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Advances in Controlling Bacterial Mastitis in Dairy Cows

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MASTITIS is the most world frequent and costly disease in dairy cattle. Although several infectious agents such as bacteria, *Mycoplasma*, and fungi have been associated to mastitis, bacteria are the primary important cause. Improved milking hygiene, implementation of post-milking teat disinfection, and maintenance of milking machinery are all general methods to avoid new cases of mastitis, but antibiotics are the primary treatment to control mastitis infection. Antibiotic residue and antimicrobial resistance, as well as the effect of antibiotic abuse on public health, have led to several limits on uncontrolled antibiotic therapy in the dairy industry across the world. New therapeutic techniques to substitute antibiotics in the control of mastitis have been examined by researchers. These efforts, which were aided by the significant advancements in nanotechnology, stem cell assays, molecular biology tools, and genomics, led to the creation of innovative mastitis treatment and control methods. In the present study, unique mastitis management approaches are discussed, including the breeding of mastitis-resistant dairy cows, the creation of novel diagnostic and treatment techniques, and the use of contemporary mastitis vaccines, cow drying protocols, teat sterilisation, housing, and nutrition. Besides, it include the use of nanotechnology, stem cell technologies, immunotherapy, traditional herbal medicine plants, nutraceuticals, probiotics, bacteriocins, bacteriophages, and acoustic pulse technology.

Keywords: Mastitis, control, antibiotic resistance, bacteria.

Introduction

The most frequent and economically significant infectious disease of dairy farms in the world is bovine mastitis (BM), it increases treatment, labor, and culling costs, resulting in significant financial losses [1]. Worldwide, there are an estimated 1.489 million cows, with more than 40% of them suffering from various types of mastitis [2]. Globally, the entire cost of failure due to BM is estimated to be 147$ per cow per year on average. Culling and milk production losses account for 11 - 18 % per cow annually [3]. The annual losses caused by decreased milk supply and quality in USA, which have a significant influence on animal husbandry, rising veterinarian care costs, and rising farm management costs, are in the billions of dollars [4]. BM is indeed important because of the disease's greater incidence worldwide and accompanying output losses [5], and it also has an impact on the quality of milk, which has effects that extend further than the dairy farm. BM was principally a problem of dairy farmers and processors, it has become an issue for consumers and society due to concerns about antibiotic residues, antimicrobial resistance, milk quality, and animal welfare [6].

Classically, BM is the mammary gland inflammation more commonly but not exclusively produced by an infection caused by bacteria, which is characterized by transport of leukocytes and serum proteins from the bloodstream to the udder [7]. In BM, there are 137 different

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pathogenic bacteria, with the *Staphylococcus aureus (S. aureus)*, *Escherichia coli (E. coli)*, and *Streptococcus spp*. being the most prevalent pathogens [8, 9 & 10]. Individual animals in herds and countries have different mastitis pathogen strain distribution [11]. Subclinical mastitis (SCM), clinical mastitis (CM), and chronic mastitis are the three types of BM based on the degree of inflammation. Visible abnormalities such as red and swollen udders, as well as fever and milk seems to be watery, with flakes and clots indicate CM [12]. Depending on the severity of the inflammation, CM is classified as per-acute, acute, or sub-acute [13], sometimes it can be fatal in severe cases [14]. Conversely, SCM has no apparent abnormalities in the udder or milk, while milk production declines as the somatic cell count (SCC) rises [12, 15]. Although it is difficult to assess the financial loss caused by SCM, experts believe that it causes more financial losses in the herd than in clinical cases [16]. In contrast, chronic mastitis is an inflammatory infection that lasts for months and occasionally experiences clinical flare-ups. It can include brief episodes of inflammation and abnormal milk, but is considered chronic if it lasts for longer than two months [15]. Because infections and the host immune system have the ability to co-evolve in order to recognize, respond to, and adapt to each other, the association between mastitis pathogens and the immune system of the host is complicated. In order to live, microbial pathogens have developed a variety of techniques to alter and avoid host defenses. Conversely, the host immune system is extremely adaptable with a wide range of defense mechanisms. Nonetheless, it is commonly known that individual susceptibility to the same microbial disease varies within a species. The characteristic host's genetic make-up, including innate and adaptive immune responses, particularly acquired immunological memory, as well as the microbial pathogen's characteristics, are all factors to consider to determine the heterogeneity in host–pathogen interactions [7]. The high incidence and low treatment rate of this very costly and possibly fatal disease are alarming for both the dairy industry and policymakers. Preventive measures, immunization programs and treatment of infected animals are all required for the control of bacterial infections in BM. A number of treatment and prevention regimens have been established, with varying successful rates [17].

Despite the fact that antibiotics are still the most common treatment for BM, worries concerning the rise of antibiotic-resistant bacteria are growing. This review provides an overview of preventive and control methods, with a particular focus on modern rapid and accurate tools for detecting and diagnosing bacteria that cause BM, dairy herd management, housing, nutrition, milking, drying protocols, and mastitis vaccinations. Likewise will highlight potential antibiotic-free therapy options including; natural chemicals produced from herbal or animals, probiotics, nanoparticles, stem cells therapy and acoustic pulse technology.

Bacterial Mastitis in Dairy Cows

Bovine Mastitis is triggered by a variety of factors including pathogen, host and environment [18].

Mastitis causing bacteria and global incidence

Raw bovine milk is considered as a vehicle for many infectious bacterial diseases such as Bovine Tuberculosis, Brucellosis, Leptospirosis, Q. fever and *Mycoplasma* infection. In addition to, Infectious bacteria associated with mastitis including *Staphylococcus aureus, coagulase negative staphylococci, Streptococcus dysgalactiae., Streptococcus agalactiae, Corynebacteriun pyogenes, Streptococcus uberis*, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Streptococcus pyogenes, Clostridium perfringens* and *Salmonella species* were also included [19]. Mastitis organisms differ from one area to another. Bacterial intramammary infection (IMI) is considered to be the most common cause of BM, these bacterial infections are divided into two categories based on the source: contagious and environmental [20]. Mastitis that can be transmitted from cow to cow, particularly during milking, is known as contagious mastitis. Contagious pathogens including *S. aureus* and *Streptococcus agalactiae (Strep. agalactiae)*, as well as less common species like *Mycoplasma bovis (M. bovis)* and *Corynebacterium*, colonize and grow into the teat canal on the cow's udder and teat skin [13]. Environmental infections, unlike contagious pathogens, survive in the herd's bedding and housing rather than on the cow's udder or teat skin. Opportunistic infections are the best way to describe them that seek out opportunities to infect. Environmental pathogens such as *E. coli* or *Strep. uberis* infiltrate then grow in the udder of cows, elicit an immune response from the host, and are quickly eradicated [21] . *Streptococcus spp*. (e.g. *Strep. uberis*), *coliforms species* (e.g. *E. coli, Klebsiella spp., Enterobacter* *spp.*), *Pseudomonas spp.*, and others have been documented to induce environmental mastitis [22]. Isolates of *E. coli* from animals with mastitis were identified and characterized based on their morphology, biochemically identified, and confirmed by molecular assays [23]. The first systematic review and meta-analysis of SCM and CM incidence for the entire world was conducted in a previous study, which used online and offline databases. The prevalence studies of SCM and CM published between 1967 and 2019 (a total of 222 and 150 studies on SCM and CM throughout the world, respectively) were collated, analyzed, and a meta-analysis was carried out. Worldwide, the prevalence of SCM and CM was 42% and 15%, respectively, SCM was more common in North America and CM in Europe [24].

Mastitis-resistant cow genotype selection and optimal age

Genetic factors and dairy cow breeding affect mastitis susceptibility and resistance. In comparison to medium-yielding cattle, high-yielding cattle, particularly Holstein-Friesian cattle, appear to be more genetically susceptible to mastitis [25]. Jersey cattle, for example, were shown to have a lower mastitis rate than Holstein-Friesian cattle [26]. Furthermore, due to immunoincompetence, multiparous cows are more susceptible to IMI than primiparous cows [25]. Direct and indirect genetic selection strategies are now being used to improve mastitis resistance. Most countries breed for mastitis resistance indirectly through SCC, The Nordic countries are the only exception, for more than 35 years, they've been selecting for disease resistance [27]. Systematic national genetic and genomic examinations for CM have been launched in France [28] and Canada [29]. Certain genetic mutations/genotypes had gotten a lot of interest because of their possible role in mastitis resistance. The lactoferrin BTA22 gene, which encodes a protein with bacteriostatic properties in the mammary glands, can often be used to explain the significance of these genes in mastitis resistance. BTA5 is a gene that produces lysozymes, which are enzymes that break down the bacterial cell wall and eliminate bacteria from the udder and BoLA, MHC, and BTA23 all play a part in mastitis resistance by controlling the acquired immune response to udder pathogens. [30]. "*Superior fathers sire superior sons*" this is why Sperm-competition Success (SCS) is considered so vital for evolutionary progress [31]. Mastitis resistance has a limited heritability, with values ranging from 0.02 to 0.10. SCS has a greater heritability of around 0.17 and is genetically associated with mastitis. As a result, it is used as a substitute characteristic to breed for mastitis resistance. Divergent selection experiments based on SCS have been conducted in cattle with the purpose of developing lines of animals who are resistant to IMI [7]. Likewise, cow susceptibility to mastitis was discovered to be influenced by teat shape, size and teat to floor distance, which determined by genetic predisposing variables that reflect the herd's genetic diversity. After calving, cows with big funnel-shaped teats, pendular-shaped udders, and blind quarters are more likely to develop SCM [32]. Because the teat canal becomes broader or partially open all of the time as a result of repetitive milking, aged cows are more susceptible to infections [13]. Furthermore, the permeability of the mammary epithelium of older cows regularly increase, owing to the irreversible damage induced by earlier inflammations [33].

Nutrition management

During lactation, dairy cattle have an increased requirement for energy and nutrients for the production of colostrum and milk. Cattle have negative energy balance when feed intake does not match lactation demands [13]. Negative energy balance is linked to trace elements deficiency in the diet (e.g., selenium, iron, zinc, copper, chromium and cobalt), and vitamins (e.g., A, C, E and β-carotene), amino acids (e.g., L-histidine and lysine),all these components contribute to cellular and humoral immunosuppression, increasing infection susceptibility during lactation [34]. Minerals are structural components of the animal and are important in nerve signaling, muscle contraction and correct keratosis as cofactors of numerous enzymes. Their shortages cause immune cells to become less active or teat innate defense mechanisms to malfunction, which promotes the development of BM [35].

Environmental factors and herd management practices

Environmental factors and herd management practices have a significant impact on animal health and welfare. Mastitis occurrence and severity can be reduced by keeping the herd clean and comfortable [36].

Housing

The design of a dairy farm building can assist prevent diseases from spreading to dairy cows, periparturient animals, and neonates [37]. High stocking density, polluted floors, moist bedding, poor ventilation, and a hot and humid climate might enhance mastitis pathogen growth and increased cow exposure, resulting in increased mastitis occurrence [12]. Every farm should have an isolation building (quarantine facility) where newly purchased cows' condition can be checked before they are introduced into the herd. The farm must have a biosecurity strategy that includes routine building maintenance procedures to minimise cattle interaction with wild animals and feed contamination from bird droppings or stray animal waste [38].

Five-point & Ten-point plan

BM can be prevented in two ways; reducing the bacteria present at the teat's end and improving the cow's tolerance to these bacteria [6]. Since the 1960s, the National Institute for Research in Dairying (NIRD) has used a "Five-point plan" to eliminate contagious mastitis infections. The "five points" are: (1) clinical case identification and treatment; (2) post-milking teat disinfection; (3) Dry Cow Therapy (DCT); (4) chronic cases culling; (5) routine maintenance of milking machine [21,39]. According to Hillerton & Booth, [39], since the 1960s, the relative distribution of mastitis pathogens has shifted in response to the program's efficacy, because environmental pathogens have become more important in the last few decades. Unfortunately, "five-point plan" is ineffective against environmental pathogens and must be used in conjunction with other relevant mastitis infection management measures. At the turn of the century; the National Mastitis Council (NMC) expanded the "Five-point plan" toward "Ten-point plan" with 73 sub-points. The "ten points are": (1) Setting udder health goals; (2) Maintaining 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 CM during lactation; (7) Effective dry cow management including implementation of appropriate DCT; (8) Maintaining of biosecurity measures to control contagious pathogens; (9) Regular monitoring of udder health status (e.g., monitoring of SCC, udder cleanliness, teat end hyperkeratosis, and so on); (10) Periodic review of the herd's mastitis control program.

Proper milking hygiene and good milking practices

Because harmful bacteria enter the mammary gland through the teat canal, the higher the bacterial load at the teat end, the more likely an infection will arise, highlighting the significance of a clean, dry environment and udder hygiene during milking. Proper milking hygiene and good milking practices stated by DAIReXNEThttps://dairy-cattle.extension.org (DAIRY-CATTLE, n.d.) and include the following components; (1) Milk in a clean and stress-free environment; (2) Check foremilk and udder for signs of CM; (3) Reduce water use in the milking parlour; (4) Wash teats with warm sanitizing solution, if required; (5) Apply premilking teat disinfection; (6) Dry teats thoroughly 30 to 45 seconds after applying premilking teat disinfectant; (7) Attach teat cups within one minute of cleaning; (8) Maintain a stable vacuum at the claw during peak milk flow; (9) Avoid squawking or slipping of teat cup liners during milking; (10) Adjust milking units as needed; (11) Before removing the machine, turn off the vacuum; (12) Immediately administer postmilking teat disinfection.

A-Traditional disinfectants

Providing an effective and safe disinfectant to the dairy industry is seen as a critical step in improving farm hygiene and biosecurity. The most common disinfectants used in dairy farms are Formaldehyde, Aldehydes, Sodium Hydroxide (Lye), Chlorine chloramines, Iodophors, Quaternary ammoniums, and Phenols. They are effective against Gram positive bacteria, Gram negative bacteria, and fungi. Because of the possibility of residues linked with chemical disinfectant in milk, such as Trichloromethane (TCM) and Chlorate associated with chlorine disinfectant, concerns over food safety are now more acute when using chlorine to clean milking equipment [40].

B-Modern approach to disinfection

The chemical-free disinfection used by dairy industry has raised a lot of concerns; "Biocides" like peracetic acid (PAA) and ozone (O_3) are now the best effective and harmless biocides routinely utilized disinfectant in the food industry to reduce spoilage and pathogenic microorganisms. In a previous study Megahed *et al.* [41] characterized and compared the disinfection capacity of sodium hypochlorite (NaOCl), PAA and aqueous-O3 against severely contaminated diverse surfaces with *Salmonella Typhimurium* from cow manure. They found that PAA and aqueous-O3 can reduce the bacterial population by 50% and 30%, respectively, compared to NaOCl, with posterior probability of 97 percent and 90 %, respectively. A previous study found that using guava leaf methanol extracts as a pre-milking teat disinfection reduced teat-end bacterial burdens considerably as compared to regular udder sanitization [42]. Similarly, according to Yu et al. [43], lactic acid bacteria (LAB) changed the bacterial composition of the teat, reducing the amount of mastitis-causing bacteria in particular, LAB disinfection could replace chemical disinfection as pre- and post-milking teat disinfectants. Many studies have found indications that Nanoparticles (NPs) could be ideal candidates for animal growth boosters, antimicrobials, and promising future cleaning agents [35].

Artificial intelligence and communication technology

Artificial Intelligence (AI) is "software's technique to handle problems and carry out tasks that would otherwise require human intelligence". Evolving AI and communication technology has brought developing technological advancements for the dairy industry by sustaining the health, physical, and physiological conditions of dairy cows in many ways: (1) Act as a daily information source for breeders, including helpful hints for mastitis management techniques; (2) Monitor animal health and general disease diagnosis, particularly for mastitis diagnosis and estrus detection; (3) Used as nano-biosensors with smart phone apps for quick recognition of infections in milk samples; (4) Sensors are linked to apps that provide real-time reports on udder health and milk composition on farms having an automated milking system, allowing for the detection of CM of machine control failure; (5) Collect epidemiological data on dairy animals in the area for epidemiological investigations [44, 45 & 46]. Data gathered during standard recording methods, as well as access to big datasets, have revealed the possibility of using trained machine learning algorithms to forecast cow udder health. Eight machine learning techniques were examined by Bobbo *et al.* [47] to forecast cows' udder health status using SCC (Linear Discriminant Analysis, Generalized Linear Model with logit link function, Nave Bayes, Classification and Regression Trees, k-Nearest Neighbors, Support Vector Machines, Random Forest, and Neural Network). All methods have an accuracy rate of at least 75%. On the basis of the cow's milk qualities reported on a prior test day, the Neural Network, Random Forest, and linear approaches fared the best in forecasting udder health classes at a specific test day, according to a number of metrics. These findings suggest that machine learning algorithms could be used by farmers as a tool to enhance decision-making. Monitoring processes would be enhanced by machine learning analysis, which would also help farmers identify the cows that will have a high somatic cell count on the next test day.

Recent, fast and precise diagnostic tools

Early diagnosis of new infections, establishing disease-free status, or determining the incidence of a certain disease in a herd are all reasons for developing surveillance programs [38]. Specificity, sensitivity, cost, time to provide results, and compatibility for large-scale milk sampling are all criteria that influence the suitability of a diagnostic detection method for routine diagnosis [48]. Strategies for SCM detection might be helpful in reducing the likelihood of infection propagation. Traditional field tests like somatic cell count, California mastitis test, electrical conductivity, and pH tests have many benefits since they are practical, affordable, and useful for tracking the health of cattle's udders [19].

The novel diagnostic tools in the last decades include:

Cell Counting

A-Direct Somatic Cell Count (SCC)

SCC was the gold standard for diagnosing SCM for decades, and it is an essential criterion for the dairy business since it influences the price of milk paid to the farmer. A SCC ≥ 200,000 cells/ml is the most commonly used cutoff value for defining SCM. The losses in milk production in dairy cows increase with the increase of SCC/ml [48]. SCC can be measured in two ways; directly and indirectly. Direct methods use either portable automatic cell counters or laboratory-based automatic counters [49]. SCCs are not always linked to udder infection, and they can be influenced by a variety of circumstances. There are many Factors increase/decrease Somatic Cell Count. The factors which may increase milk SCC are udder infection/injury, unhygienic/incomplete milking, unhygienic cow surrounding, increase in stage of lactation, change in housing/feed, an increase in parity, keeping sick cows with healthy cows, hot humid climate, and any other stresses. Meanwhile, the factors which decrease milk SCC are healthy udder/animal/milker, hygienic milking practices, clean animal surroundings, regular udder screening, feeding antioxidants, treating infected cows/culling chronic mastitis cows, post milking teat dipping, awareness about mastitis/proper dry cow therapy and selection against mastitis [50].

B-California Mastitis Test (CMT)

CMT is a widely used indirect method of determining SCC. It's a detergent made out of Alkyl Aryl Sulfonate (3%) and NaOH (1.5 %) added to a high-cell-count milk sample, which promotes cell lysis, nucleic acid release, and the development of a "gel-like" matrix. CMT's main advantages are that it is rapid, inexpensive and easy, and it may be used as a "cow-side" test [51 , 52].

C-Wisconsin Mastitis Test (WMT)

WMT is a laboratory test used to diagnose mastitis in milk. The scores can be used to estimate the amount of somatic cells on average. The same reagent is used as in the CMT, but the reaction is determined by gel level in a tube rather than estimated, yielding a more precise result than the CMT [48].

Milk electrical conductivity (EC)

Mastitis causes variations in ion concentrations due to a rise in sodium, potassium, calcium, magnesium and chloride levels, resulting in changes in milk's EC. Because of the significant number of false positives, although EC change can help detect mastitis, it is not a reliable or sensitive sign for making a definitive diagnosis on its own. EC testing is currently the most widely used automated mastitis diagnosis approach in milking robots [53].

Mastitis biomarkers

Invading bacteria come into contact with somatic cells in milk and lining epithelial cells triggers the innate immune system in the teat cistern, which is followed by an increase in cytokine production, which attracts neutrophils to the inflammation site. Some of these cytokines induce the acute-phase response, which is characterized by fever, leukocyte mobilization, and increased synthesis of acute-phase proteins (APP). APPs are now widely used as diagnostic biomarkers in veterinary medicine. In ruminants, the main APPs are serum amyloid A (SAA) and haptoglobin (Hp). This milk protein's concentration has been considered as a sensitive indication of mastitis in dairy cows. There is substantial evidence that measuring milk amyloid A (MAA) in milk during SCM is important, this might possibly be a rapid and accurate indicator of inflammation. The low or even undetectable level of MAA in healthy cows' milk, which is unaffected by anything other than mastitis, gives MAA an advantage over other SCM diagnostic indicators [54].

Advanced molecular biological diagnostic assays

Variety of molecular typing methods have been utilized to investigate the epidemiology of BM. Comparative typing approaches based on (1) electrophoretic banding patterns, (2) library typing methods based on the sequence of chosen genes, (3) virulence gene arrays, and (4) whole genome sequencing projects, are only a few examples [55]. However, as polymerase chain reaction (PCR) and PCR-based techniques improved in sensitivity and specificity, they became the new gold standard [56]. The invention of PCR and PCR variations made it possible to diagnose diseases quickly, cheaply, accurately, and sensitively, as well as to determine the responsible genotype and the existence of antibiotic resistance genes [57 & 58]. Despite the fact that up to 30% of CM samples do not grow in bacterial culture, PCR analysis can detect growth-inhibited bacteria, lowering the probability of false-negative results [59]. Since it requires a compact portable cycler, the progress of a novel promising version of the PCR specifically; Recombinase Polymerase Amplification (RPA) and its variants, Multiplex RPA and on-chip RPA, facilitated field molecular diagnosis of mastitis in situ for the first time. Unlike PCR, in RPA cycler programs, the samples do not need to be cooled or heated, and the test can be done in the field. Other molecular techniques, such as new-generation sequencers or loop mediated isothermal amplification (LAMP), which is a fast, efficient, and cost-effective method, are now accessible for normal commercial diagnostic purposes [56]. Also, detection of some circulating endogenous non-coding micro RNA (miRNA) is an enhanced method for detecting udder invading infections and diagnosing mastitis early [57].

Nanotechnology in the diagnosis of mastitis

Nanotechnology's rapid advancement has resulted in enhanced diagnostic assays for the early and accurate diagnosis of mastitis. A unique class of analytical methods for the diagnosis of mastitis is "nano-biosensors". The use of it is one of these main diagnostic assays. Biosensors are devices featuring bioreceptors particular for the antigen or molecule being studied, as well as a physical nanotransducer (sensor). These sensors use electric signals to report the presence of particular biological molecules [60]. Nanotechnology, for example, enabled the creation of a real-time mastitis sensing diagnostic based on the detection of hepatically generated acute-phase proteins (e.g., haptoglobin). The diagnostic immunoassay is a chemiluminescence-coupled magnetic nanoparticles (NPs) opto-chemical technique. The bioassay may detect haptoglobins (a mastitis biomarker) in mastitic milk samples extremely early and with a high sensitivity level [61].

Vaccination

Vaccine development against common udder pathogens has progressed in recent decades. In dairy herds, both commercial vaccines and herd-specific autovaccines (Local strains obtained from cows infected with mastitis in the herd are locally prepared and subsequently applied to the whole herd) are commonly used [25]. Vaccines against *S. aureus* and *Strep. agalactiae* contained either the entire organism (cellular lysates, inactive, and attenuated vaccines) or subunits (toxins, surface proteins, and polysaccharides) such as clumping factor A DNA vaccines, live attenuated *S. aureusaroA* gene vaccines, capsular polysaccharide (CPS) protein conjugate vaccines, and recombinant *S. aureus* mutant enterotoxin type C vaccines [11 & 1]. Vaccines could also be categorized as monovalent or polyvalent depending on how many pathogens they contained [25 & 30]. Commercial vaccines like "Somato-Staph/Lysigin™" vaccine which is a polyvalent whole cell vaccine that comprises five phage kinds of culture lysates and "MASTIVAC-1™" against *S. aureus*, which is comprised of three different field strains of *S. aureus*, demonstrated a significant increase in serum immunoglobulin and a reduction in milk SCC, as well as promising outcomes in field studies [11]. Vaccines against coliform mastitis, "Mastiguard and J Vac™", "J-5 Bacterin™" which is made out of the J5 mutant *E. coli* strain, are commercially available. "Startvac ™ (Hipra)" is a vaccine that protects against coliform bacteria, coagulase-negative staphylococci, and *S. aureus*. Under field conditions, these vaccines resulted in a significant reduction in clinical mastitis cases [62 & 63]. Vaccination should be paired with other control strategies such as clean milking, antibiotic therapy, diseased cow culling, and so on to reduce the incidence and duration of BM cases [25]. Ghobrial *et al.* [64] investigated the efficacy of vaccines and antibiotics in controlling *S. aureus* mastitis in Egyptian dairy cattle and discovered that a combination of *S. aureus* bacterin (Lysigin) and an extended antibiotic (Marbocyl 10%) was effective in eliminating 53.8 % of BM caused by *S. aureus*. Also, Kotb *et al.* (2021) [1] developed and analyzed locally manufactured inactivated *S. aureus* vaccines using several adjuvants "Montanide ISA-206", "Montanide ISA-70", and "alum hydroxide" gel. The Montanide ISA-206 and ISA-70 vaccines were found to be safer and more efficient than the alum adjuvant vaccine, stimulating the humeral immune response in vaccinated heifers, resulting in a high level of serum antibodies which result in reducing the incidence and severity of clinical cases of mastitis. As previously stated, mastitis is caused by a variety of bacterial infections, different bacterial strains differ not only in their site of infection in the mammary gland, but also in their virulence features and immunogenic capacities; thus, the lack of vaccination efficacy in some circumstances attributed to the multi-etiological character of BM [65]. Within a herd and within a single cow, there might be many strains, thus finding a vaccination that can protect against a wide variety of strains is critical [66].

Antibiotic therapy

Despite numerous trials to replace antibiotics in the treatment of BM, antibiotics are still the primary treatment. Penicillin, ampicillin, tetracycline, gentamycin, ceftiofur, novobiocin, cloxacillin and other antibiotics can be administered through intramammary infusions, intramuscular injections, or intravenous injections to treat mastitis [67, 68].

Dry Cow Therapy (DCT)

All dairy animals must go through a non-lactating phase 6-10 weeks before calving (typically once a year). The cow is vulnerable to develop intramammary infections at this time, especially shortly after 'drying off,' or cessation of milking, and around calving [20,69]. Dry cow therapy is one of the most effective treatments for mastitis control and prevention. The main benefit of DCT is that it allows bacteria to be treated and eliminates the need to discard milk. Furthermore, antibiotics can persist in the udder for up to 20-70 days at high concentrations necessary to execute infectious bacteria, depending on the antibiotic formulation used. Furthermore, prolonged antibiotic exposure speeds up the healing of intramammary bacterial diseases. However, in the case of invading pathogens forming biofilms, this can be inhibited [70]. Teat sealants (bismuth subnitrite) are increasingly being used as part of dry cow therapy or as an organic alternative to intramammary antibiotic infusions at the start of the dry period. The cows were inspected for any signs of mastitis before being dried off; the antibiotic was then injected into the cow's udder through the teat canal, followed by the application of teat sealant, which replicates the keratin plug and provides a physical barrier to bacterial invasion and milk loss, shortly after the last milking [67,71]**.** Its ingredients have been improved in several ways to improve its effectiveness to prevent BM. The teat bio-sealant made with *Weissellacibaria*, a probiotic LAB, exhibited antimicrobial activity against a variety of bacterial infections [72].

Milking Cow Therapy

Antibiotic residues in milk are a major issue whenever mastitis is diagnosed during lactation. When a cow has an active mastitis infection, the first step is to segregate the cow from the rest of the herd, and totally milk it out to remove bacteria, milk clots, debris, and any toxins generated by the bacterium. The antibiotic is subsequently administered by intramammary infusion to ensure that it reaches the udder as well as the blood circulation [68]. As well, it is important to consider where the infecting organisms have the largest concentration, since this can assist determine the chance of successful intramammary treatment, the length of treatment required, and whether systemic antibiotics are required: (1) Milk and lining epithelial cells e.g. *Strep. agalactiae, Strep. dysgalactiae* and *coagulase-negative staphylococci* (*CNS)*; (2) Deep gland tissue e.g. *S. aureus, Strep. uberis,* and *Acinetobacter pyogenes*; (3) Infection of other body organs at the same time e.g. *coliforms* [67].

Because getting the cow back to milking is the primary priority, long-acting antibiotics are not ideal for mastitis diagnosed during lactation. As a result, it is critical to obtain an understanding of the pathogen present in order to identify an effective remedy for infected cows. Antibiotic susceptibility of bacterial isolates usually carried out on "Mueller-Hinton agar" using the disk diffusion technique against different commercially available antibiotics; e.g. ciprofloxacin, norfloxacin, ofloxacin, levofloxacin, amoxicillin-clavulanic acid, amoxicillin, penicillin, neomycin, cloxacillin, oxacillin, gentamycin, cefquinome, and rifampicin [58]. Mastitis with lameness, calving, and metritis are the most common disorders and diseases that cause pain in dairy cows. Inflammation and pain associated with mastitis are reduced by taking nonsteroidal anti-inflammatory drugs (NSAIDs). Cows given antibiotics and NSAIDs in the mammary gland exhibited lower cell counts, higher cure rates, and higher fertility than cows given antibiotics alone [73]. The overuse and misuse of antibiotics in the treatment of BM have caused some problems in the dairy sector, notwithstanding the high cost. In general, owing to the possibility of allergies and drug resistance brought on by antibiotic residues, antibiotic-treated milk must be thrown after a withholding time because it cannot be consumed by the customer [66].

Modern trends for non-antibiotic therapy

Mastitis cannot be completely eradicated from a herd, although it can be reduced to a minimum. While antibiotics remain the primary treatment method, their efficiency is limited, and the growth of antibiotic-resistant pathogen strains has become a major obstacle in antibiotic therapy [74 , 75]. Furthermore, as public health concerns about antibiotic resistance grow, the milk industry is being pressured to minimize the use of antimicrobial medications. As a result, it is necessary to look for alternatives to antibiotic therapy, particularly those originating from natural sources such as plants and animals [76 , 77].

Plant extracts

Plants have long been a valuable source of components in traditional medicine, and researchers are increasingly interested in using them to treat cow mastitis. Plant-derived chemicals have the advantage over antibiotics in that they do not cause resistance even after prolonged exposure, the low toxicity of plant-derived chemicals is another advantage [78]. Depending on the type of formulation, herbal therapy could be administrated with different routes. The most widely used procedures are topical, oral, and intramammary [72]. However, with oral administration of plant extracts, the recommended doses for mastitis treatment are extremely large, as a result, the expense of therapy will be costly and labor-intensivem [77]. *Ocimumbasilicum* and *Parapiptadeniarigida* were the two plant species employed in the intramammary approach in bovine mastitis [79]. To prevent digestion and breakdown in the gastrointestinal tract, plant extract might be transported on lipid-based nanocarriers (nanophytosomes), or it could be combined with antibiotics to achieve synergistic effects [30]. Several plants with antibacterial, anti-inflammatory, antioxidant or immunomodulatory properties are being studied, with encouraging results. Sedky *et al,* (2022) [80] recorded that, due to their chemical composition, antioxidant capability, and efficacy against Gram-positive and Gram-negative mastitis-associated bacteria, *Balanitesaegyptiaca* fruits and *Curcuma longa* powder extracts can be utilized as an alternative to traditional mastitis therapy. The combination of *Artesunate* (extraction from the Chinese herb *Artemisia Annua*) and antibiotics could be a promising candidate for treating methicillin-resistant *S. aureus* isolated from BM [81]. Because of its ability to build biofilms, *Strep. epidermidis* is one of the most common causes of BM. In vitro investigations on the effect of *Oxytropisglabra* (a Chinese herbal) indicated possible inhibitory mechanisms that might be further investigated in the development of innovative medications to treat biofilm-associated infections [82].

Probiotics

According to the Agriculture Organization of the United Nations/ World Health Organization (FAO/ WHO), the word "probiotic" means "for life," and it is now used to refer to bacteria that have health benefits for humans and animals [83]. LAB of the *Lactobacillus* and *Bifidobacterium* genera are the most used and studied bacteria. Actually, intramammary probiotic infusion has emerged as a promising option for controlling and treating BM, particularly during the dry-off season. Probiotics could be used at drying-off, during the dry period, around parturition, or while nursing, depending on the lactation cycle. It could be given orally, intramammary through the teat canal, or at the teat apex by teat dipping, depending on the application site or route [84]. LAB present in honey has been discovered to be a novel source of antibacterial agent. On tested BM isolates, a mixture of 13 species of LAB previously isolated from honey, from the genera *Lactobacillus* and *Bifidobacterium*, showed antibacterial activity [85]. Lactococcus lactis subp. lactis CRL 1655 and Lactococcus perolens CRL 1724, both derived from cow's milk, have also been demonstrated to inhibit BM bacteria. Because these species can adhere to the teat canal, they may play a role in preventing BM during the dry period [86]. In addition to milk, LAB isolated from bovine mammary microbiome has udder-friendly characteristics. In bovine mammary epithelial cells (bMEC) triggered by *E. coli*, nine of the LAB species identified showed an anti-inflammatory response. Furthermore, both *L. brevis 1595* and *1597*, as well as *L. plantarum 1610*, had strong colonization capabilities towards bMEC, indicating that they could be ideal candidates to compete with pathogens in mammary gland colonization [87].

Lactoferrin

Lactoferrin is an iron-chelating glycoprotein that can be present in milk, colostrum, and other exocrine secretions including saliva and tears [55]. It plays a vital function in the innate immune system's psonisation of microorganisms for phagocytosis as an immunomodulator [89]. It was found to have antibacterial properties against *S. aureus, Strep. Agalactiae*, *E. coli*, and *Pseudomonas aeruginosa (P. aeruginosa)* owing to its iron-chelating capability, which inhibits biofilm formation by iron sequestration [88]. Some researchers believe that intact lactoferrin alone lacks sufficient antibacterial action, thus they've looked into different ways to boost its effectiveness. The cure rates were improved with combination of lactoferrin with different antibiotics [90]. Lactoferrin combined with Penicillin G gave higher cure rate (33. 3%) against bovine mastitis caused by β –lactam resistant *S. aureus* bacteria comparing with Penicillin G alone (12.5%) [91]. Combination of Lactoferrin with Cefazoline gave higher cure rate (81%) for drying cows' clinical mastitis infected by *S. aureus* [92]. Moreover, in another study, the combination of Lactoferrin with Novobiocin increased the antibacterial activity against *E. coli* [93].

Bacteriocins

Bacteriocins, which are antimicrobial peptides produced by bacteria, have been proposed as a possible BM therapy [94]. Nisin, a lantibiotic generated by *L. lactis* and having 34-amino acid residues, is one of the most researched bacteriocins against BM. Nisin forms a compound with the bacterial cell wall, limiting the production of the cell wall, the complex then forms a pore in the bacterial membrane after aggregating and incorporating into the cell wall [95]. Mastitis has been treated with medicinal formulations using bacteriocins generated by *Strep. equinus HC5*. Pilot investigations revealed that they can limit the growth of more than 80% of the tested staphylococcal and streptococcal strains [96]. Furthermore, combining nisin with dioctadecyldimethylammonium bromide NPs enhanced *Staphylococci* susceptibility to nisin [97]. Because nisin resistance has been documented in *Staphylococci*, novel bacteriocins, either by itself or combined with nisin, are very necessary [98]. "Wipe Out", a teat wipe, contains nisin as an active ingredient [99].

Bacteriophages

Bacteriophages are bacteria-specific viruses that are safe for humans, animals or plants; as a result, bacteriophage and their derivatives (i.e., endolysin, exolysin, and depolymerase) are being hailed as valuable antimicrobial alternatives with the capability to minimize antibiotic use in agricultural food production while also improving the productivity of animals and protecting the environment. Furthermore, when engineered with genes of interest, bacteriophages have been shown to be suitable for vaccination, making them beneficial against bacterial and viral infections [100]. One of the most significant benefits of utilizing bacteriophages and phage endolysins are their capacity to eradicate antibiotic-resistant infections [101]. Guo et al. [102] isolated three lytic phages; they were identified from sewage of a dairy farm and named vBEcoM SYGD1 (SYGD1), vBEcoP SYGE1 (SYGE1), and vBEcoM SYGMH1 (SYGMH1). The three phages have a wide host range and a high bacteriolytic effectiveness against *E. coli* strains from various sources. However, because of their narrow host range and inability to reproduce in raw milk inside the udder, their efficacy in mastitis treatment has been varied [103].

Immunotherapy

Immunotherapy is an alternative treatment for BM that is based on immunology. By immunising chickens with formaldehyde-killed bacteria in a long-term immunisation response, *E. coli* and *S. aureus* specific IgY can be generated. Immunoglobulin extracted from yolks increased phagocytic activity against mastitis-causing bacteria, suggesting that it could be used as a therapeutic agent in the treatment of BM [104]. Leitner et al., [105] compared treatments with sulfadiazine + trimethoprim, procaine penicillin + streptomycin or NSAID with "Y-complex" (microbead that contains specific anti-mastitis bacteria antibodies as well as a phagocytosis enhancer) on mastitic cows naturally infected with *E. coli*, *Strep. dysgalactiae*, or coagulase negative staphylococci (CNS). In terms of eradicating bacteria, the Y-complex was safe and effective, and it could be used to treat mastitis in the future, although, Y-complex-treated cows rejected considerably less milk than antibiotic-treated cows. Although trials using pathogen-specific antibodies to treat mastitis yielded fewer encouraging results, this could be due to the antibodies' limited therapeutic spectrum, which makes this approach impractical for field use. A new Ab against *S. aureus* (scFv-Fc Ab) was reported by Wang et al. [106], the scFv Ab was fused to the Fc fragment of a bovine IgG1 Ab to create the scFv-Fc Ab. Bovine scFvs-Fc Abs were successfully expressed in *E. coli* cells using goat-anti-bovine IgG (Fc) Ab coupled with horseradish peroxidase. Purified bovine scFvs-Fc Abs displayed good binding activity to *S. aureus* and efficiently suppressed bacterial growth in culture media, and bovine scFvs-Fc Abs increased *S. aureus* phagocytosis by neutrophils isolated from peripheral blood in a dose-dependent manner. The total effective percentage of bovine scFvs-Fc Abs for treating S. aureus-induced bovine mastitis was 82 %. These new bovine scFvs-Fc Abs could be effective as therapeutic candidates for *S. aureus*-induced cattle mastitis prevention and therapy.

Nanotechnology

Nanoparticles are frequently employed in human and veterinary medicine for therapeutic purposes in addition to diagnosis. Therapeutic active materials are nanosized to make them more active and soluble, allowing them to pass through various body's different physiological barriers. These preparations become more effective, have a longer release time, and are less likely to cause side effects. The use of NPs as a suitable alternative to antibiotics is recommended by meta-analysis and collective data. They have broad-spectrum antibacterial activity and do not promote bacterial resistance development [107]. Chitin is the most prevalent aminopolysaccharide polymer found in nature, and it is the building block that provides crustaceans, insects, and fungus their exoskeletons and cell walls their strength [108]. Chitosan is a polysaccharide derived from chitin that has been shown to have antibacterial activity against fungus and bacteria, it constrain *Staphylococcus spp.* from causing BM by inhibiting their development and biofilm formation [109]. Chitosan NPs have better antibacterial and anti-biofilm properties than natural chitosan [110]. Pilot studies on the potential of metal NPs such as silver, copper, or silver–copper NPs were successful, and the first commercial products are now available [107]. Commercial NPs are also utilized to boost animal productivity, immunological response, and reproductive health. Nano-ZnO, for example, can improve udder health in dairy animals with SCM and lower the milk SCC. Their strong capacity for eradicating mastitis-inducing bacteria, as well as their safety for mammalian cells and low cost, make them attractive candidates for mastitis therapy [111].

Green gold nanoparticles (Au NPs) were successfully synthesized using aqueous leaf extract of *herba-alba* and *M. alba* as bioreducing agents. The biosynthesized Au NPs showed antibacterial activity against multidrug-resistant (MDR) pathogenic *E. coli* and *Salmonella* species [23]. Because the use of NPs in the treatment of mastitis is not yet fully established as a viable alternative to the traditional antibiotic technique in the field, many researchers opt for a transition period in which they utilize a combination of NPs and antibiotics [112]. Pilot trials revealed that the combination NP-Antibiotic mix was extremely effective in treating mastitis. The therapeutic efficiency of intramammary infusions of nanosilver cream with ceftiofur is up to 93.33 percent in some circumstances. When given 2 weeks before calving, the formula could also be utilized as a protective against mastitis [113].

Mammary stem cells

Stem cells are classically defined as "Cells capable of self-renewal through replication and differentiation into specific lineages". Self-renewal, differentiation of progenies, expression of specific molecular marker(s), and clonal testing are all characteristics of progenitor cells. Aside from that, stem cells have the unique ability to act as an internal repair mechanism, dividing virtually indefinitely to restore other cells on condition that the animal is a live [17]. The adult mammary epithelium is made up of two types of cells: an internal layer of luminal epithelial cells that create milk during lactation and an external layer of myoepithelial cells that push milk through the ductal network to the teat cistern. The principal stem cell types are epithelium and myoepithelial stem cells, which have significant therapeutic value in mammary gland tissue because they can aid in the development of the mammary glands vascular network (endothelial and smooth muscle cells) [114-116]. The adoption of mesenchymal stem cells (MSc) rather than epithelial and myoepithelial progenitor cells, on the other hand, is promising. Recent studies have found that mesenchymal stem cells derived from bovine adipose tissue have an additional therapeutic benefit, MSc could be used to treat mastitis in cattle by promoting wound healing and reducing microbial infection [117]. The antinflammatory, antibacterial, and immunomodulatory activities of MSc are linked to their activation of innate immunity [118], MSc boost the expression of the bBD4A and NK1 genes, which code for antibacterial peptides (APs) such as cathelicidin, indolamine 2,3-dioxygenase, and hepcidin, all of which have broad-spectrum bacteriostatic and bactericidal capabilities [119]. Furthermore, Sharma et al. [120] looked into the ability to insert transgenes into bovine mammary stem cells and get them to produce desired proteins. The goal of the research was to clone the bovine *lactoferricin* (*LFcinB*) gene into the *PiggyBac* Transposon vector, examine its expression in bovine mammary epithelial stem cells (*bMESCs*), and test the antibacterial properties of recombinant *LFcinB* against BM causing bacteria *S. aureus* and *E. coli*. The evaluation of antimicrobial activity revealed solid evidence of significant antibacterial activity. The possibilities of bovine mammary stem cell therapy offer significant potential for tissue regeneration that can potentially replace/repair diseased and damaged tissue in the udder with minimal risk of rejection and side effects by differentiating into epithelial, myoepithelial and/or cuboidal/columnar cells [17].

Acoustic Pulse Technology (APT)

APT has been successfully utilised to treat inflammatory diseases in people for more than 20 years; it now aims to develop the optimal solutions for BM. The biological effect of low-intensity APT has been used to develop a new treatment option for BM in dairy cows, this treatment affects cells by mechano-transduction, promoting and modifying the creation of new arterioles, and enhancing blood supply and oxygenation, which together assist in faster healing and consequence in anti-inflammatory benefits [121 , 122]. APT is a short positive pressure pulse (1–3 µs) followed by a negative pressure pulse, resulting in the generation of microbubbles and cavitation that disappear in a few hundred microseconds [123 & 124]. A total of 400 acoustic pulses on a frequency of 2.7 Hz (~2.5 min/treatment) were delivered during an APT therapy session. The maximum depth of penetration into the affected gland was 350 mm, with a tissue volume of 2.6 L shielded [124]. To treat mammary infections in dairy cows, the European Union (EU) funded the APT project (April 2020 - June 2022) under INDUSTRIAL LEADERSHIP - Innovation with a total cost of €3,207,685(https://cordis.europa.eu/project/id/946330) to develop innovative non-invasive; antibiotics free therapies based on acoustic pulse technology. The APT-X device created by (Armenta Ltd.) was designed exclusively for the treatment of dairy cows. It is completely safe for the animal, has no influence on the milking practice, and produces no wasted milk. On commercial dairy farms, treatment of CM and SCM with the APT-X has been shown to achieve in >70% recovery, reduced culling by >70%, and enhanced average milk yield [125].

Conclusions

Mastitis is a multifaceted condition with three major risk factors: the causal agent, host resistance and environmental factors, all of which must be taken into account when developing prevention and control strategy. Effective mastitis control strategies emphasize prevention over therapy. Animal biosecurity is the sum of all activities performed by an entity to prevent disease pathogens from entering a certain area. The advancement of molecular biological diagnostic assays has made it a new gold standard for early mastitis diagnosis and pathogen identification. Its potential for use in control measures is also promising. For enhancing farm cleanliness and biosecurity instead of chemical disinfectant "Biocides" like ozone "O_3", peracetic acid, guava leaf extracts, lactic acid bacteria should be used consistently as an alternative disinfectant because of their minimal residues and high reactivity. Antibiotic therapy is still an important part of mastitis control regimens today, they are frequently used with other medicines, yet their effectiveness is still insufficient. As a result, new therapeutic alternatives must be sought, a wide range of natural compounds derived from plants, animals, and microbes have been studied and found to be effective in the treatment of BM. Because several strains can survive in a herd and even inside a single cow, designing a vaccination program that protects against a broad variety of strains is desired, and vaccination must be used in conjunction with other control methods. Nanotechnology, stem cells, immunotherapy, natural products and acoustic pulse technology, among other advanced biological and physical techniques must be used in dairy farms as alternatives to antibiotic therapy, but, prior to commercial applications, field research should be taken into consideration to ensure the effectiveness of alternative medicine.

Conflict of interest

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الحديث فى مكافحة التهاب الضرع البكتيرى فى الأبقار الحلابة (بحث مرجعي)

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التهاب الضرع هو المرض الأكثر شيوعًا والأكثر تكلفة في العالم الذى يصيب قطيع الأبقار الحلابة. على الرغم من أن العديد من العوامل المعدية مثل البكتيريا والميكوبلازما والفطريات قد ارتبطت بالتهاب الضرع، فإن البكتيريا هي السبب الرئيسي للإصابة. يُعد تحسين نظافة المحلب، و تطهير الحلمة بعد الحلب، وصيانة آلات الحلب كلها طُرقًا عامة لتجنب حالات التهاب الضرع الجديدة، ولكن تظل المضادات الحيوية هي العلاج الأساسي المُستخدم حتى الأن للسيطرة على عدوى التهاب الضرع. وقد أدى وجود بقايا المضادات الحيوية ومقاومة مضادات الميكروبات، فضلاً عن تأثير إساءة استخدام المضادات الحيوية على الصحة العامة، إلى العديد من القيود على العلاج الغير منضبط بالمضادات الحيوية في مزارع الألبان في جميع أنحاء العالم. و قد تم البحث عن تقنيات علاجية جديدة لتحل محل المضادات الحيوية في علاج التهاب الضرع من قبل الباحثين. أدت هذه الجهود، التي ساعدتها التطورات الكبيرة في استخدام تقنية النانو، و تقنية الخلايا الجذعية، وأدوات البيولوجيا الجزيئية، وعلم الجينوم الى ابتكار طرق حديثة لعلاج التهاب الضرع والسيطرة عليه. و تناقش الدراسة المرجعية الحالية مفاهيم جديدة للتحكم في التهاب الضرع مثل تربية أبقار الألبان المقاومة لالتهاب الضرع وراثياً، وتطوير إجراءات تشخيصية وعلاجية جديدة، واستخدام لقاحات حديثة لمقاومة الإصابة بالتهاب الضرع، وتطبيق بروتوكولات تجفيف الأبقار، وتطهير الحلمات، و توفير السكن المناسب، و توفيروسائل الإتصال الحديثة، والتغذية المتوازنة المناسبة لمقاومة المرض. ولتطبيق هذه المفاهيم تم مناقشة الحديث فى استخدام تقنية النانو، وتقنيات الخلايا الجذعية، و الأعشاب الطبية، و تأثير المغذيات، وبكتريوسينات البروبيوتيك، و العاثيات (الفيروسات آكلة البكتريا)، وتكنولوجيا النبض الصوتي كبدائل حديثة و فعالة للمضادات الحيوية من اجل السيطرة على عدوى التهاب الضرع البكتيرى.

الكلمات الدالة: التهاب الضرع ، السيطرة ، مقاومة المضادات الحيوية ، البكتريا.