very rare, intramammary infection (IMI) may also occur due to hematogenous spread.

Doherr et al. [22] from Switzerland reported that the risk factors associated with subclinical mastitis in dairy cows in Switzerland were dry cow therapy, nutrition and poor milking practices. Elbers et al. [3] working from the Southern Part of The Netherlands identified trampled teats, no disinfection of the maternity area after calving, consistent use of post-milking teat disinfection, use of a thick layer of bedding in the stall, and the stripping of foremilk before cluster attachment as the major risk factors. Oliver et al. [23] from Tennessee identified calving time, milking practices, age at first calving, presence of pathogens on the body as significant factors. Fadlelmoula et al. [2] working on large scale dairy farms in Thuringia-Germany mentioned early stage of lactation, summer calving, udder cleanliness, milk yield and peri-parturient diseases as predisposing factors for developing mastitis. Plozza et al. [1] working on mastitis and its associated risk factors on dairy farms in New South Wales revealed that wearing gloves, using paper towels, and feeding after milking were the risk factors associated to mastitis prevalence whereas dipping teats was not significantly associated.

3. Types and causes of mastitis

In addition to the foregoing classification, mastitis can also be divided into contagious and environmental mastitis [8]. Bacteria involved in the pathogenesis of contagious mastitis include *Staphylococcus aureus, Streptococcus agalactiae, Corynebacterium bovis* and *Mycoplasma bovis* [17]. Environmental causative agents include the streptococci, *Streptococcus uberis* and *Streptococcus dysgalactiae* and the coliforms *Escherichia coli, Klebsiella* spp.*, Citrobacter* spp.*, Enterobacter* spp.*, and Pseudomonas* spp. [5].

Minor pathogens include coagulase-negative *Staphylococcus* spp. and the list of uncommon pathogens is endless: *Trueperella pyogenes, Nocardia* spp., *Pasteurella* spp., *Mycobacterium bovis, Bacillus cereus, Serratia marcescens*, anaerobic bacterial species, fungi and yeasts [17].

The source of mastitis is therefore either contagious pathogens or environmental pathogens. Infection of each mammary gland occurs via the teat canal, the infection originating from either an infected udder or the environment. In dairy cattle the infection originating from infected udders is transmitted to the teat skin of other cows by milking machine liners, milkers' hands, wash cloths, soils, bedding, contaminated milk, washing water, drying cloth/paper, udder and any other material that can act as an inert carrier [24].

With mastitis, the danger of bacterial contamination from affected milk rendering it unsuitable for human consumption looms large. Coupled with this is the potential for food poisoning or interference with manufacturing process or, in rare cases, a mechanism of spread of disease to humans and potentially antimicrobial resistance to humans [25].

4. Mastitis detection

The diagnosis of clinical mastitis is not difficult if careful clinical examination of the udder is done as part of the complete examination of a cow with systemic clinical findings. Examination of the udder is sometimes omitted in a recumbent animal only to find later that severe mastitis was present [26]. The diagnosis of mastitis depends largely upon the detection of clinical abnormalities of the udder and gross abnormalities of the milk or the use of an indirect test like California Mastitis Test to detect subclinical mastitis [27].

The detection of subclinical mastitis can be either by surveillance of the herd through periodic examination of the udder health by evaluation of milk at the herd level or at the individual cow level. This is done by examination of either bulk tank milk or individual cow tank milk or individual cow composite milk samples using indirect tests for evidence of subclinical mastitis.

There are many chemical methods for mastitis detection at farm level and these include pH, chloride test and the CMT [28]. The majority of the chemical methods depends upon the demonstration of abnormalities in milk composition and is therefore indirect tests for mastitis. Abnormal changes may not appear with regularity in the milk of all cows having an udder infection. In most cases a positive test indicates an infected quarter, but a negative test does not indicate that the quarter is not infected [28].

The most commonly indirect tests used for the existence of mastitis include the use of chemicals. Milk from affected udders is abnormally alkaline with the degree of alkalinity depending upon the severity of inflammation. Abnormal milk may have a pH as high as 7.4; where as normal milk has a pH of 6.4–6.8. The reaction of milk may be determined by several different methods, the most common of which is the use of indicators that change color at near the normal milk pH. The pH should be determined on freshly drawn milk although milk held at refrigerator temperature for 24–48 hours may be used [28]. Due to the influence of fat on the result reading, it is necessary to use milk drawn at the beginning of milk removal. The test is of a little value for cows in late lactation because will result in false positive alkaline reaction [28].

The recent technologies that can used in field settings include portable devices such as Draminski®, PortaSCC® test, California Mastitis Test (CMT) and the UdderCheck® test [14] all these tests for presence of indicators of inflammation of the quarter/udder.

5. Mastitis control strategies

The disease cannot be eradicated but can be reduced to low levels by good management of dairy cows and its environment. After each milking the teats should be dipped or sprayed with disinfectant teat dip and keep cows standing for at least 30 minutes after milking (e.g., offer them food). Treat clinical mastitis using antibiotics in intra-mammary infusion and/or injection. It is also highly recommended to give intra-mammary infusions or injection under veterinary supervision.

Hygiene and milking best practices such as wash dirty udders before milking with clean water and dry thoroughly, wash and disinfect hands before milking each cow, foremilk all teats into a strip cup and check the milk for mastitis, milk cows affected by mastitis last and discard the milk and treat all functional quarters of cows at drying off with specifically designed infusions of antibiotics will reduce the incidence of mastitis. Cows with repeated clinical mastitis should culled.

When *hand milking* is used; practice proper milking hygiene such wearing milking gloves, wash hands and equipment thoroughly, etc. When *milking machine* is used it has to be properly designed, operated and maintained. Keeping the cows healthy such as improved immune function of the udder would prevent mastitis even if bacteria enter the teat canal (**Figures 1** and **2**).

Figure 1.
Teat dipping after milking.

Figure 2.
Antibiotic infusion in infected teat.

5.1 Standard operating procedures for mastitis control and prevention

The following routine will reduce the number of infected cows and clinical mastitis by at each milking: adopt good cow management practices as the essential basis for a mastitis control routine (e.g., feeding, housing, hygiene). Reduce exposure to pathogens by cleaning thoroughly all equipment used when milking, hygiene of milkers, avoid housing cattle under dirty conditions preferably change organic bedding materials regularly, wash dirty udders before milking with clean water preferably with the hand, a disposable paper towel or a disinfected cloth and dry thoroughly.

Teat dipping/spraying using appropriate products (e.g., Chlorine, Chlorhexidine, hydrolyzed fatty acids, Iodine, Quaternary ammonia based, etc. Adopt practices that prevent the occurrence of teat lesions and always milk cows affected by mastitis last and discard the milk. Additional benefits can be obtained by disinfecting hands before milking each cow, using individual paper udder cloths, dipping teat cups in disinfectant before each cow is milked.

Reduce mastitis in non-lactating cows in the dry period by avoid using low lying grazing land and damp wooded areas where flies are common, use good fly control measures and treat cows at drying off with antibiotics recommended by veterinarian.

Three key control measures include teat dipping by appropriate variant, practice dry therapy program and effective treatment of sick animals.

5.2 The do's for hygienic milker

Milkers may spread mastitic pathogens and other diseases e.g., typhoid and paratyphoid fever, tuberculosis, dysentery, scarlet fever, septic sore throat, diphtheria and cholera are milk-borne and enter the milk from infected workers causing disease infection to consumers.

Therefore, milkers should be in good health and their hands free from any infections. Hands with infected wounds can add bacteria to milk and cause milk contamination and subsequent human infections. Have a medical check for diseases such as typhoid, tuberculosis, dysentery, diphtheria and cholera regularly from a medical center is mandatory.

Wash your hands thoroughly before milking each cow to avoid any contamination and disease's spread. Wear clean clothes and cover your head or have short hair to prevent loose hairs falling into the milk.

Ideally, milkers should use disposable milking gloves that are maintained clean and regularly disinfected during milking, particularly between cow.

Milk quickly and quietly in a stress-free environment and milk the cows at regular times daily is very important.

5.3 Standard operating procedures for a good environment in hygienic milk production

The milking shed should be constructed on windward side of roads to avoid dusty conditions in the shed. The floor should be constructed of cement with a strong concrete finish. The milking shed must be kept clean and dry and well-ventilated house to allow sufficient supply of fresh air. Avoid buildup of dung, urine or excreta as this may cause floors to be slippery and create suitable environment for bacteria growth. Avoid using muddy pens which are contaminated with feces as this causes outbreaks of mastitis.

Waste storage areas for example: manure heaps should be sited away from milk shed avoiding possible pollution of watercourses, lakes, reservoirs, wells, boreholes and underground water. When bedding material is used in the pens it should be changed frequently, preferably daily. A good supply of clean water is required for cleaning the floors.

5.4 The do's of the milking cow

The cow should be in health condition and regularly examined for diseases such as mastitis and zoonotic diseases.

The body of the cow should be free of soil, dirt and manure and contamination of milk from external sources such as animal hairs and dirty water dripping from the cow's body should be avoided. Use regular milking routine and proper milking techniques which do not strain the teats. Use teat dip disinfectant after each milking to reduce udder infection by bacteria. Renew bedding materials frequently, avoiding sawdust where bacteria proliferate.

5.5 Standard operating procedures for cleaning utensils in hygienic milk production

Dairy utensils should be of approved type, seamless with close fitting lids. They should not be used for any other purpose. Do not use containers that previously contained paint, paraffin, herbicides and other chemicals because traces of these substances can taint your milk reducing its quality. After using utensils, they must be rinsed with clean cool or warm water. Scrub the utensils using a detergent, such as washing soda solution (1.5 tablespoons washing soda in 5 liters of water) and a disinfectant such as bleach (2 tablespoons bleach in 4.5 liters of water). In developed countries specialized washing acid and alkaline products are used.

Rinse several times with clean hot water to remove any remaining detergent and disinfectant. When disinfectant is not available, after scrubbing the equipment in hot detergent solution, disinfect the utensils by immersing it in hot (above 75°C) water for at least 3 minutes.

Milking buckets, cans and measuring jugs should be stored turned upside down on a rack to keep them free from dust.

5.6 Standard operating procedures for hand milking in hygienic milk production and mastitis control

Good hand milking is a skill which can be learned. Good hygiene is of the utmost importance because the level of hygiene influences the quality of the milk. There are a number of general rules which should always be followed:

Maintain clean and healthy cows. Keep a clean milking environment. Keep milking routines and times consistent and regular. Milk in the correct way to avoid the damage of the teats and udder. Wash hands with soap and clean water before milking and between milking of cows. Wash the udder with warm water and dry the with a clean dry cloth. Make the first draw into a strip cup to check for mastitis and throw away from the milking area even if it appears clean. Use clean containers for milking. Cows with mastitis should be milked last and their milk discarded. The milker should not: (a) have long nails, (b) sneeze or cough, (c) smoke.

Milk from cows under antibiotic treatment should not be sold until, at least, three days after the last treatment or as advised by the veterinarian.

After every milking, dip/spray the teats into an “antiseptic dip.” Release the cow from the milking area as soon as milking is finished.

Milk filtering (to reduce contamination) using clean sieve/filter cloth. After milking and filtering, cover the milk to avoid contamination and move the milk to a clean and cool area. To reduce the dust content of the air in the shed avoid: sweeping the milking area before milking, handling hay and feeds before and during milking, brushing the cow immediately before milking, dusty bedding and accumulation of dirt and dust in the pens.

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Chapter 5

How to Control the Increased Bulk Milk Somatic Cell Count in Dairy Cows

Majid Mohammad-Sadegh

Abstract

Subclinical mastitis in dairy cattle covers a much larger population than cows with clinical cases. To remedy this type of mastitis, it is necessary to pay attention to the number of somatic cells in bulk milk, the history of control measures against mastitis (dry cow therapy, post-milking teat dipping, increasing the level of immunity of livestock and the herd, reducing stress, and increasing mammary health), and necessary actions are divided into two groups, fast and gradual. In the rapid group, ensuring the pre-milking striping during milking, throwing away the flake or clots containing milk, ensuring the effectiveness of the treatment of clinical cases and reducing recurrent cases from the treated ones, culling or isolating some cows from the herd (cases with very high or chronic SCC, high days in milk, reduced milk production, lack of pregnancy, history of recurrent complication, old age, etc.) are included. In the group of gradual measures, ten mastitis control and prevention measures would be implemented. It is better to use intramammary antibiotic therapy only to eradicate *Streptococcus agalactiae*, and treat the rest of the infected quarters at the time of drying the cow. Because BMSCC is dynamic, the efficacy of actions needs to be monitored.

Keywords: subclinical, bovine, mastitis, combat, intramammary antibiotic

1. Introduction

Bovine mastitis is considered to be the most expensive infectious disease in the dairy industry [1]. Therefore, control (prevention and timely and effective treatment) of mastitis plays an important role in economic efficiency in the dairy industry. Techniques for detecting and fighting bovine subclinical mastitis are very diverse, but finding methods that are both new and practical is still ongoing.

Herd bulk milk testing, composite milk evaluation, and obtaining information from milking machine sensors that identify subclinical mastitis in modern herds are some of the methods that monitor the quantity and quality of milk, or the effectiveness of the measures being implemented to fight mastitis in the herd.

The purpose of this research is to investigate and introduce new methods and more importantly the common methods of combating this type of mastitis.

2. Definition of mastitis

Mastitis is an infectious disease that is diagnosed based on observation of an inflammatory response to an intramammary infection (IMI) [2]. Mastitis is characterized by a range of physical and chemical changes in the milk and pathologic changes in the udders [3]. When clinical signs caused by inflammation of glands or ducts or breast parenchyma appear in the milk (subacute or Grade 1) or udder (acute or Grade 2) or the cow itself (per acute or Grade 3), mastitis is called clinical type [2]. But, if there are no clinical signs of this inflammation, and the inflammation can only be detected directly (such as SCC), or indirectly by looking for changes caused by the inflammation (e.g., California Mastitis Test, CMT, CMT or testing electrical conductivity), it is called subclinical mastitis [4].

3. Mastitis is a multifactorial disease

Three categories of factors work together to cause mastitis, which include environmental and management conditions, the virulence of the pathogen, and cow (especially the udders) conditions (immune system, metabolic status, and antioxidant) [5, 6]. Some of these factors are considered primary factors and some as risk, predisposing, or virulence factors. Physical factors such as teat end lesions (e.g., frostbite or improperly functioning milking machine), chemical factors such as nonstandard teat dip disinfectants, and microbial factors play a role in causing mastitis. However, the role of bacteria as mastitis pathogens is the most prominent among microbes [2, 5].

4. Definition of subclinical mastitis

To differentiate types of mastitis, the clinical signs caused by mastitis in milk (such as pus, wateriness, and bleeding), udder (pain, swelling, warmth, serum leakage, edema, swelling, and redness), and systemic signs in cows (grounding, dehydration, fever, decreased activity of the rumen, and increased heart rate and breathing) have been used [7]. However, if no inflammatory signs are visible, but an increase in SCC is seen, the quarter is considered to have subclinical mastitis [4]. A geometric mean bulk milk somatic cell count (BMSCC) lower than 400,000 cells/mL has complied with milk export [8]. Also, financial incentives were provided to milk producers to achieve and maintain very low BMSCC (<200,000 cells/mL) [9]. Today, the number of somatic cell counts (SCC) in bulk milk of a herd and SCC of the composition of milk of more than 200,000 (Cells/mL), and individual SCC of each quarter of more than 100,000 (Cells/mL), are an indicator of subclinical mastitis. An SCC scoring system that divides the SCC of composite milk into 10 categories from 0 to 9, known as the somatic cell score (SCS) (originally called the linear score), is becomings more widely used (**Table 1**). The number of somatic cells often does not have a normal distribution, but the score of somatic cells has a normal distribution and makes statistical calculations and comparisons easy. Each one-unit increase (or decrease) in SCS is associated with a doubling (or halving) of the SCC. The goal of monitoring linear score is to be less than 4 in 85% of the cows and 90% of first lactations. In total, herd cows should be less than 3.3 [10, 11].

Somatic cell count SCC (×10^3)	Somatic cell Score (SCS)(Linear score)
25	1
50	2
100	3
200	4
400	5
800	6
1600	7
3200	8
6800	9
Adopted from Refs. [10, 11].	

Table 1.
Calculating somatic cell score (previously called the linear score) from the somatic cell count.

5. Consequences of subclinical mastitis

5.1 The outcomes in dairy products

In cases of subclinical mastitis, there is a decrease in herd milk production per head of a cow (**Table 2**), the amount of cheese produced from such milk, the milk is not being curdled in the yogurt production process, and the precipitation of milk during heating are some of the issues faced farmers or milk technology engineers in dairy factories. Increasing the microbial count in milk is a consequence of subclinical mastitis, which, in addition to increasing lipase, plasmin, and other harmful enzymes, reduces the growth of useful yogurt-producing bacteria during any other milk fermentation process [10]. The increase of bacteria prevents not only the production of yogurt but also reduces the stability of milk and reduces its quality.

5.2 The outcomes in involved cows

A decrease in conception rate, an increase in loss of embryo and fetus, and an increase in cases of anestrus and ovarian cyst are other morbidities that frequently occur in herds involved in subclinical mastitis [12].

Bulk tank milk somatic cell count (cells/mL)	Infected quarters in heard (%)	Production loss (%)
200,000	5	0
500,000	16	6
1,000,000	32	18
1,500,000	48	29

Table 2.
The estimated prevalence of infection and losses in milk production associated with bulk milk SCC.

6. Bacteriology of subclinical mastitis

Based on the location of the reservoir of bacteria, these microorganisms have been divided into two groups, infectious and environmental. In the contagious group, the reservoir is the cow (udders, teats, skin of the udder or mouth, and oral lymph nodes) and that can be spread by milking machines and the hands of milkers. In the environmental group, the reservoir is the cow husbandry, such as bedding and the environment under the cow's feet. Although the distinction between contagious and environmental pathogens is still a useful learning tool, the distinction is not always clear; for example, *Streptococcus uberis*, and *Streptococcus dysgalactiae* display both "contagious" and "environmental" properties [13]. Common contagious organisms include *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae,* and *Mycoplasma* spp. Environmental organisms include *Enterobacter aero-genes, Escherichia coli, Klebsiella pneumoniae, Proteus* spp.*, Pseudomonas* spp.*, Serratia* spp., *T. pyogenes, Corynebacterium bovis* and other gram-negatives, environmental streptococci, yeast or fungi, and *Proto-theca, zofeia*. Some authors put coagulase-negative staphylococci (CoNS) in the teat skin opportunistic group [14–16].

Of about 140 to 150 species of microorganisms that have been identified [17], the main etiological agents include *Staphylococcus aureus* (coagulase-positive bacteria), CoNS, *Streptococcus agalactiae*, environmental streptococci, *Escherichia coli* and other coliforms, and *Corynebacterium bovis* [18, 19]. On the other hand, mastitis-causing bacteria are also divided into two major and minor groups. Most of the mentioned bacteria are of the major type. Only coagulase-negative staphylococci (*S. hyicus, S. chromogenes*, *S. xylosus, S. sciuri*, *S. warneri, S. simulans,* and *S. epidermidis*) and *Corynebacterium bovis* are included in the minor group. The minor bacteria are generally opportunistic and rarely cause clinical mastitis, but they increase the number of somatic cells to more than acceptable levels and may prevent some mastitis caused by other bacteria. Coagulase-negative staphylococci have a protective effect against colonization of the teat duct and teat skin by *S. aureus* and other major pathogens, except *E. coli* and the environmental streptococci. But, *Corynebacterium bovis* prevents clinical mastitis caused by the major bacteria [13, 15]. Among the above-mentioned pathogens, *Mycoplasma bois* is usually not isolated in milk culture by usual methods, but it increases the BMSCC greatly [20].

7. Common subclinical mastitis detection methods with a view to the future

After receiving a warning from milk processing factories that the number of milk somatic cells is high or observing traces of this problem in the herd (such as a decrement in milk production), various laboratory techniques are used at the farm or laboratory level to confirm the presence of this problem [21].

California Mastitis Test (CMT) [22], the electric conductivity of an individual quarter [23] or ultrasonography of mammary glands, precision technologies with in-line sensors; [9, 24] as an on-farm test, and SCC of a quarter, composite four milk or bulk milk cell count, *N*-acetyl-β-d-glucosaminidase (NAGase) test of composite or quarter samples, the concentrations of L-lactate, glucose, lactose, and acute phase proteins (serum amyloid A and haptoglobin) are of current test to identify subclinical mastitis in cows. Among the various tests, the somatic cell count is considered the golden test. The CMT, as an indirect indicator of the level of SCC is practical and usable as an on-farm or cow-side test. If the CMT is used to minimize the false negative rate and achieve the highest level of sensitivity, then the test should be read as negative

(CMT = negative) or positive (CMT = trace, 1, 2, or 3). If the CMT is used to minimize the false-positive rate and achieve the highest level of specificity for culling decisions, then the test should be read as negative (CMT = negative or trace) or positive (CMT = 1, 2, or 3) [22, 23]. It should be noted that some of these tests indicate more damage to the mammary epithelium than to inflammatory cells. Therefore, their combination with inflammation monitoring tests such as SCC will have excellent results [25].

7.1 Portacell; portable somatic cell measurement device

In some new on-farm devices such as Portacell (made by Portacheck, USA), the amount of color created in the chemical reactions between the diesterase enzyme and the corresponding substrate is the basis for counting the number of somatic cells. In a farm test study to determine the efficacy of PortaSCC, 68 milk samples from bulk tanks were collected. Somatic cells of samples were counted with a light microscope and then with a PortaSCC on-farm set (Portacheck company, Moorestown, New Jersey, USA). Then, milk samples were cultured, and counted cells with two different methods were compared in each isolated bacteria to elucidate the effect of the kind of bacteria on diesterase enzyme-dependent PortaSCC activity. As a result, the Pearson correlation test showed a direct correlation between two different tests ($r = +0.828$, $P < 0.01$). Sensitivity, specificity, and observed overall accuracy were 73.3, 94.4, and 0.785 respectively, when microscopic somatic cells cut-off was considered to be 2.5×10^5/ml. However, the sensitivity and specificity of PortaSCC were 81.6 and 84.2%, respectively when the Rock curve determined 1.6×10^5 cells in Porta SCC as a cut-off point, and microscopic SCC was considered as a golden test. McNamara test ($P < 0.01$) and kappa coefficient (0.537) showed that PortaSCC could be a substitute for microscopic somatic cell count. Pearson correlation test showed that there was a direct correlation between TBC and SCC counted with two different methods ($r = 0.506$, $P < 0.01$). Fisher exact test showed that PortaSCC counted cells were significantly more than microscopic counted cells in *Bacillus cereus* infected samples. *Bacillus cereus* had a significant effect on the activities of the Porta SCC set ($P < 0.05$). It is concluded that Porta SCC is a reliable and valuable screening test to identify subclinical mastitis [26].

7.2 Echo morphometric findings via ultrasonography

Ultrasonography has been used to diagnose or confirm the diagnosis of subclinical mastitis [27]. The epithelium diameter of alveoli, teat, and udder cistern would be measured in this method. Zanjirani and Mohammadsadegh [28] to determine the efficacy of ultrasonography in identifying subclinical mastitis, 30 Holstein cows in the Garmsar area, which were often in the third and fourth parity, were selected based on the CMT grade ≥ 1, and positive milk culture results, and considered as the experimental group. Then, the diameter of the teat, the diameter of the cistern, and the diameter of the alveoli were measured with a 5 MHz ultrasound probe. The healthy udder and contralateral to the same Cartier after confirmation of health by the CMT and negative culture results were considered as a control group and examined with the ultrasound probe. Results of that study showed that only alveolar epithelium diameter significantly increased in cases of subclinical mastitis (**Figure 1**). In this case, the best cut-off was estimated at 8 mm in the epithelial diameter of the alveoli. The sensitivity, specificity, and predictive value of all cases was 66.7%, and the predictive value of the negative cases was 100%. However, the kappa disagreement coefficient was 0.33 ($P < 0.05$). In examining the relationship between alveolar epithelium diameter and

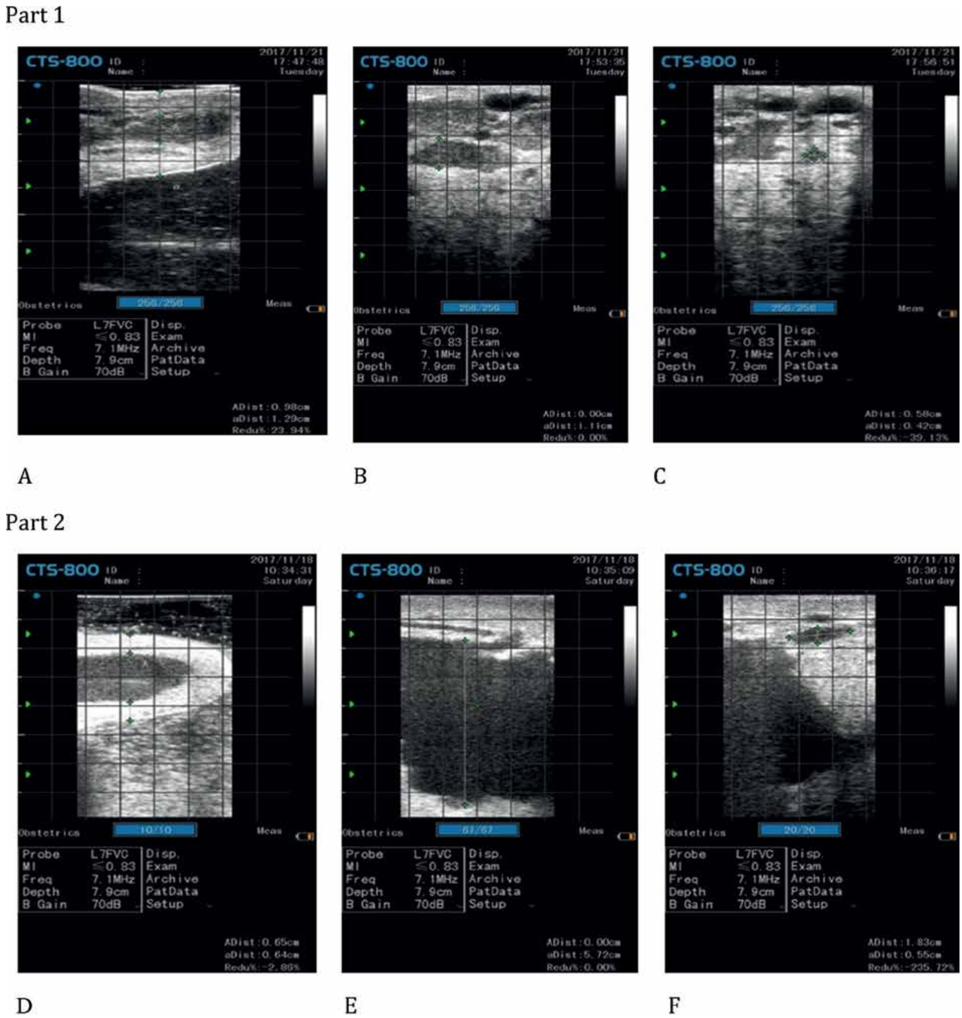


Figure 1.
Ultrasound findings in the mammary gland of cows. Part 1; subclinical mastitis; A = teat cistern, B = udder cistern, and C= alveoli. Part 2; a normal cow; D = teat cistern, E = udder cistern, and F = alveoli [28].

SCC, SCS, the level of lymphocyte, neutrophil, and the degree of hyperkeratosis, the correlation was only significant with SCS. ($P = 0.049$, $r = 0.36$) [28].

7.3 Micro RNA

Diverse miRNAs may play an important role not only in the diagnosis but also in the treatment of mastitis in cows. MiRNAs are posttranscriptional regulators that bind to complementary sequences on target mRNAs, usually resulting in translational repression in mammals [29–31]. It is estimated that each miRNA regulates on average 200 target genes through an interaction between the seed sequence and the complementary target sites [32].

Li *et al.* [33] attempted to identify and characterize novel and differentially expressed microRNAs in peripheral blood from healthy and mastitis Holstein cattle by deep sequencing, and found that the patterns of miRNAs expression differed significantly between the peripheral blood from healthy and mastitis Holstein cattle, which provide important information on mastitis in miRNAs expression.

Saenz-de-Juano et al. [34] tried to determine extracellular vesicle properties and miRNA cargo variability in bovine milk from healthy cows and cows undergoing subclinical mastitis and showed that the miRNA profile and particle size characteristics remained constant throughout consecutive days, suggesting that miRNAs packed in EVs are physiological state-specific. In addition, infected quarters were solely affected while adjacent healthy quarters remained unaffected. Finally, the cow-individual miRNA changes pointed toward infection-specific alterations (**Table 3**). In addition to microRNA 21, other microRNAs that are related to the increase of somatic cells are: bta-miR-223-3p, bta-miR-142-5p, bta-miR-146b-5p, bta-miR-2890, bta-miR-2284ab, bta-miR-22-3p. Meanwhile, the presence of some microRNAs has been associated with a decrease in the number of somatic cells and may be effective in resistance to mastitis. Such hairs are: bta-miR-19b-3p, bta-miR-29c-3p, bta-miR-374b-5p, bta-miR-339a-5p, bta-miR-141-3p, etc. [34].

7.4 Bulk tank analysis

Using a sample of milk from the whole milk collected in the herd, which is called a bulk tank milk sample, facilitates the analysis of findings at the herd level. The proper

High SCC vs. Low SCC			
miRNA	**Log2 FC**	**FDR**	**High SCC**
bta-miR-223-3p	9.5	6.1E-11	↑
bta-miR-142-5p	7.4	3.4E-09	↑
bta-miR-146b-5p	2.9	3.1E-07	↑
bta-miR-2890	2.4	1.5E-04	↑
bta-miR-2284ab	1.4	3.3E-02	↑
bta-miR-22-3p	0.7	1.8E-02	↑
bta-miR-21-5p	0.7	7.0E-03	↑
bta-miR-27b-3p	−0.4	1.8E-02	↓
bta-miR-181a-5p	−0.4	3.3E-02	↓
bta-miR-10,174-3p	−0.4	1.2E-02	↓
bta-miR-29a-3p	−0.6	1.2E-02	↓
bta-miR-29b-3p	−0.8	4.3E-02	↓
bta-miR-2285bf	−0.9	2.8E-02	↓
bta-miR-141-3p	−1.0	2.8E-02	↓
bta-miR-339a-5p	−1.1	3.0E-03	↓
bta-miR-374b-5p	−1.1	2.4E-02	↓
bta-miR-29c-3p	−1.2	2.0E-02	↓
bta-miR-19b-3p	−1.5	3.3E-02	↓

Table 3.
List of differential miRNAs in milk EVs from High SCC versus Low SCC quarters. FC: Fold change; FDR: False discovery rate.

method of sample preparation and the factors affecting its changes by different is to be discussed [35]. Sometimes, instead of one sample from the whole herd, several samples are prepared, each of which represents a specific group of cows in the herd, and it is called string sampling [36, 37]. Somatic cell count and the total number of bacteria in tank milk are usually checked to assess the state of the herd.

Bulk tank cultures may detect high numbers of specific contagious organisms (*S. agalactiae*) and indicate a herd problem that requires individual cow cultures. Usually, it is not enough to culture the tank milk once [38]. *Mycoplasma* spp. and *S. aureus* may be shed intermittently in milk by a small percentage of the herd and may be detectable only after repeated cultures. Except for cases of *S. agalactiae* and possibly mastitis due to other *Streptococcus* spp., standard plate count (SPC) are general indicator of milking and management problems [35].

An increase in the total number of milk bacteria can occur both in cases of mastitis and in cases of contamination of milk. The total number of milk bacteria is placed in three groups; standard plate count (SPC > 5000–10,000/mL), pre-incubation count (PIC>40,000/mL), and pasteurized laboratory count (PLC > 180–200/mL) [35–37]. Normally, the PIC level is less than four times the SPC, and cases that are more than four times usually increase the level of mastitis, especially subclinical, although with a lower probability, that it can be caused by milk contamination. If mastitis cases increase PIC, the number of SCCs will be more than 200,000. If the PIC was less than four times the SPC, but the SCC was more than 200,000, the presence of mycoplasma SPP is suspected (**Table 4**) [36]. The number of somatic cells is more than 200,000/mL in conventional herds and 150,000/mL in organic herds, indicating an increase in cases of subclinical mastitis and the loss of large amounts of milk due to this crisis. A cell counts greater than 400,000 usually indicates a condition that requires intervention and correction, but not everywhere.

The advantages and disadvantages of the polymerase chain reaction (PCR) method in identifying clinical mastitis bacteria or herd tank milk have been explained by different researchers [39]. Despite the high cost of this method and not separating live bacteria from dead bacteria (it is important in the pathogenesis of bacteria), there is a lot of hope in its development, especially in identifying the causative agents of mastitis. However, the need to prepare a specific primer for each

SPC > 5000			
PIC<4 × SPC (due to mastitis)		**PIC>4 × SPC(Low hygiene in bedding, udder, and machine)**	
Bulk tank SCC		**Bulk tank SCC**	
<200 × 10^3	**>200 × 10^3**	**200 × 10^3 >**	**>200 × 10^3**
High environmental and contagious mastitis pathogen	High LPC and high environmental pathogen	High environmental and contagious mastitis pathogen	High coliforms and LPC
Urgent control and prevention of mastitis	Prevention of environmental pathogens and evaluation of the milking process	Milk quality should be checked	The entire milking path and the milking machine must be checked and cleaned

Adopted from Ref. [36].

Table 4.
A schematic diagram for identifying current and potential milk quality and mastitis problems in a herd.

potential bacterium is one of its disadvantages, especially in the identification of bulk tank milk bacteria.

Analyzing a bulk tank milk sample is not enough to make a decision and determine the necessary action, and it is more logical to judge based on several samplings and preparation of the geometric mean, in herds with seasonal changes, the use of rolling SCC [35–37]. If the milk collected in the herd is stored in several refrigerators, as is done in large herds, it is recommended to use the milk from the refrigerators instead of the milk from the bulk milk tank of the herd, which is called string sampling. It is easier to search and find the group of animals involved in this method. In using bulk milk to monitor herd SCC, it should be remembered that subclinical mastitis is not the only cause of SCC increment. In addition to subclinical mastitis, not separating quarter's milk with mastitis, mixing quarter's milk being treated, and mixing quarter's milk which is apparently treated but bacteriologically involved is the common cause of increased bulk milk SCC.

7.5 Composite milk analysis

One of the useful indicators in the evaluation of subclinical mastitis is the determination of composite milk somatic cell count. To prepare it, they mix the milk of four quarters from each cow. Estimating the geometric mean of the composite milk cell count is a good alternative to the bulk tank. A number greater than 200,000 for each cow indicates subclinical mastitis [4, 40]. It seems the degree of agreement between the number of somatic cells in tank milk and composite milk makes a difference according to the type of pathogen in cows' udders and the herd, the median, average, and especially the standard deviation of the number of bulk tank somatic cells, the number of cows that spread large amounts of milk and somatic cells in the herd milk (the author).

As an abstract at the end of the research subclinical mastitis detection methods, it should be emphasized that the most reliable laboratory detection method is somatic cell counting and the most reliable on-farm or cow-side detection method is the case of CMT.

8. Decision-making to combating

In the absence of regular herd testing and precision technologies with in-line sensors, a general recommendation is to take a milk sample from the herd bulk tank or string bulk tank milk every month, and in cases where an unacceptable increase is seen that requires treatment, a composite milk sample or weighted cell count should be prepared from all the cows in the herd. In many developed dairy countries, there is a possibility to use the last herd test data or request an urgent herd test and obtain data at a cow level. Finally, as a field measure, the entire herd can be tested using CMT or other ancillary tests for the detection of inflammation. Some practitioners find it sufficient to use a composite milk sampling medium. In this way, you can find cows that need attention. Before determining the method of combating subclinical mastitis, common measures are introduced.

8.1 Short-term or long-term methods

The strategies aimed at the reduction of the number of somatic cells in cow or herd level milk can be divided into two groups: Short-term and long-term efforts.

Prompt and compliant treatment of clinical cases, shortening or prolonging the dry period, treatment of clinical cases that respond positively to the CMT test in 2 to 4 weeks after treatment [41, 42], pre-milking stripping and throwing away contaminated milk, and treating it if necessary are among the measures that reduce the herd level of somatic cell count very quickly and in a short time.

The rate of response to the treatment of subclinical cases caused by many bacteria, except for *Strep agalactia* during lactation, is very low and sometimes disappointing [29]. Treatment of involved quarters during lactation is recommended only in cases of *Streptococcus agalactia* in the form of BLITZ or mini-BLITZ, and the response to the treatment is relatively fast [43]. In the case of other bacteria, it is either not implemented at all or after the implementation of other measures [29].

Preventive measures include pre- and post-milking teat disinfection, blanket or selective therapy at the dry period, the use of teat sealants after placing dry cow ointment at the beginning of the dry period, disinfection of the milking machine, reduction of bacterial load in the herd, udder, and teats, increasing the level of immunity, and decrement the stress level of the cow in the herd [44, 45] are placed in the group of long-term measures.

8.2 Herd level or individual strategies

The fight against subclinical mastitis is carried out at two levels: 1- the herd level aiming to decrease bulk milk SSC, and 2- the individual level.

8.2.1 Heard level strategies

In addition to the standard measures to prevent and control mastitis at the herd level, efforts to increase the health of cows and udders during the dry and lactation periods, improve the immune system, improve the food supply, and many other such measures are carried out at the herd level that plays an important role in the control of subclinical mastitis. Determining the priority of necessary actions is somewhat difficult and is discussed by Shukken et al. [46].

8.2.1.1 More effective treatment of clinical cases

An incidence rate of 15 new cases per 100 cows per lactation is achievable in good herds and whenever this number is exceeded further investigation is warranted [7]. To achieve this goal, faster identification of new cases and more efficient therapy is needed.

8.2.1.2 Identification and disposal of involved milk

Pre-milking striping of all quarters and discarding the involved milk (stinky, diluted, warmer, or purulent) from apparently normal involved (red, painful, swollen, and sweaty) quarters can rapidly decrease bulk milk and somatic cell count. Persistence of clot for at least four pre-milkings, and the use of CMT solution is necessary to confirm milk contamination in mild cases of clinical mastitis [7, 22].

8.2.1.3 More complete treatment of clinical cases

In many milk-producing herds, the treatment is stopped after the signs of mastitis disappear, while if a bacteriological sample is prepared from the milk, active bacteria are still present in many cases. Such a situation increases not only the cases of return or recurrence of mastitis after treatment but also during the period of incomplete treatment and the presence of active bacteria after treatment, the number of somatic cells of quarters will be high. To reduce this situation, ideally, after the signs of the disease disappear and before stopping the treatment, a bacteriological sample should be taken from the milk. Since such a protocol is very expensive, and there is a good correlation between CMT and IMI [42, 47], it should be recommended that 3 to 4 weeks after stopping the treatment of mastitis, all the involved quarters should be tested with a CMT solution, and if they were involved to subclinical mastitis, intramammary treatment should be performed in one or two next milkings according to the CMT results. Such additional treatment is very useful, but as it is assumed, these quarters will increase the number of somatic cells in the bulk milk tank of the herd within 3 to 4 weeks of the mentioned gap. For this reason, it should be recommended that when mastitis signs disappear after a period of treatment, an additional intramammary treatment be given in the next milking (8 to 12 hours later).

8.2.1.4 Isolation or segregation strategy

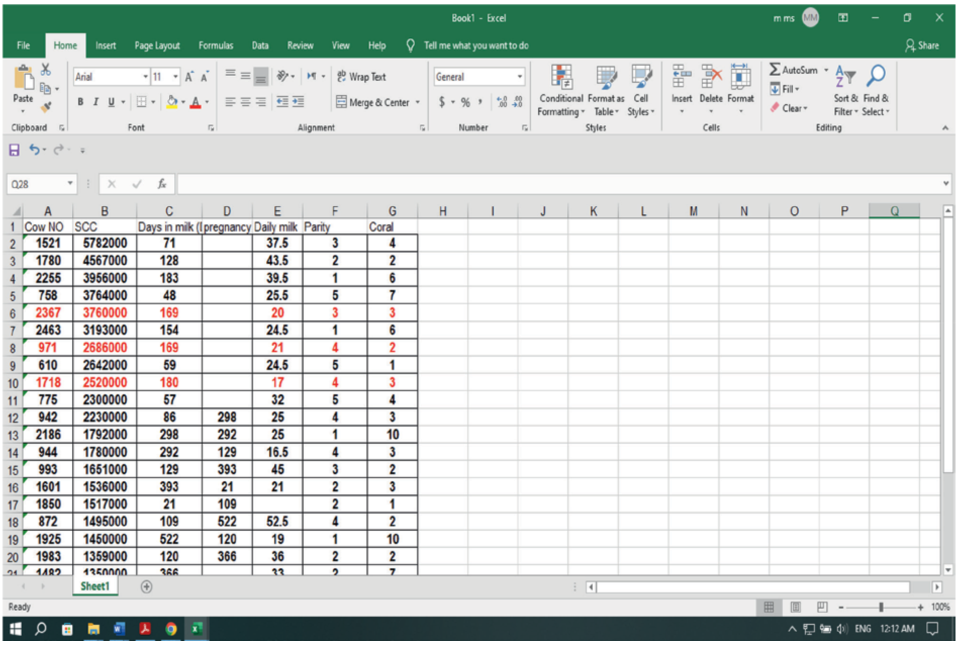
In many herds especially in non-developed countries, more than 50% of cows that have an initial clinical case of mastitis will have a repeat clinical case in the same lactation. A 30% repeat case rate is considered an acceptable level 38]. It has been shown that 6–8% of herd cows are the cause of 40–50% of all clinical cases in the herd [48]. Therefore, if they can be found as quickly as possible and separated or removed from other cows in the herd, not only in the rest of the herd will there be a drastic reduction in cases of contagious mastitis but also the number of somatic cells in the herd will decrease rapidly.

8.2.1.5 Controlling the length of the dry period

An increase in the length of the dry period causes the development of intramammary infection and subclinical and clinical mastitis caused by coagulase-negative *Staphylococci, Strep uberis*, and mycoplasmas. Reducing the length of the dry period will be associated with the reduction of intramammary infection caused by *Escherichia coli* and *Staphylococcus aureus* [24].

8.2.2 Individual level strategies

To draw a plan to combat subclinical mastitis and determine the priorities of the fight before any action, factors such as composite milk of cows, DIM, the amount of milk production in the current lactation peak, the amount of milk production in the previous lactation, pregnancy status, and age of the cow should be estimated, and placed in an Excel sheet (**Figure 2**). Then, based on the number of somatic cells, they are sorted from maximum to minimum. In many livestock evaluations, many cows are found to be unacceptably past their delivery and initiation of lactation (DIM >180 d), are not pregnant, and have been given a small amount of milk in the current and previous lactations. After finding the above cows (usually many of them have already received a culling suggestion), they should be culled or segregated from other cows and their milking place should be separated from the milking of the herd so that their milk is separated from the others. This will quickly and significantly reduce the number of somatic cells in the herd.



	A	B	C	D	E	F	G
1	Cow NO	SCC	Days in milk (I	pregnancy	Daily milk	Parity	Coral
2	1521	5782000	71		37.5	3	4
3	1780	4567000	128		43.5	2	2
4	2255	3956000	183		39.5	1	6
5	758	3764000	48		25.5	5	7
6	2367	3760000	169		20	3	3
7	2463	3193000	154		24.5	1	6
8	971	2686000	169		21	4	2
9	610	2642000	59		24.5	5	1
10	1718	2520000	180		17	4	3
11	775	2300000	57		32	5	4
12	942	2230000	86	298	25	4	3
13	2186	1792000	298	292	25	1	10
14	944	1780000	292	129	16.5	4	3
15	993	1651000	129	393	45	3	2
16	1601	1536000	393	21	21	2	3
17	1850	1517000	21	109		2	1
18	872	1495000	109	522	52.5	4	2
19	1925	1450000	522	120	19	1	10
20	1983	1359000	120	366	36	2	2
21	1482	1350000	366		33	2	7

Figure 2.
Somatic cell number analysis sorted from high to low along with other data (pay attention to the red rows).

In some cows, it can be seen that they are given a small amount of milk, they are pregnant, but the time has not yet come to dry them; however, it may be better to dry them depending on the type of bacteria and herd policy,

8.2.2.1 Treatment strategy

It is generally considered not advisable to treat subclinical mastitis during lactation [19, 34]. However, it is important to consider the causative organism (*Streptococcus agalactiae* is easily treated), the age of the cow (better in younger), days in milk (before the peak of lactation is good), the number of intramammary infections for the cow (lower is better), history of the quarter in the previous lactation, history of the resent clinical or subclinical mastitis, pregnancy state (nonpregnant may be culled), milk production levels in previous and present lactation (low milk producers may be culled), and the udder health status of the herd.

8.2.2.2 Should subclinical mastitis caused by all bacteria be fought in the milking period?

It should not be forgotten that all bacteria cause both clinical and subclinical mastitis, but subclinical mastitis caused by minor bacteria is usually not treated during the lactation period due to their supportive role. Minor bacteria such as *Corynebacterium bovis* and CoNS prevent the occurrence of primary bacterial mastitis with a slight increase in the number of somatic cells [5].

All major or minor bacteria, common or uncommon, gram-negative or gram-positive, and environmental, contagious or opportunistic cause subclinical mastitis, but only some of them are easily treated. Coliform infections caused by *Escherichia coli* and *Klebsiella pneumoniae* are usually short-lived. Although the number of somatic cells caused by *E. coli* is very high, their subclinical cases are not considered important [49]. Environmental streptococci such as *S. uberis* and *S. dysgalactiae* show poor response when treated during lactation, and after possible treatment, the probability of infection returning is high. Therefore, their subclinical cases are not considered suitable for treatment [50]. Subclinical mastitis caused by *Staphylococcus aureus* causes a relatively persistent infection in the udder and increases the number of somatic cells to a moderate extent, and due to poor response to treatment during the lactation period; treated only in young cows, when of shorter duration of infection and lower SCC, days in milk less than 100, in non-chronic and repetitive and high producing cows [51, 52]. In cases caused by *Mycoplasma bovis*, the number of somatic cells increases drastically, but the total number of bacteria in the bulk milk tank does not change. Its subclinical cases are not treated, and an attempt is made to cull or segregate affected cows [50].

Briefly, milk from CMT-positive quarters is aseptically sampled and submitted to the laboratory. Intramammary treatment is limited to quarters infected with gram-positive pathogens (streptococci and, in some herds, first- or second-lactation cows with new *S. aureus* infections). Milk from cows with no growth or other pathogens is managed by other means, including no intervention, segregation, dry-off, or “killing” of the quarter [9].

8.2.2.3 When the herd’s somatic cells should not be reduced

1. Somatic cells in the range of 300,000 to 400,000 indicate the activity of environmental bacteria in the herd. For this reason, in such a situation, although reducing somatic cells to 200,000 will increase milk production and achieve other benefits of SCC decrement, it will increase clinical cases caused by environmental bacteria [53, 54]. Therefore, in such a situation, it should be reduced only when it is ensured that the level of bacteria is low, the bedding is dry, the level of immunity of herd cows is high, and the level of stress in the herd is low. The total antioxidant capacity can be estimated from the imine level in cattle.

2. Never select a quarter or a cow that shows an increase in SCC in a single sampling for intramammary treatment to reduce SCC. Many increases in SCC may be temporary and resolve on their own [55].

3. Never choose a quarter or a cow that has shown an increase in SCC in more than five sampling times for intramammary treatment to reduce SCC. Such cases are considered chronic mastitis, which does not respond well to treatment, especially during the lactating period, and it is better to cull them. If it is difficult to cull them, such cows should be segregated and milked separately, so that, their milk does not mix with the milk of the rest of the herd. In cases where such cows remain in the herd, they may recover by being treated at drying and receiving dry cow (DC) ointment [55].

4. *Corynebacterium bovis* and *CoNS* should never be selected for intramammary treatment to reduce SCC unless the efficacy of post-milking teat dip and dry period therapy are acceptable. After ensuring the effectiveness of the post-milking teat dip and dry period therapy, intramammary treatment can be used to accelerate the recovery of the quarters from the above bacteria [45].

8.2.2.4 Classification of cows with subclinical mastitis before treatment

Schocken 2008 suggests two types of increase in somatic cells. In one type, the number of cows whose cells are more than the herd targets is less than 2% [46]. In such herds, chronic cases (they have been infected in more than five consecutive samplings are more important than cases of sudden and temporary increase. For this reason, bacterial culture and identification, culling or isolation, early drying off, and treatment during the dry season are more important than treatment during lactation.

In the herds where more than 2% of the cows in the herd are responsible for increasing the number of somatic cells from the predetermined goals in the herd, the heifers are encountered as the same as cows, and the affected cows are divided into three groups. If the rate of a new infection is more than 8%, the following efforts should be checked: Heifer health, milking procedures, purchased cows, days in milk (with increasing the cell count this indicator increases), and the seasonality of mastitis. In cases where the chronic infection is more than 5%, attention should be paid to the pattern of increasing the number of somatic cells. The patterns of SCC increment may be as follows: High- low- high or sudden increase (spikes), or chronic-high [34]. Segregation and isolation, culling, cessation of lactation, and rarely intramammary antibiotic treatment are considered finally. In cases where more than 15% of fresh cows are infected, the following matters are checked: Their health, treatment during the dry period, seasonality of mastitis, and food ration.

8.2.2.5 When should be subclinical mastitis treated?

There are several situations in which lactational therapy of subclinical mastitis may be indicated:

1. When the main cause of subclinical mastitis in a herd is *Streptococcus agalactiae*, all CMT-positive quarters in the herds in BLITZ strategy, or quarters with SCC > 5×1000^5 in mini-BLITZ strategy would be treated with intramammary antibiotic therapy [43].

2. In the situation where cow husbandry has received the warning of high SCC (usually with bulk milk tank more than 400,000/mL) and does not have much time to reduce SCC, and *S. agalactiae* in the herd is not isolated, and so, BLITZ therapy cannot be performed, all CMT-positive quarters from which environmental streptococci or exceptionally *S. aureus* has been isolated in milk culture, and the cows are in their first or second lactation, and the days in milk (DIM) is before the milk peak, they are treated with an intramammary antibiotic for at least three times (usually once every 24 hours). To ensure the result, it is recommended to repeat the treatment up to eight times in the case of *S. aureus*. Cure rates of cows with subclinical mastitis caused by *S. aureus* during lactation are much lower. Reported cure rates following intramammary therapy are between 15 and 60% [51, 52].

3. When the cow suffering from subclinical mastitis with any bacterial agent following this type of mastitis, involved in other important reproductive diseases such as anestrus, ovarian cysts, or repeat breeding, the involved quarters should be treated.

4. In cases of positive CMT test in 2 to 4 weeks after stopping the treatment of the mastitis case due to the remedy and mitigate of clinical signs, the quarters should be treated.

5. CoNS are the main bacteria in the intramammary infection of heifers and are the common cause of clinical mastitis in heifers after parturition, so it may be necessary to diagnose and treat heifers before parturition. In this context, intramammary treatment with cloxacillin (200 mg) and cephapirin sodium (200 mg) has been useful. Intramammary or parenteral tilmycosin may also be helpful [3].

8.2.2.6 Antibiotic of choice

Lactational therapy of subclinical *S. aureus* mastitis using intramuscular penicillin along with intramammary amoxicillin infusion, compared with the intramammary infusion alone, and increased the cure rate to 40% (Almost twice as much as the other group) [29, 56]. In staphylococci sensitive to penicillin, the best antibiotic is penicillin, but due to the possibility of mixing in the author's experience, it is usually ceftiofur sodium (injection solution in the form of 0.125 mg/100 ml intramammary or intramammary ointment is used. In herds with a high prevalence of *S. agalactiae* mastitis, BLITZ therapy followed by good sanitation procedures can be used for eradication of the pathogen, increased milk production, and reduced penalties for high SCCs. Erythromycin (30 mg), cloxacillin (500 mg), and ampicillin (250 mg) can be used for this purpose [29]. Extracts of various traditional medicinal plants have been used and effective in the treatment of mastitis, especially the subclinical type [57–59]. Some probiotics, such as *Bacillus subtilis*, have been effective in the treatment of subclinical mastitis, similar to antibiotics [60].

8.2.2.7 Non-steroidal anti-inflammatory drugs (NSAD)

The response of the immune system to the inflammatory stimuli of microbes [61], physical stimuli such as frostbite, or chemicals such as non-standard teat dip causes an increase in the number of somatic cells [62]. Therefore, in all cases of this type of mastitis, the use of non-steroidal anti-inflammatories will be effective. However, in such cases, anti-inflammatory drugs are usually not used unless the treatment strategy chosen by the veterinarian is to use these drugs, especially in cases where antibiotics are not prescribed. Meloxicam, ketoprofen, caprofen, and to some extent, flunixin meglumine have been recommended in such cases. The use of dipyrone and phenylbutazone has not been successful. Carprofen and meloxicam are cyclo-oxygenase-2 (COX-2) selective, single-dose, long-acting NSAIDs to treat bovine mastitis [63]. One-time use of long-acting drugs such as meloxicam and caprofen seems to be enough [63]. Systemic administration of these drugs is common. Intramammary NSAD is not available, and if necessary, intra-mammary ointments containing steroid anti-inflammatories such as hydrocortisone, prednisolone, and dexamethasone are used. The use of these medicinal products in pregnant cattle is not allowed because it causes abortion in cows pregnant for more than 4 months [64].

8.2.2.8 Warnings

It is also important to ensure that standard mastitis control procedures, such as post-milking teat disinfection and dry cow therapy, have been implemented.

Treatment of subclinical mastitis cases in herds where the principles of control and prevention are not observed is associated with treatment failure or disease recurrence.

It is very important to pay attention to the bedding. Never use wet organic materials bagasse, or molasses, under the cow's feet after intramammary treatment. Fungal infections after intramammary treatment are common. It is recommended to change the bedding, keep it dry, and burn the surface of the cow's place before intramammary treatment, especially BLITZ therapy.

9. Conclusion

The somatic cell count of tank milk or string samples should be prepared every month. If there is a need to make some efforts and control the number of somatic cells (usually in the number of somatic cells more than 300,000/mL), a composite milk sample or weighted sampling is prepared and the information is placed in an Excel file and sorted from top to bottom. Cows with a cell count higher than 200,000/mL, and in most cases, more than 400,000/mL may need intervention, which includes treatment or other measures (separation of quarter milk with infected pre-milking, segregation of some cows, or cessation of lactation in some quarters).

The pregnancy status, chronicity and the level of high intensity of somatic cell count, days in milk, age and parity, and milk production level (at the time of sampling and the peak of lactation) should also be determined and the treatment method (culling, segregation, etc.) should be implemented based on all of the mentioned factors. Meanwhile, intramammary antibiotic therapy should be the last measure. Before intramammary treatment, it is necessary to pay attention to several health measures (Collecting manure, drying and disinfecting the bedding, not using the bedding containing organic substances such as bagasse, using effective post-milking teat dipping, and effective treatment during cessation of lactation).

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Chapter 6

Harnessing Nanominerals for Managing Subclinical Mastitis in Cattle: An Innovative Approach

Duraisamy Rajendran, Partha Sarathi Swain, Ayyasamy Manimaran, Muniswami Shobha, Subhashree Tripathy and Chinmayee Sahu

Abstract

Mastitis is a problem associated with mammary gland and results in drop in milk production. The significance is more in milch animals as milk is the primary product. Use of antibiotics for treating mastitis is not only adds to expenses but also raises the concern of antimicrobial resistance among the consumers. Use of nano-minerals may be a good alternative for treating mastitis in domestic animals. Nano-minerals can be defined as minerals of 1-100 nm range. The nano-minerals have unique properties as compared to their counterparts. Due to the biocidal properties, mineral nanoparticles of Ag, Au, Se, Cu, Zn, etc., in the diet as feed additive can control or reduce the subclinical mastitis, and thus can be a potential alternative of antibiotics. The nano-minerals act efficiently against mastitis causing agents, thanks to their anti-microbial, anti-oxidant, anti-inflammatory, and immunomodulatory properties. Though the results are encouraging, use of nano-minerals as a preventive and curative to subclinical mastitis is in its infancy. Further studies are warranted to validate the route of administration and evaluate its efficacy in long-term use in varied animal species. Moreover, the side effects of application of nano-minerals have to be studied before recommending in commercial scale.

Keywords: antimicrobials, bovine, clinical nutrition, mastitis, nanominerals

1. Introduction

Mastitis is the most prevalent and expensive condition that affects dairy cattle in India [1] and worldwide [2], causing severe losses in the dairy industry. Next to foot and mouth disease, mastitis is regarded as one of the costliest and economically devastating diseases influencing the profitability of Indian dairy farmers and industries [3] through reduced milk production, milk quality, treatment, and culling cost [4]. Mastitis poses the risk that the bacterial contamination of milk from affected cows will render it unfit for human consumption by causing food poisoning or interfering with the production process or, in rare situations, providing a route for disease transmission to people [5].

The inflammation of the mammary gland is called mastitis, which can be observed in all mammals. This is an important disease in dairy animals as it affects their productivity with the potential to affect the health of humans and animals consuming it. In the majority of cases, an infection caused by bacteria causes this condition. Attempts are being made worldwide to manage bovine mastitis because of its significant impact on cattle and public health and the altered composition of milk from mastitis-affected cows. These could have an adverse impact on the suitability of milk processing as well as the quality of processed goods made from it. The loss in the dairy industry does not only refer to economic issues including milk quality and quantity, antibiotic usage, or extra labor but also in addition to the disease significantly affecting animal welfare and public health [6].

Mastitis may have different etiological factors, namely, bacteria, viruses, fungi, and algae-like infectious factors [7] coupled with the genotype, environmental conditions, immune status, and feed composition (dietary supplement addition) such as noninfectious factors [8]. Furthermore, the noninfectious factors may contribute directly or indirectly to the occurrence and severity of mastitis [6]. Depending on the source of infection, mastitis can be divided into two subcategories: contagious and environmental. Environmental mastitis is caused by pathogens from the environment, whereas contagious mastitis spreads from other infected quarters [9]. Among the bacteria, the most common bacteria that cause intramammary infection are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, and *Streptococcus uberis* [6, 10–12].

Among the noninfectious factors contributing to the onset of mastitis, mineral deficiencies are forerunners. Any nutritional deficiency will result in a weakened immune response, which will increase the risk of udder inflammation, and thus contribute as a predisposing factor for udder inflammation. Minerals are a group of nutrients that have been reported to influence udder health status since they strongly impact the immune system. Consequently, these deficiencies result in weakened immunity, which increases the risk of any infectious disease. The minerals such as Cu, Zn, Se, and Mn play a vital role in enhancing the immunity of cattle. Moreover, deficiencies of some minerals are associated with metabolic disorders such as milk fever, hypophosphatemia, and hypomagnesemia. Every mineral deficiency leads to immunosuppression [13] that can predispose the cows to mastitis [6]. Furthermore, cows with sound immune system can deal with microbial invasion and avoid the inflammation process, and thus prevent the occurrence of mastitis [6].

2. Types of mastitis

Mastitis can be classified into two types: clinical and subclinical form. In the clinical form, signs may vary depending on the causative agent; whereas in the subclinical form, there are no visible changes in the aspect of the udder or milk, although it can limit milk output. However, subclinical mastitis is regarded to be the most economically significant type of mastitis for many cattle farms because of its high prevalence (19–78%), being a chronic source of infection for herd mates, its difficulty of detection and possibility to convert into clinical form [4, 14]. In India, Varshney and Naresh [15] reported that subclinical mastitis (SCM) had a higher incidence (10–50%) than clinical mastitis (1–10%). It reduces the milk production and causes changes in the composition and quality of the milk, as well as shortens lifetime of the affected cows and causes significant damage of the milk secretory cells [16]. Monitoring the number of somatic cells is a widely used practice in the European Union (EU) for assessing milk quality [17, 18] that increases in mastitis.

3. Economic implications

The economic implication of mastitis can be attributed directly through the loss in milk production and indirectly through the cost of treatment of animals [19–21]. Moreover, in subclinical mastitis, the owners fail to detect the disease; thus, there is an obvious delay in treating the cows. Despite the possibility of the consumption of infected milk causing health issues in humans and animals, it is still being consumed. Nevertheless, the prevention and treatment of mastitis, as well as the costs associated with preventive measures, also incur additional expenses for the farmer. To create the right economic interventions to prevent and cure mastitis, accurate information of the disease status is required, allowing feasibility of preventive measures for one dairy cow or for the entire herd [22].

Again, the costs of mastitis are divided into two broad groups: those that occur directly and those imparts indirectly. Veterinary services, diagnoses, therapies, additional labor needs, and milk wasted during treatment are considered as direct expenditures. The term "indirect costs," often known as "hidden costs," refers to expenses that are not always apparent to the producer of milk. Many farmers are unaware of the indirect costs associated with subclinical mastitis (SCM), which include decreased milk production, premature culling losses, and decreased quality premiums [23]. Education is essential in this area, since failing to detect indirect losses could make it harder to introduce mastitis control measures [22].

Most economic expenditures associated with mastitis are believed to be primarily attributable to SCM. Besides, the Indian diary industry lost more than 2.37 billion rupees each year as a result of mastitis, with SCM responsible for roughly 70%. According to some research, SCM is more common and causes greater economic loss in India than CM (28–42%: 1700–3000 crores annually vs. 58–72%: 4150–4365 crores; [24]). Rathod et al. [25] reported that depending on the cow's health, the overall economic losses caused by SCM each lactation period ranged from INR 21,677 to 88,340. Singh et al. [26] reported that high-yielding crossbred cows suffered greater financial losses per lactation than native cows and buffaloes (INR 868.34 and INR 1, 272.36, respectively).

4. Problems in the recent treatment regimen

In bacteria, *Staphylococcus aureus, Streptococcus agalactiae, Escherichia coli,* and *Streptococcus uberis* are predominant pathogens attributed towards mastitis [6, 10–12]. Thus, antibiotics are used for treating mastitis. However, indiscriminate use of antibiotics threaten the emerging issues such as antibiotic resistance in humans and cattle consuming such contaminated milk (**Figure 1**). Similarly, Pol and Ruegg [27] stated that mastitis is the prime cause of antibiotic use in dairy animals, and this can be a potent threat to the public's health in terms of antibiotic resistance development. For instance, antibiotics are used to treat *S. aureus* infections causing mastitis, and beta-lactam antibiotics, that is, methicillin has been reported ineffective as a result of resistance to methicilin [28], known as methicillin resistant *S. aureus,* which has the *mec* gene [29].

There are many diagnostic tools for the detection of mastitis in cows. However, their efficacy and time required for the characterization and identification of mastitis causes the significant difficulty and delay in the effective treatment of diseases. The majority of diagnostic procedures are often used widely, even though many lack the required accuracy. Some are time-consuming and expensive, whereas others can only identify the clinical mastitis when the cow is severely affected. For example, CMT has been used as a cowside test for a long time [30]. Errors can occur while interpreting

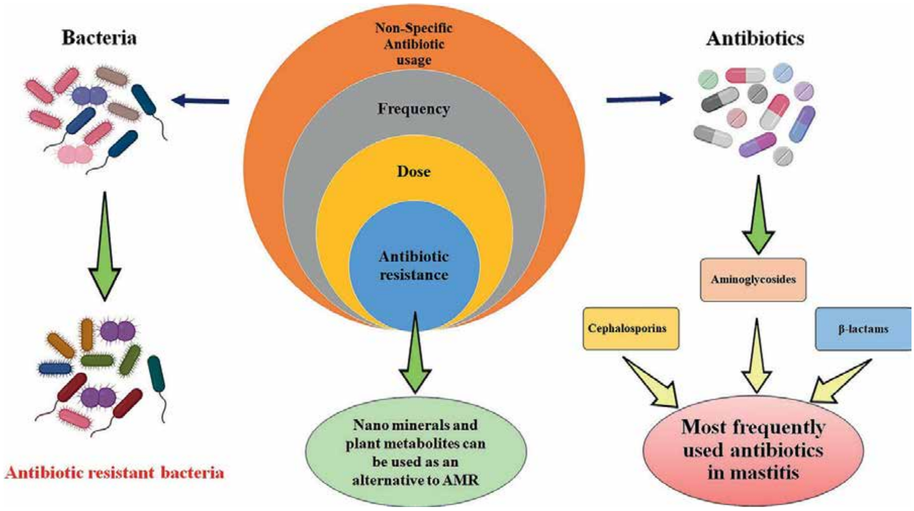

Figure 1.
Compact antibiotic resistance by the use of nanominerals and phytochemicals.

SCC data because of the influence of several factors such as the presence of bacteria, diurnal variation, age, stage of lactation, and storage of the milk sample [31].

Because of the rise in antibiotic resistance and the public demand for high-quality milk, mastitis in cows need to be properly prevented and treated with non-antibiotic alternatives. For instance, in a study in the Transylvanian area (Romania), *Staphylococcus aureus*, isolated from the milk of the mastitis affected cows, were resistant to penicillin and tetracycline [32]. The detection of subclinical mastitis and adequate treatment are added challenges for veterinary professionals, dairy farmers, and scientists. Some reports suggest that nanominerals could be a good alternative to antibiotics for treatment (**Figure 1**) and prevention of mastitis in dairy cows [33, 34]. Though scanty, the literature indicates a lot of promise in the utilization of nanominerals, particularly Ag, Se, Zn, and Au, used to control mastitis in cows.

5. Nanotechnology

The era can be conferred as the era of nanoscience, thanks to its use in various fields such as science and technology. However, nanotechnology keeps surprising the researchers by virtue of its new phenomenon and unexpected outcomes. Though not limited to medicine, cosmetics, agriculture, scarce science, nanoscience has proved its mantle in many fields. The use of nanotechnology in veterinary medicine and as an animal feed supplement is relatively new but found promising applications [35–38]. The quantum of research in nanotechnology, particularly nanomineral feeding, indicates its essence and opportunities in the field of veterinary science [4, 37, 39, 40]. Nanotechnology is a potential option for novel treatment for bovine mastitis [33, 35].

The science dealing with the materials studied in nanoscale is referred to as nanotechnology. More precisely, the materials having a size in the range of 1–100 nm scale are called nanomaterials [41]. For instance, nano-zinc denotes zinc particles that are engineered and processed to have dimensions in the nanoscale, which can potentially improve its bioavailability and cellular uptake compared to traditional zinc supplements.

6. Preparation and properties of nanominerals

The huge opportunities in nanominerals have popularized the technology, which is evident from the quantum of research done on nanominerals. The huge requirement warrants the scientists to standardize and make economical the synthesis of targeted nanominerals. The nanominerals can be synthesized by either physical, chemical, or biological methods (**Figure 2**). An application of physical forces such as ball mill to reduce the particle size is physical synthesis, whereas the use of chemicals to reduce the particle size is a chemical method of nanoparticle synthesis [42]. The use of live organisms to reduce the particle size is a biological synthesis of nanominerals [42]. The biological methods are reported to be environmentally friendly. However, chemical synthesis of nanominerals is economical and tends to produce uniform nanominerals [42].

In another way, Neculai-Valeanu et al. [35] classified the synthesis of nanominerals into top-down (e.g., laser ablation, ball milling and chemical etching) and bottom-up approaches (e.g., sol-gel process, chemical vapor deposition, spray pyrolysis, green synthesis). In the top-down method, the bulk material is converted into nanometer-sized structures using different reagents and physical treatments. In the bottom-up approach, nanoparticles are developed to a specific size and shape from simpler molecules [35]. The size of nanoparticles can be altered to produce nanoparticles of the desired shape and size by altering pH, temperature, and reaction time [43, 44].

Nanominerals are synthesized with a size ranging from 1 to 100 nm [41]. Nanominerals are reported to portray novel physical, chemical, and biological activities [42, 45], and thus produces unexpected biological responses when fed to cows [38, 39, 46]. Thanks to the superior bioavailability of nanominerals as compared to their conventional inorganic counterpart, they result in better cow health and production at even reduced doses [34, 47]. Being nanometer size, zinc nanoparticles are easily absorbed in the gastrointestinal tract of animals, and thus

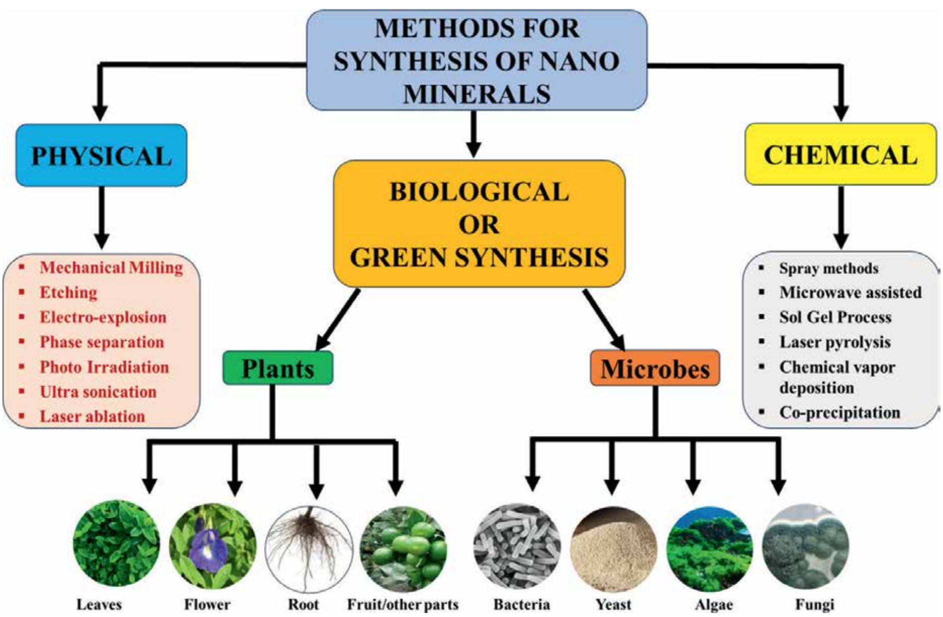

Figure 2.
Preparation of nanominerals by various methods.

are more effective than the larger size ZnO even at lower doses [41] and simultaneously less toxic as compared to the conventional inorganic salts [48]. Nanominerals had the capability of crossing the animals' small intestines and entering easily into blood, brain, lung, heart, kidney, spleen, liver, intestines, and stomach [49]. However, nanoparticle absorption and metabolism are affected by many factors, namely, size, shape, zeta potential, other ligands, surface chemistry, age and species of animal, intestinal health, and dose of use [50, 51]. Desai et al. [52] reported that the translocation of 100-nm nanoparticles is 15–250 times more than that of micromolecules. Nanominerals interact more effectively with organic and inorganic substances in animal bodies that may be attributed to their large surface area [53]. Similarly, Rosi and Mirkin [54] stated that the chemical, catalytic, or biological effects of nanominerals are highly influenced by the particle size of the mineral. For instance, CuO nanoparticles are transported quickly into cells compared with CuSO4 and CuO microparticles [55].

7. Nanominerals as alternatives to antibiotics

Among the beneficiary effect of nanominerals, the antibiotic effect is realized and documented by many researchers [36, 46, 56–58]. The incidence of mastitis is found to reduce by the application of nanominerals. This may have been achieved either by directly killing bacteria and/or by improved immunity of the animal [36, 57]. Elkloub et al. [59] reported a decrease in *E. coli* count as a result of supplementing birds with Ag nanoparticles, demonstrating the antimicrobial effect of Ag nanoparticles.

Mineral nanoparticles of Zn, Ag, and Au can be used as a replacement for antibiotic drugs and without any drug residues in milk and animal products. Reports [36, 57] denote the antimicrobial effect of Nano Ag at the intestine level along with the immunomodulatory effect. Similarly, the somatic cell count in mastitis milk of Holstein Friesian cows was greatly reduced by the supplementation of Zn nanoparticles [34]. Moreover, Zn nanoparticles are found to be effective against both Gram-positive and Gram-negative bacteria [56]. Rosi and Mirkin [54] reported that the nanoparticles are effective against spores that are resistant to high temperature and high pressure.

Antibacterial activity means the reagent that locally kills the bacteria or slows down their growth, without being toxic to surrounding tissues [58]. The antimicrobial effect of ZnO is related to their electromagnetic effects as microorganisms are negatively charged, and thus get attracted towards positively charged metal oxides, resulting in oxidization and subsequent death of microbes [56]. The large surface area of metal nanoparticles can be the reason behind the antimicrobial effect as compared to the large-sized particles [60]. Minerals in nanoform also retard bacterial adhesion and biofilm formation [61], which may be the reason behind the antimicrobial effect. Padmavathy and Vijayaraghavan [61] studied the effect of various nano-Zn particle sizes (20–40 nm, 12 nm, 45 nm) against *E. coli* and observed that nano-Zn has better bactericidal activity than bigger ZnO particles. These results contribute to the abrasiveness and the surface oxygen species of ZnO nanoparticles promoting its bactericidal effects. According to Rajendran et al. [62], ZnO nanoparticles inactivate the proteins responsible for nutrient transport; hence, decreasing the membrane permeability leads to cellular death. Similarly, Au nanoparticles promote innate immunity at <1 mg/kg dose in chicken [63].

Negatively charged bacterial membranes are pulled to positively charged metal nanoparticles resulting in leakage and bacterial cell lysis Gahlawat et al., [64].

Kim et al. [65] discovered that Ag nanoparticles could stop the development of yeast isolated from a case of bovine mastitis and hemorrhagic enteritis-instigating *E. coli* O157:H7 with an estimated MIC value of 3.3–6.6 nmol/L and 6.6–13.2 nmol/L, respectively. Mineral nanoparticles act as biocides that may be ideal alternatives to antibiotic drugs and prevention of contagious diseases, including mastitis, limiting the burning issue of antimicrobial resistance in animals and humans [66].

8. Nanominerals and mastitis

Bacteria are the main cause of mastitis in dairy cows, and the agents that act against them will also be helpful in the prevention and cure of mastitis. Thanks to the antimicrobial effects, nanominerals may be good and healthy alternatives of antibiotic drugs in treating and controlling mastitis. The antimicrobial effects of metal nanoparticles are mainly because of the release of metal ions, disruption of the cell membrane and cell wall, reactive oxygen species production, and inhibition of appropriate DNA replication [35]. Many researchers [67–69] have found metal nanoparticles to be effective against bovine mastitis pathogens [70, 71] and bacteria that are resistant to methicillin as well [35]. Similarly, Rajendran et al. [34] observed an improvement in milk production by the supplementation of nano-ZnO in cows affected with subclinical mastitis. The increases in milk production were attributed to the suppression of subclinical mastitis evident from the reduction of somatic cell count [33, 34]. The use of nanoparticles as a therapeutic alternative for bovine mastitis controls the gaining importance attributed to their improved antimicrobial activity and low cytotoxicity and opens the window for organic farming [35, 70, 71]. Furthermore, Se nanoparticles prevent the growth of common mastitis-causing bacteria such as *Pseudomonas aeruginosa, Staphylococcus aureus,* and *E. coli* at a concentration of 1 mM [72]. Studies conducted by Soni and Yadav [73], Krishna et al. [74]; Mohsenabadi et al. [75], and Vasile et al. [76] have documented the efficiency of nanogels against intracellular pathogens such as *Staphylococcus aureus,* and thus opened new possible method to address the therapeutic challenges caused by mastitis in animals. Debata et al. [72] studied the *in vitro* antibacterial activity of nanominerals on mastitis-causing bacteria using the well diffusion method and reported that nano-Se particles were able to inhibit *Escherichia coli* and *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Zhang et al. [77] postulated that the antibacterial activity of nanoparticles may be because of the generation of reactive oxygen species, malondialdehyde (MDA), and leakage of proteins and sugars in bacterial cells. Wernicki et al. [78] and Kalinska et al. [79] observed that silver and copper nanoparticles exhibit the highest antimicrobial activity against bacteria isolated from inflamed udders. Thus, antimastitic effect of nano-minerals is an additional benefit along with its environment friendly effect [40, 80], augmentation of animal productivity [58, 81] and improved mineral retention [40, 82] which may be proved vital in maintaining the productivity and profitable farming.

9. Conclusions

Mastitis has long been a problem that has not been fully resolved. This issue also affects organized farms that are run efficiently as well as unorganized farms. Mastitis not only reduces the production of animals but also incurs financial loss directly though treatment and reduction in milk production. Even though in a smaller number of incidences, mastitis threatens to cease the milk production completely, and thus directly governs the economic importance of dairy animals. Apart from that, the use of nonspecific use of antibiotics to control and treat mastitis in dairy animals is a potential reason for the development of antibiotic resistance in human as well as animals. Hence, in this context, the literature survey indicated that the use of mineral nanoparticles of Ag, Au, Se, Cu, and Zn in the diet as feed additives can control or reduce subclinical mastitis, and it can be used in place of antibiotics, thanks to their biocidal properties. The mechanism of action is not only restricted to antimicrobial properties but also as a potent antioxidant, anti-inflammatory, and immunomodulatory agent that acts collectively to prevent subclinical mastitis. The antibacterial properties particularly in mastitis animals are studied for so long, and encouraging responses are also recorded by many. However, more systematic studies are warranted to establish their effect as a substitute for antibiotic drugs. Moreover, the duration of application and probable toxicity to the animal under treatment and, in a remote sense, those who are consuming the products keep knocking the minds of the researchers and are to be addressed.

Author details

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Chapter 7

Approaches of Milking Biosecurity and Milking Parlour Hygiene in Dairy Farms

Georgeta Stefan and Stelian Baraitareanu

Abstract

Control of diseases in dairy farms is based on various management factors, such as separation of dairy farms from other domestic and wild animal species, control of human circulation and contact with cows, cleaning and disinfection of vehicles, equipment, surfaces, and other unanimated secondary sources of contamination, feed and water hygiene, waste management, and management of technological processes (e.g., calving management, colostrum management, and milking management). In dairy farms, the milking parlour surfaces should be cleaned regularly and disinfected twice daily to avoid the multiplication of pathogens. Some biosecurity measures that can prevent the spreading of mastitis in dairy farms are the use of personal protective equipment (e.g., milkers' gloves and milker overall), treatment of all infected quarters at the end of lactation (blanket dry cow treatment), removal of udder hair (shaving or singeing), and washing unclean udders. This chapter details biosecurity and hygiene solutions in the cattle milking parlour.

Keywords: biosecurity, risk management, health management, milking hygiene, dairy farming

1. Introduction

Despite the continuous involvement of governmental and non-governmental institutions in developing and promoting milk quality standards and good milk handling practices in dairy farms, the negative attitudes and low knowledge of some processors and dairy farmers are still affecting the prevention and control of zoonoses and antibiotic residues, with a real impact on public health [1, 2].

In dairy farms, control of diseases is based on various management factors, such as separation of own cows from other domestic and wild animal species, control of human circulation and contact with cows, cleaning and disinfection of vehicles, equipment, surfaces and other unanimated secondary sources of contamination, feed and water hygiene, waste management, and management of technological processes (e.g., calving management, colostrum management, and milking management) [3].

Consumers must have access to clean and safe dairy products, and all supply chain actors must accept and apply good milk practices, regulations and standards [2].

The main biosecurity measures that can prevent the spreading of mastitis in dairy farms are the use of personal protective equipment (e.g., milkers' gloves and milker overall), treatment of all infected quarters at the end of lactation (e.g., blanket dry cow treatment), removal of udder hair (shaving or singeing), and washing dirty udders. However, the safety of milking and raw milk is influenced by a group of management and control measures applied throughout the entire farm, designed to reduce the microbial load in the environment and raw milk (e.g., dairy herd health control, good milking practices, and milking parlour hygiene control) [4].

2. Milking biosecurity in cattle dairy farms

Milking biosecurity measures should take into account all sources of germs that might infect the udder and contaminate the milk, beginning with the environment and the cows being milked, then milkers and milking equipment, and finally milking and water [4, 5].

2.1 Milking environment

The production of good-quality milk is mainly influenced by the sanitary condition of the milking area [6]. Maintaining the good sanitary condition of barns and parlours is an important task of internal biosecurity on dairy farms. Also, clean and dry bedding is important to reduce the growth and transfer of microorganisms to the exterior of the teats and from here to the teat canal and milk [6, 7]. Therefore, identifying the practices that expose the teat end to wet and muddy pens, faeces, and dung and favour the growth and transfer of microorganisms will decrease the risk of occurrence of mastitis and milk contamination.

2.2 Cows

Cows are a major source of microorganisms that can infect the udder and contaminate the milk. All cows with mastitis must be identified before the spreading of microorganisms to other cows, and indicators of udder infection should be used in all dairy farms. Somatic cell count (SCC), which comprises leucocytes (75%), such as neutrophils, macrophages, lymphocytes, and erythrocytes, as well as epithelial cells (25%), is a good indicator of udder infection. When there is a bacterial infection, tissue damage, or stress, leucocytes grow and defend the body of the cow and combat pathogens. The quality of raw milk is negatively impacted by increased SCC in milk. However, low milk supply, changes to milk consistency, a decreased chance of appropriate milk processing, a lack of protein, and a significant risk for milk hygiene since it may potentially include pathogenic organisms are all characteristics of subclinical mastitis [8].

Even when the mammary gland is free of culturable pathogens, milk contains its own resident microbial community, the vast majority of which are not related to mastitis [9]. Falentin et al. discovered that 76 milk samples from healthy quarters included a high number of the *Clostridia* class, the *Bacteroidetes* phylum, and the *Bifidobacteriales* order [10]. Kuehn et al. investigated bacterial DNA diversity in 10 mastitic, culture-negative milk samples using pyrosequencing of bacterial 16S rRNA genes [11]. The microbiota of milk samples taken from healthy quarters of the same cows was also characterised in this study for comparative reasons. *Ralstonia*, *Pseudomonas*, *Sphingomonas*, *Stenotrophomonas*, *Psychrobacter*, *Bradyrhizobium*, *Corynebacterium*, *Pelomonas*, and *Staphylococcus* were the most prevalent genera. *Pseudomonas*, *Psychrobacter*, and *Ralstonia* genera were substantially greater in healthy samples than in mastitic samples [11]. Oikonomou et al. investigated the microbial diversity of 144 bovine milk samples collected from clinically unaffected quarters throughout a wide range of somatic cell count values [12]. *Fecalibacterium*, *Lachnospiraceae*, *Propionibacterium*, and *Aeribacillus* were found in all healthy quarter samples and might be regarded part of the milk core microbiota. *Bacteroides*, *Staphylococcus*, *Streptococcus*, *Anaerococcus*, *Lactobacillus*, *Porphyromonas*, *Comamonas*, *Fusobacterium*, and *Enterococcus* were among the other species discovered in majority of the milk samples with very low somatic cell counts. *Lactobacillus* and *Paenibacillus*, for example, have been linked to better udder quarters [12].

As a result of the research on raw milk microbial ecology, –omics methods are currently being applied to the sensu stricto milk microbiota of dairy ruminants. As a result, its effects on the physiology and health of the breastfeeding mother and her suckling progeny are becoming increasingly clear [13]. The discovery of a milk microbiome linked to desired production qualities such as high milk output and low SCC, as well as protection against infectious mammary infections, would be critical in reducing antibiotic use on dairy farms [14]. Furthermore, there is a rich array of bacteria in bovine milk that play critical roles in promoting gastrointestinal tract development and aiding immune function maturation in offspring [15]. Because the bovine milk microbiome promotes early-life gut development by boosting intestinal microbiota and immunological functions, removing this microbiota may do more harm than good in cow herd health management. Drinking water, milking equipment, bedding, skin, faeces, and the barn environment are all external microbial sources of bovine milk microorganisms [9, 15, 16]. Endogenous transfer routes include entero-mammary, rumen-mammary, and mammary resident microorganisms [15, 17, 18]. Therefore, the application of -omics sciences to the milk microbiota is anticipated to increase our understanding of open questions and challenges, including the aetiology and dynamics of sub-clinical and culture-negative mastitis, the effects of farming management choices on the health of the mammary gland and the offspring, the function of the intestine as a mastitis pathogen reservoir, and the dynamics of drooling in dairy ruminant farms [13].

A milking biosecurity plan must include the udder cleaning before milking (to increase the quality of cleaning, the udders of the lactating cows should be clipped). In the evaluation of milking biosecurity, it is important to know how the teats are cleaned before milking. The following ways of cleaning were described: pre-foaming, dry cleaning with separate towels, wet cleaning and drying afterward with separate towels, and wet cleaning but not drying afterward. Also, it is important to know if the foremilk is examined during fore-stripping if the teats are disinfected after the teat cups are removed, and if cows are kept upright for a period after milking. To prevent the spreading of mastitis, cows must be milked in a specific order: it is recommended that the cows with mastitis and/or a high SCC be milked last. Also, mastitis prevention can be done by culling chronically infected cows.

Periodical hygiene and udder health evaluations should be considered on dairy farms. To prevent the spreading of udder pathogenic bacteria (e.g., *Staphylococcus aureus*, *Mycoplasma* spp., *Corynebacterium bovis*, and *Streptococcus agalactiae*), it is required a regular bacterial examination of the udder of all cows (i.e., minimum once per year—mastitis control programs designed for systematic identification of the prevalent bacteria), regular cleaning of the udder of all cows before milking, removal of soil particles, bedding material, and manure from the udder and flanks, and a regular clipping of the tails of the lactating cows [19–23].

The studies of Zigo et al. revealed that the interplay of effects between methods of prevention that offer protection against the emergence of new infections and disease control methods, which significantly shorten the duration of the infection, leads to unique and continuous progress in the reduction of mastitis by the implementation of a mastitis control program. The incidence of clinical mastitis in the herd can be reduced to a minimum via constant observation of the hygiene practices in milking, treatment of dairy cows with efficient treatments for cows with clinical mastitis, and disposal of cows with the chronic form [24].

2.3 Milking personnel

The health experts involved with the dairy herd may not always be able to explain why preventive or treatment programs based on established risk factors for mastitis fail and the milk cow becomes infected [25]. Also, during milking, cooling, storage, and processing, sterile milk from the udder of a healthy cow becomes infected. A possible explanation is that not only cows, equipment, and milking routines are involved in milk contamination, and herd health plans designed for better milk quality must consider milking personnel as one of the factors [26]. Pathogens can be introduced directly into milk by milk-handling workers (milkers), especially if they are careless, ignorant, or deliberately negligent, Farm workers should be in good health and understand the value of hygiene because organisms can be present on the milker's hands, nasal cavities, mouth, skin, and gastrointestinal tract [4].

2.4 Milking equipment

In the management of dairy herd, milking is a fundamental component that can affect both dairy herd health and milk production [27]. Milking systems can be classified into conventional milking systems (CMS) and automatic milking systems (AMS). The type of milking system installed on a dairy farm is determined by several factors, such as the number of cows, the cost and quality of manual labour, the level of milk production, and the availability of spares and services.

In small dairy farms, the recommended CMS are trolley systems (one or two milking groups are housed on the trolley, transported inside the barn, and managed by one operator) and the bucket milking system (BMS). In BMS, one operator can control up to three milking buckets connected to the vacuum pipeline. A disadvantage of trolley systems and BMS is that the operator must transfer the milk from the buckets to the transport bulk tank and move it out of the stable. However, trolley systems and BMS are very useful for milking a fresh cow, a sick cow, or a cow separated for treatment. Among the advantages offered by BMS on a traditional small dairy farm are: obtaining clean, completely untouched, high-quality milk, increased farm profitability and productivity, reducing dependence on labour, the possibility of developing the farm, and increasing the size of the herd [28].

The biosecurity risk associated with milking in small dairy farms should be considered in the following tasks of the routine of trolley and bucket systems: (a) bucket or trolley positioning; (b) pre-dipping; (c) foremilk inspection; (d) pre-milking udder preparation; (e) teat cups attaching; (f) removing of the teat cups; (g) post-dipping; (h) emptying of bucket or container; and (i) relocation of trolley. For each task, the risks associated with udder infection (dairy herd health) and/or milk contamination (public health) must be considered in the farm's biosecurity plan.

On small and medium dairy farms, the recommended CMS are milk lines (two fixed pipes for milk and vacuum connected at a milk room and a machine room, located near the barn). The milk line system can be used with or without automatic cluster removal [28, 29]. The biosecurity risk associated with the milk line system should be considered in the following tasks: (a) milking group positioning; (b) pre-dipping; (c) foremilk inspection; (d) pre-milking udder preparation; (e) teat cups attaching; (f) teat cups removing; (g) post-dipping; and (h) group removal. As in the case of trolley and bucket systems, in farms with the milk line system, the biosecurity plan must consider the risk of infectious agents spreading during milking by analysing each task.

On large dairy farms, the recommended CMS are milk lines, parlours and AMS. The biosecurity risk on dairy farms with parlours should consider the following tasks of routine milking: (a) cow entry; (b) pre-dipping; (c) foremilk inspection; (d) pre-milking udder preparation; (e) teat cups attaching; (f) post-dipping; and (g) cow exit. Common types of milking systems used in dairy parlours are parallel, tandem, rotary, and robotic. Both CMS and AMS have benefits and disadvantages for milking biosecurity, and the conversion from conventional to automatic systems is not always accepted by farmers. The main benefits of AMS on a dairy farm are reduced labour (with the consequent reduction of the risk of introducing and disseminating pathogens through farm workers), a better social life for farmers, and improved milk yields due to more frequent milking [30]. Neijenhuis et al. showed that, in farms with automatic milking, the risk factors for mastitis are more or less comparable with those found in conventional milking [31]. However, the main physical performance indicators of CMS and AMS (e.g., milk production per cow, milk production per hectare, pasture grazed per hectare, or milk solids per full-time equivalent) are similar [32]. Unfortunately, high-technologized milking parlours come with some disadvantages, like periodic planned services and regular maintenance, changing of milking liners, and other milking system parts. In large dairy farms, inefficient or incomplete milking, poor milk quality, teat trauma, and mastitis infections are frequently associated with poorly serviced, maintained, and operated milking equipment.

In developing countries, the source of many microorganisms can be poorly cleaned and sanitised milking utensils [33], but this risk remains on all dairy farms where biosecurity measures are not respected or when malfunctions or accidents associated with milking equipment are not identified and reported immediately. Because milk is an excellent medium for the growth of a variety of bacteria, equipment and utensil surfaces with milk residues will facilitate the growth of numerous microorganisms, including pathogens [34]. Therefore, milking equipment should be made with easy-to-clean materials (e.g., stainless steel) and maintained in good condition (regular revisions) [4].

Milking equipment cleaning and sanitation are a mix of chemical, thermal, and physical procedures that require a short reaction time to be successful [25]. Pre-rinse, washing phase, and post-rinse are the three primary phases of an automated cleaning procedure. The pre-rinse phase is critical for removing the majority of milk remains. Alkaline or acid detergents should be used during the washing phase. Alkaline detergent aids in the removal of organic residues like milk protein and fat. Mineral deposits in water and milk are removed regularly using acid detergent [35, 36]. There are several milking machine cleaning solutions on the market, both caustics and acids, although sodium hypochlorite and sodium hydroxide are the most popular active components of caustic detergents, while phosphoric acid is the most popular acidic product. However, because milking machine distributors frequently stipulate which sanitizers are permitted, the usage of milking machine detergents is heavily impacted by the milking equipment [36].

2.5 Milking

In milking biosecurity, a good milking routine is very essential. In certain underdeveloped nations, most farmers do not wash their cows' udders before milking, therefore, milking practices should be modified to promote better sanitary conditions [37]. However, udder cleaning before milking should be viewed as a method of removing mud but not of removing germs from the cow's skin.

The milking routines used in European dairy farms with milking parlours should guarantee that the product obtained is of the greatest possible quality (e.g., hygienic removal of milk from the udder and prevention of mastitis), the work practices used are safe (e.g., milking operators should have a best practice milking course and use safe cow handling practices), and the time spent milking cows is used efficiently (e.g., milking row times of less than 9 minutes). An efficient milking routine has the following components: (1) parlour preparation, (2) row filling, (3) milking preparation, (4) batch preparation and milk let-down maximisation, (5) cluster attachment, (6) cluster removal, (7), teat disinfection, (8) row exit, and (9) parlour hose down [38].

The waiting time in the collecting yard before milking should be kept to a minimum in order to prevent cows from becoming anxious and dirty before being milked and to minimise the risk of foot injuries (e.g., solar ulcers). Parlour preparation should consider rinsing the collecting yard floor and walls, checking the availability of teat dip, and ensuring that the milking plant has been washed and is ready for milking. To avoid overstressing cattle during row filling, allow the cows to join the row without leaving the pit.

Farmers use a variety of methods for pre-milking udder preparation. A proper pre-milking cleanliness regimen can lower the cow infection ratio by lowering not just udder bacterial contamination from the environment, but also bacterial contamination from other diseased cows [39]. Disposable nitrile gloves (rinsed and disinfected routinely during the milking) and a clean parlour suit should be used to avoid the spread of mastitis and to keep the operator clean and free of discharges. Teats should also be washed (e.g., with a dry wipe), dried (e.g., with a paper towel), and fore milked 90 seconds before the cluster attaches (it aids in the early detection of mastitis and guarantees that optimal milk let-down occurs when the cluster is connected shortly thereafter) [38]. All pre-milking procedures, including wet washing and manual drying with paper towels, will result in the lowest bacterial levels [40, 41]. Udder dryness at the time of machine attachment is a critical part of pre-milking udder care [40]. Allowing a gap of about 90 seconds between batch preparation and cluster attachment enhances milking efficiency and maximises milk let-down. Preparing each cow reduces the amount of time spent walking up and down the parlour [38].

Many farmers now dip teats pre-milking with different disinfection solutions such as iodophor solution, iodine-based gel, sodium hypochlorite, dodecylbenzene sulfonic acid (DDBSA), chlorine, chlorhexidine, phenolics, and alcohol instead of washing and drying them [41–45]. Therefore, preventing mastitis begins with reducing the bacteria count in the teats before milking. Before cluster attachment, for efficient oxytocin release and milk ejection, 15 seconds of pre-stimulation followed by a brief latency interval should be performed [46]. The cluster attachment should be with the hand closest to the cow's exit side (typically the closest to the cow), keeping the pulse and milk tubes on the exit side of the cow and out of the way when going to the next group. In this way, handling time is reduced and the risk of contamination of the cluster is reduced. Manual cluster removal should be done with the hand that the milker intends to attach to the next cow. If cluster attachment and removal are automated, then removing the clusters should be done at the proper flow rate. Allowing the unit to become limp on the udder reduces the danger of an air blast happening after cluster removal, lowering the risk of mastitis and teat-end injury [38].

Post-milking teat disinfection aimed at getting total coverage of the teat will destroy the most germs and utilise an emollient-containing disinfectant to enhance teat condition. Iodine and lactic acid are commonly used in post-milking disinfection to lower the occurrence of clinical mastitis [38, 47]. After removing clusters and post-milking teat disinfection, the milker should open the row exit gate. This also ensures easy access to and exit from the milking parlour and reduces filling delays between rows. After the last row of cows has exited the parlour, a prompt washdown and plant sterilisation should be performed to reduce faecal contamination of the milking areas [38].

2.6 Water

Bacteria-contaminated water can also raise milk bacterial levels, compromising food safety [43, 48]. In farms with a low biosecurity plan, water can serve as a primary source of contamination, mainly if it is obtained from an inadequate open water supply [37, 49]. Unsafe drinking water is a serious health issue in many poor nations, contributing to high morbidity and death rates [48]. Therefore, a steady supply of clean, cold water is required for the production of high-quality milk. Water used in cleaning and rinsing milk equipment and handling containers must be of the same safety and purity as drinking water. Milking tools that have not been fully cleaned and sanitised may be the source of numerous germs that turn high-grade milk into an unsatisfactory product [4, 50].

3. Milking parlour hygiene

3.1 The hygiene of milking parlour

Hygienic situations vary depending on the production system, appropriate practices, level of awareness, and availability of resources. To prevent pathogen proliferation in dairy farms, milking parlour surfaces should be cleaned and disinfected twice daily. The cow milking parlour is a separate location for collecting the dairy farm's final output—raw milk. The milking parlour's design, location, milking equipment, proper hygiene, and working practices must all work together to reduce the danger of milk contamination through the environment and milk contact surfaces. The milking area must be located and built in accordance with food safety regulations to maintain sanitary conditions during milking.

To limit the possibility of contamination, the milking parlour should be designed with adequate sanitation and a high degree of pest control (flies, birds, or other animals). There must be appropriate separation from the premises where the cows are housed. Cleaning the milking parlour is part of the dairy farm's normal program, and all surfaces and component elements of this must be washable and resistant to the action of disinfection agents in order to achieve an ideal degree of cleanliness. The surfaces of the doors, walls, and floor must be easy to clean, smooth, and composed of waterproof materials. To minimise contamination during milking, the floor must allow for appropriate drainage of the remaining water.

After each milking, all components of the milking station should be cleaned. A supply of potable water must be provided to guarantee adequate water pressure and temperature. The circulation of air and ventilation velocity inside the milking parlour is critical for avoiding contamination from moisture [51].

3.2 Milking equipment hygiene

The design of milking equipment must minimise the danger of milk contamination. The milk contact surfaces must be simple to clean, disinfect, and keep in good shape. For these goals, they must all be made of washable, non-toxic materials that are resistant to the action of disinfection agents [52]. Cleaning the milking equipment is done to avoid microbial contamination of the milk. The cleaning technique must be conducted after each milking to remove milk residues, organic residues (fat, protein, lactose), and mineral residues (milk stone, limescale) from surfaces, as well as killing or decreasing bacteria to an appropriate level (disinfection) [53]. To prevent milk residue from drying on equipment, the entire milking system must be cleaned off at the end of the milking stage. Water washing readily removes the majority of milk residue (95%). Organic milk traces (fats, proteins) and/or the milk stone might remain on the cleaned surface, potentially protecting microorganisms by forming a biofilm [54].

Many factors impact cleaning performance, including water hardness, water temperature, flow velocity of water, chemical component concentration, and contact duration. Water is a critical component in the cleaning of milking equipment since it is part of the cleaning solution, has a mechanical impact on the surfaces to be cleaned, and ensures the ejection of milk residues (discarded water). The mineral composition of the water (calcium, magnesium, iron, and so on) as well as other dissolved components affect the performance of cleaning and disinfection chemicals. Water hardness is an essential quality indicator of water in terms of cleaning action performance. Water hardness affects the cleaning ability of detergent agents and allows the production of biofilm on surfaces if it is too high. The biofilm provides excellent protection for thermoduric bacteria.

Cleaning agents are divided into two types: detergents and disinfectants. To save time, the detergent can be used with disinfectants such as chlorinated alkaline or peracetic acid. Cleaning agents are alkaline chemicals that dissolve organic milk residues and acid compounds that dissolve mineral milk residues to prevent calcium and magnesium cations from creating milk stone. Enzymatic detergents are non-alkaline detergents that contain enzymes that work on a particular substrate (organic milk residues). Typically, alkaline detergent is used as the primary detergent. Once a week, acid detergents might be used to eliminate milk stone. They must all be authorised for usage in the food sector [54].

Disinfectants are used to destroy germs, reducing the number of bacteria on milk contact surfaces to a negligible level. Cleaning chemicals may contain adjuvants such as sequestering and chelating compounds, which interact with various water minerals such as calcium and magnesium to prevent them from precipitating out of solution and creating milk stones on the contact surfaces of milking equipment. Wetting agents, or surfactants, are another effective adjuvant because they reduce the surface tension of the cleaning solution and allow contact with the milk leftovers to remove them.

In dairy practice, the combination cleaning chemical (detergent-disinfectant) is most commonly used. The cleaning of milking equipment is divided into three stages: prewashing, main wash, and final rinse. All direct contact surfaces (hoses and other elements of the milking equipment) must be prewashed (first rinsed) with fresh warm water (40–maximum 50°C) immediately after milking to eliminate the majority of milk residues. This cycle should be repeated until the wasted water has a clear appearance (physical cleanliness). The temperature of the water is the most critical aspect at this step to avoid the solidification of milk lipids on the surfaces (when the temperature is too low) or the "baking" of milk protein (when the temperature is too high).

The primary wash (circulation cleaning) is a thermal and chemical treatment designed to remove milk residues (mineral and organic residues) and decrease bacteria on pipes to negligible levels. It uses hot water and a cleaning solution.

The capacity of milk residues to dissolve and emulsify is affected by the temperature of the water. Detergents are more easily dissolved in warm water. To avoid the production of fatty coatings (when the temperature is below 40°C) and protein denaturation (when the temperature is too high), the temperature of the washing water should be 65–80°C at the start and 40–50°C at the end of this stage.

The alkaline washing cycle is used to remove organic milk residues (e.g., lipids and proteins) that are breaking down and floating in the wash solution. The effectiveness is proportional to the alkalinity of the cleaning solution, which must be between 250 and 500 ppm (e.g., Na_2O) [54].

On the other side, the acid washing cycle is performed to eliminate the mineral residues from the washing water and from the contact surface of the milking equipment with acid solutions (pH 3–3.5). Efficiency, as the frequency of the acid washing is in direct relation to the quality of the water. For this step, warm or lukewarm water could be used. The water rinse step shall be set between chemical cleaning cycles to remove residual cleaning chemicals and to avoid the mixing of alkaline and acidic substances. The last rinse is used to eliminate any cleaning solution residues (chemical cleanliness). It employs cold or lukewarm water for this purpose. The wasted water is drinkable until the end. "The last glass of (post-rinse) water coming out of your system should be drinkable!" [55].

Two additional stages, such as a rinse stage after washing with detergent and subsequently a disinfection circulation, must be set up for cleaning systems that use detergents and disinfectants separately. A final rinse will be performed after the disinfection. As part of appropriate hygiene practice, milking equipment is disinfected or sanitised to reduce germs to negligible levels or eradicate microorganisms that may persist on surfaces (the microbiological hazards). This is a preventative strategy to avoid milk contamination during the subsequent milking stage, and it is designed to be used on clean surfaces (no milk residues). Because of their effectiveness against Gram-positive and Gram-negative bacteria, as well as some viruses, chlorine compounds such as calcium hypochlorite and sodium hypochlorite are the most commonly used. Because active chlorine interacts with and is inactivated by milk organic residues, disinfection with chlorine compounds must be undertaken on clean surfaces. Except for the cleaning procedure, which employs a combination of cleaning product detergent-disinfectant, chlorine disinfection is frequently set up as a pre-milking phase [56].

Acid disinfectants, such as acid-anionic surfactants (often an anionic detergent and phosphoric acid) and organic acids (e.g., propionic acid, acetic acid, and lactic acid), work as both milk stone removers and disinfectant agents. Potential organic milk residues on surfaces do not affect their functions. There are several advantages to utilising acid cleaning chemicals, including the fact that they are most effective on stainless steel surfaces, are heat stable up to 100°C, and have an efficient action against a wide variety of vegetative Gram-negative and Gram-positive bacteria.

Cleaning systems might be operated manually or mechanically. In a manual cleaning system, the operator is responsible for the whole operation and management of the cleaning process, including the preparation of the cleaning solution, controlling the volumes of hot and cold water, and determining the duration of the circulation cleaning phase. In comparison, the cleaning unit ensures the majority of activities in an autonomous cleaning system, with just a tiny portion of these still requiring manual intervention. Clean-in-place (CIP) is a typical way of cleaning pipes and other elements of milking equipment, including the bulk tank. A suitable cleaning system maintenance program is established regularly, at least twice a year, to guarantee effective cleaning with an impact on the sanitary quality of milk.

After the final rinse, the whole milking pipeline, including the supply line, is drained. Gravity generally provides drainage since milking tubes are typically too short to have a sufficient slope. Gravity action may be inadequate to provide an effective drain in rotating systems such as round-the-shed milking parlours; thus, a supplemental means, such as sponges pushed down the pipes to drive the remaining water out of the system, is required. The sponges are injected manually or mechanically into the system and must be withdrawn manually at the end. The final element of the cleaning program for the round-the-shed milking parlour is drying, which is accomplished by blowing air through the whole pipeline system [56].

The milk cooling tank is cleaned using the same techniques as the milking equipment: prerinse, primary cleaning, final rinse, and disinfection. The greatest danger is psychrophilic bacteria, which can grow quickly at temperatures ranging from 1 to 10°C). The milk transporter is used for lukewarm water rinses that occur shortly after the milk is removed; rinse water temperature should range from 30 to 50°C. During the primary wash cycle (thermal and chemical processes), the cleaning solution temperature is set at 50°C. The final washing of the cooling tank with cold or lukewarm water is critical to ensuring the elimination of all residues. It is finished with acidified water, which neutralises and eliminates detergent and mineral residues. Disinfection is done soon before the next milking, allowing enough time for disinfection agents to drain before filling. Depending on their size, cooling tanks can be cleaned manually, with CIP or mechanical methods, or both.

The robotic milking systems (the stationary or mobile AMS) are designed apart from the cow house area to safeguard the entire installation from contamination. The AMS platform is constructed, maintained, and cleaned in such a way that waste accumulation is minimised. It has a solid floor and a plumbing drain trap connected to a wastewater system for this function.

All utilities are required to ensure good manufacturing and hygienic practices, such as proper lighting to perform equipment checks and maintenance, sources of hot and cold potable water with an optimum flow rate, proper ventilation, and effective pest control to prevent birds from nesting. All direct milk contact surfaces and AMS exterior surfaces must be cleanable. The AMS platform is outfitted with essential facilities and supplies for hand hygiene (washing, disinfection, and drying), as well as cleaning utensils and the surrounding space (hoses). Because an automatic milking system replaces the milker, it includes automated cleaning and milking gear. The automated milking system is thoroughly cleaned (including disinfection) at least three times every day, at around 8-hour intervals. All contact surfaces of the automatic milking system, including the cleaning agents, are cleaned in accordance with the manufacturer's cleaning guidelines. As a precaution, it established a brief rinse cycle between two consecutive milkings to limit the danger of infection transmission from cow to cow [57]. It initiates a water washing cycle for all milk contact surfaces of AMS and hoses that have been inactive for more than 40–50 minutes, followed by emptying.

The AMS buffer tank is cleaned promptly after each emptying, and it must be cleaned or sanitised again within a few hours of the next milk pickup. The outer surfaces of AMS, including the floor and milking platform, are cleaned regularly and maintained clean. The milking equipment maintenance program is critical to ensuring the proper operation of AMS, including cleaning procedures. A proper maintenance program must always be followed to ensure the proper operation of an AMS [53].

3.3 The efficiency of the cleaning process

Cleaning milking equipment might be regarded as a significant element in terms of raw milk hygiene [58]. Given that milking is a regular habit (at least twice per day), visual inspection is the measure used during and after cleaning to ensure that the overall efficiency of the cleaning and associated routines is at a satisfactory level. The monitoring technique comprises measuring water temperature, assessing water flow, and evaluating surface cleanliness. The temperature of the washing water may be verified using a conventional thermometer (for manual cleaning) or a temperature sensor and a display for the cleaning machine. Only transparent portions of the milking equipment, such as milk metres, clusters, pipes, and the receiver, are used for visually inspecting water flow.

The visual assessment of surface cleanliness, as a preoperational control before milking, detects leftover residues in the case of insufficient washing, or it may reveal a probable fault of the machine. In most circumstances, insufficient cleaning may be feasible in certain areas designated as difficult-to-clean. The sanitary quality of milk can be impacted by improperly cleaned milking equipment, inadequate milk chilling, inappropriate teat and udder washing, the health of the udder, and so on. As a result, other methods of assessing cleanliness must be established, such as the Standard Plate Count (SPC), the colony count of mesophilic bacteria growing under aerobic conditions, which is used to determine the bacterial quality of bulk tank milk but is not as useful in identifying the source of bacterial contamination. A more specific parameter, which is directly related to cleaning efficacy, is the identification of the presence of thermoduric bacteria on the surface of milk equipment, particularly in areas with a cracked surface (old, cracked rubber), and the survival of milk pasteurisation if cleaning is inadequate [58, 59].

It has been demonstrated that failing to maintain sufficient hygiene contributes to milk contamination with undesired or pathogenic microorganisms as well as chemical or physical dangers. Poor cleanliness contributes to more germs, causing milk to deteriorate quicker. To keep raw milk fresher for longer, practice proper cleanliness both while milking and when handling the milk afterward [60]. The efficiency of the cleaning process can be influenced by the maintenance of equipment, milking technique, and milking management.

3.3.1 Maintenance of equipment

A static (without milking cows) and dynamic (while milking cows) test should be done on the milking equipment once a year. A dynamic test assesses the milking process by machine and farmer, and hence only this test provides a comprehensive picture of the milking process's functionality. The frequency with which teat cup liners should be replaced varies according to the type: rubber and silicone teat cup liners should be replaced after 2500 and 10,000 milkings, respectively [61].

3.3.2 Milking technique

Before milking, the farmer should wash, sanitise, and disinfect his or her hands and/or use gloves. Teats should be thoroughly cleansed with a clean towel before milking. Teats should be dried following disinfection if they are also cleaned before milking. After removing the teat cups, the teats should be sanitised. A visual examination of the foremilk is recommended. After milking, the milking equipment and parlour should be cleaned. Milking equipment should be sterilised between cows, ideally using steam or water heated to 75°C. After milking, the milking equipment and parlour should be cleaned [61].

3.3.3 Milking management

Cows should be milked in the most comfortable and hygienic conditions possible. Rubber mats or a similar surface should be included in resting places when cows are kept in stables with slatted floors to prevent cows from lying down on the slatted flooring. Cows with chronic subclinical mastitis should be removed from the herd, and a microbiological investigation of all cows' udders should be performed at least once a year. All lactating cows should have their flanks, udders, and tails clipped. To reduce stress, the cows' hierarchical order should be followed, although unwell cows in lactation (e.g., mastitis) should be milked last. The teat holes stay open for 30 to 60 minutes after milking. It is thus advisable to leave the cows standing for at least 30 minutes after milking. This can be aided by supplying new feed at the feeding fence [61].

4. Conclusions

Whether the cows are milked by hand, mechanically, or automated, biosecurity and good hygiene are necessary. Milking biosecurity should be a part of a farm biosecurity program designed to increase herd health, welfare, and production. The milking biosecurity plan must be monitored and assessed in order to identify all risks of udder and milk contamination. The milking biosecurity plan should be decision-focused and tailored to the individual circumstances of each dairy farm. Many of the difficulties experienced can be avoided or mitigated with the assistance of veterinary services. Also, staff and visitors should be taught about biosecurity precautions used on the farm. Milking parlour hygiene necessitates that the milker's hands and clothes be clean, he or she be in excellent health, and the milking machine and milk storage equipment, such as milk churns, be maintained clean and in good working condition.

Conflict of interest

The authors declare no conflict of interest.

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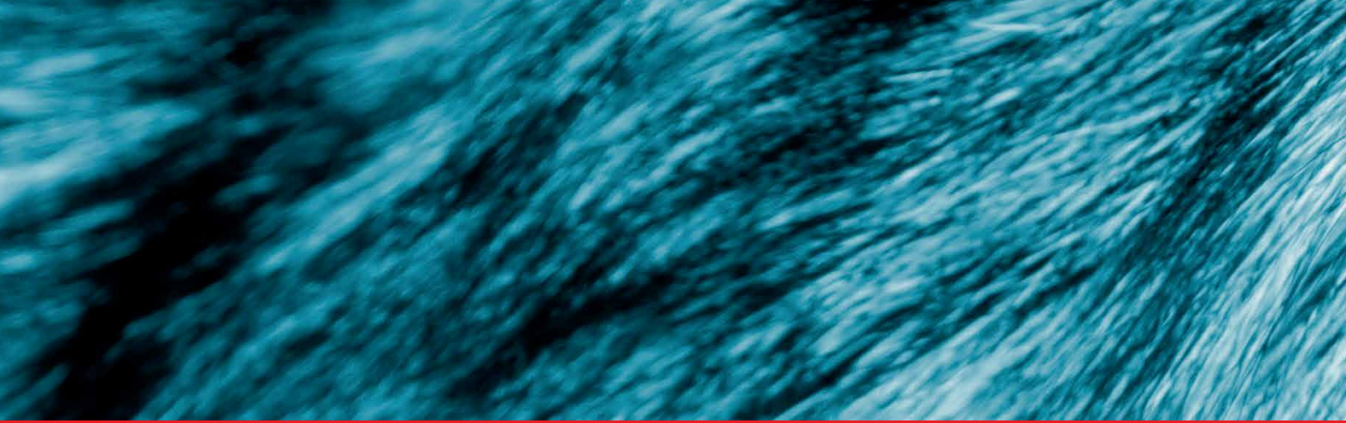

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Bovine mastitis is a problem well known to anyone who works within or is involved with the dairy cattle industry. Progress in the understanding and approaches to treatment and control is ongoing and rapid. Following the most recent literature is difficult for busy professionals. Therefore, a useful, comprehensive summary is always welcome. This book provides that summary to all farmers, veterinarians, students, scientists, and anyone else who is interested to learn about or update their knowledge of the treatment and control of bovine mastitis. Chapters address such topics as traditional and alternative treatment strategies, traditional and novel control strategies, and biosecurity and food safety. They also discuss the two main forms of mastitis: clinical and subclinical bovine mastitis. The book provides literature reviews and novel findings related to bovine mastitis, providing readers with a comprehensive resource.

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