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BACTERIOCINS – EXPLORING ALTERNATIVES TO ANTIBIOTICS IN MASTITIS TREATMENT

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ABSTRACT

Mastitis is considered to be the most costly disease affecting the dairy industry. Management strategies involve the extensive use of antibiotics to treat and prevent this disease. Prophylactic dosages of antibiotics used in mastitis control programmes could select for strains with resistance to antibiotics. In addition, a strong drive towards reducing antibiotic residues in animal food products has lead to research in finding alternative antimicrobial agents.

In this review we have focus on the pathogenesis of the mastitis in dairy cows, existing antibiotic treatments and possible alternative for application of bacteriocins from lactic acid bacteria in the treatment and prevention of this disease.

Key words: mastitis, antibiotic, milk, bacteriocin, food safety

MASTITIS

The general health and well being of individuals depends largely on meeting basic nutritional needs. Milk and fermented milk products such as cheese, cultured milks and yoghurt have formed an important part of daily nutrition, and the variety of products produced from milk has increased dramatically over the years, as modern food processing technologies have improved. An increase in global population coupled with the increasing demands for milk as an economic food and as an industrial raw food product has necessitated an increase in production by dairy farmers.

Current statistics indicate that the annual milk production in South Africa has increased steadily over the last 20 years from approximately 1700 million litres in 1985 to an estimated 3400 million litres in 2009. Consumption of dairy products has also increased at similar levels with a sharper increase in recent years, due primarily to a larger personal income base for individuals (46).

In a commercial milking environment, dairy cattle need to be in perfect physical condition to maintain a high level of milk production. The risk of lesions and infections that develop in modern dairy farming has consequently increased. Low milk production has been attributed to a large extent to the control of diseases in dairy cattle, of which mastitis accounts for the largest economic losses on dairy farms in many countries in the world, including the USA, United Kingdom, Europe, Australia and South Africa (29, 63).

Improving udder health and decreasing the incidence of udder infection and inflammation in dairy herds, will result in increased milk production as huge losses are directly or

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indirectly incurred through loss of milk during treatment periods, culling of cows and death of clinically infected cattle. Mastitis control programmes addressing various aspects of dairy farming such as feeding practices, animal husbandry, hygiene and general health care can contribute towards reducing the incidence of udder infections. Treating infection with antimicrobials can, in conjunction with good farming practices, assist in this endeavour to eliminate, or at least decrease, the incidence of mastitis infection within a dairy herd.

"Mastitis" describes an inflammatory reaction in the mammary gland. The term comes from the Greek derived word elements *masto-* referring to the mammary gland and *-itis* meaning – "inflammation" (6). Although "mastitis" could technically be used to describe any udder injury that may result in inflammation, it is generally accepted that the causative agents for the inflammatory reaction are microorganisms that have gained entry into the teat canal and mammary tissue (65). The extent of the infection that occurs as microorganisms multiply and proliferate within the mammary tissue determines the type of mastitis affecting the cow udder.

Mastitis-causing pathogens

The main etiological agents responsible for mastitis infections can be divided into different groups of organisms depending on the source of the organism involved. These include contagious pathogens, environmental bacteria, opportunistic bacteria and other organisms that less frequently cause mastitis less frequently (65).

Contagious organisms

Contagious microorganisms are usually found on the udder or teat surface of infected cows and are the primary source of infection between uninfected and infected udder quarters, usually during milking. The organisms that fit into this category include: *Staphylococcus aureus* (coagulase-positive staphylococci), *Streptococcus agalactiae* and the less common sources of infection caused by *Corynebacterium bovis* and *Mycoplasma bovis* (65, 67).

Environmental organisms

Environmental pathogens are found in the immediate surroundings of the cow, such as the sawdust and bedding of housed cows, the manure of cattle and the soil. Bacteria include streptococcal strains other than *S. agalactiae,* such as *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Streptoccous bavis*, *Enterococcus faecium* and *Enterococcus faecalis* and coliforms such as *Escherichia coli*, *Klebsiella pneumonia* and *Enterobacter aerogenes* (67,79). Mastitis caused by environmental organisms is essentially opportunistic in nature and becomes established if the immune system of the host is compromised or if sanitation and hygiene is not adequately practiced (80).

Opportunistic organisms

Opportunistic pathogens result in mild forms of mastitis and include coagulase-negative staphylococci. The coagulase test correlates well with pathogenicity and strains that are coagulase-negative are generally regarded as non-pathogenic (67). These staphylococci occur commensally and may be isolated from milk but usually illicit a minor immune response in cattle and infections caused are slight. They include *S. epidermidis*, *S. saprophyticus* (23,67), *S. chromogenes* (20) and *S. simulans* (23).

Other organisms

Many other bacteria and even yeasts may be responsible for causing mastitis, but are less common and occur if conditions in the environment change to increase exposure to these organisms. A condition known as "summer mastitis" occurs mostly in European countries in the summer months when wet, rainy conditions prevail. The source of infection is usually traced to an increase in exposure of the cows to flies in pastures that transmit infecting *Arcanobacterium pyogenes* and *Peptostreptococcus indolicus* strains and is more common in non-lactating cows (67, 84).

Mastitis caused by *Pseudomonas aeruginosa* is often traced to contaminated water sources and will result in a condition similar to coliform mastitis infections where

endotoxemia occurs (65, 67).

Nocardia asteroides causes severe cases of mastitis resulting in fibrosis and permanent damage to mammary tissues (67). Treatment is usually ineffective and a high mortality rate occurs. The source of the infection caused by *Nocardia asteroides* is usually from the soil and could be prevented by ensuring that effective sanitation measures are enforced before treatment with intramammary infusions (65).

Less common causes of bovine mastitis include *Bacillus cereus,* resulting in peracute and acute mastitis and also the human pathogens *Streptococcus pyogenes* and *S. pneumonia* that causes acute mastitis and is accompanied by fever symptoms in the host (67).

Current aetiology of mastitis

Contagious organisms have usually been responsible for the highest incidence of both clinical and sub-clinical cases of mastitis. Bradley (8) sites the changes that have occurred in the United Kingdom from 1967, where *S. aureus* and *S. agalactiae* were primarily responsible for the highest number of clinical mastitis cases in dairy herds. Three decades later in 1998, after the implementation of control strategies in the late sixties, the number of incidences of contagious pathogens responsible for clinical mastitis decreased significantly, accounting for only 10 % of cases. *E. coli* and Enterobacteriacae, however, were responsible for 34.7 % and 40.9 %, respectively, of all cases (9).

Adequate mastitis control strategies have thus played a key role in reducing contagious cases of mastitis. It would appear however, that as contagious pathogens were reduced, opportunistic and environmental pathogens seemed to play a greater role in causing persistent infections (8). The importance of the correct diagnosis and identification of the aetiological agent causing inflammation in the udder tissue is essential in determining the treatment strategies. It is also important to understand the history of mastitis incidence within a herd over a period of time and to understand the different periods when a cow may be at higher risk for infection. For example, cows are especially susceptible to mastitis during the periparturient period (just before and after calving) and at drying off - due to structural changes occurring in the mammary gland. A decrease in the number and functionality of white blood cells caused by interactions with specific hormones during these periods results in a compromised defence system (61,95).

Infection

Mammary structure is composed of the milk-producing tissue or alveoli that lead into the lactiferous ducts, gland cistern, teat canal and finally the teat opening or duct. The alveoli are lined with epithelial cells that become specialised during the gestation period, before calving, and after calving. These specialised cells produce colostral and lacteal secretions and finally, milk. Connective tissue and muscle cells support the alveoli glands and contract and squeeze milk from the alveoli during milking (29, 65).

Table 1 summarises the type of mastitis infection that occurs when pathogens invade the teat canal and mammary tissue. Some pathogens are well adapted for the udder tissue environment and are the primary source for recurrent intramammary infections, especially contagious mastitis caused by *S. aureus* and *S. agalactiae*. Most microorganisms, including *S. uberis* (2), *S. dysgalactiae* (3) and *E. coli* (21,22) adhere to and internalise into epithelium cells. Persistence of the pathogen in the tissue may vary, some are easily destroyed by the host immune system while others such as *S. aureus* are well-adapted and cause serious injury within the mammary tissue, producing virulence factors that disarm the host immune systems cells (2, 36).

E. coli and other coliform pathogens are not only able to adhere to and invade epithelium (22) but are also able to multiply rapidly in the gland cistern, which elicits a rapid inflammatory response that destroys a large number of the invading pathogens. However, upon cell lyses endotoxins are released causing severe toxaemia in the blood stream of the cow (65, 67).

Mastitis control strategies

The “five point plan for mastitis control” has been the gold

standard for control strategies for many years (29), and has been successful in reducing the incidence of mastitis. The strategy addresses areas where the risk of infection is the greatest and promotes the use of treatment at specific times. The five points listed by Giesecke *et al*. (29) include: (A) Teat disinfection after milking; (B) Proper hygiene and milking procedures and adequate milking equipment; (C) Culling of chronically mastitis cows; (D) Antibiotic dry-cow therapy; (E) Prompt treatment of clinical mastitis during dry period and during lactation.

Table 1. Characteristics of common mastitis-causing pathogens, invasiveness and infection

Pathogen	**Type of mastitis**	**Infection**
S. agalatiae	Mostly subclinical, but also clinical, recurrent and chronic if treatment is not effected soon enough	Highly contagious. Primarily infect duct system and lower portion of the udder on the surface of epithelium. Causes injury and scarring to duct system and clogging results in accumulation of milk in ducts and reduction in milk production. Involution occurs (65).
S. dysgalactiae	Clinical acute	Environmental source. Bacterium can adhere to and be taken up into cells without losing viability and therefore persist in tissue and may be protected from antibiotic therapy. Bacterium does not cause severe permanent injury to epithelial tissue (13).
S uberis	Clinical acute	Environmental source. Able to adhere to and is taken up by epithelium cells and persist intracellularly for extended periods. Responsible for chronic infection but does not cause severe tissue injury. One of the most commonly isolated organisms during non-lactating period (90)
S. aureus	Subclinical, clinical or chronic, in severe cases gangrenous mastitis	Highly contagious. Bacterium adheres invades the deeper tissue of the alveoli where it becomes encapsulated by fibrous tissue and abscesses form, thus walling-off the bacterium. Involution occurs. In severe cases, toxins can cause blood vessel constriction and clotting cutting off blood supply to tissue resulting in gangrenous mastitis (65).
E. coli and other coliform bacteria	Acute clinical (toxaemia) mastitis, may develop chronic mastitis	Environmental, fairly common due to high incidence of bacteria on host and environment. Bacteria invade tissue in teat and gland cistern. Tissue damage occurs in teat cistern, gland cistern and large ducts. Large influx of somatic cells through damaged tissue results in formation of clots in the milk. Usually no long-term effects to alveoli occur and host immune system often clears up infection. (65)

Farm management

A strategy to control mastitis must be practical and economical. The primary goal would be to reduce the rate of new infections and the duration of current infections within a herd. It would also be essentially important to maintain normal udder health ensuring that the natural immune response in the cow can resist and fight disease while still producing the required level of milk (65).

Control strategies need to target every facet and process of dairy farming and can begin with maintaining good hygiene practices in the environment. The holding yards or stalls should be kept clean and dry. The water supply should be adequate and free of coliform bacteria and equipment should be maintained and sanitised between milking (29). The welfare of animals is becoming increasingly important in modern dairy production as consumers become more concerned about the manner in which farm animals are treated. The Farm Animal Welfare Council in the UK has defined "the five freedoms" of animals, which highlight issues relating to the treatment and management of animals. The advantage of implementing such quality control measures within the herd would ensure that dairy cows are free of a stressful environment, injury, pain, hunger and discomfort, which in turn would promote a healthy immune system and udder health in general (77).

The milking practice is of paramount importance as this is most often the route of infection. The udder should be prepared before milking by washing the teats, followed by disinfection and drying with clean paper towels. If the teat area is dripping with water from run-off of areas that were heavily soiled it could lead to pathogens gaining access to the teat canal. Milker's hands should also be disinfected to prevent the transfer of pathogens. Post milking treatment is also important and all cows should be treated with a teat dip disinfectant to reduce the risk of infection (29, 65).

Monitoring SCC on a regular basis and follow-up investigations give an indication of the success of good animal husbandry and hygiene practices. It therefore forms an integral part of mastitis control strategies and assists in diagnosis and treatment.

The elimination of mastitis in a herd may require the culling of cows that are incurable or are so severely infected that the mammary tissue has been scarred and damaged to the extent that the tissue no longer functions (29).

Treatment

A cow may spontaneously recover from mastitis, but this will usually occur in mild cases of subclinical mastitis. Theoretically, the mechanism by which a cow recovers from infection without treatment can be capitalised upon to produce a vaccine (65). Research in this area continues and some vaccines such as *E. coli* J5 can reduce the number and severity of coliform mastitis cases by 70 – 80 % (17). Recent technology has focused on a DNA vaccine that expresses virulence factors *in vivo* and is primarily targeted against *S. aureus* mastitis, as antibiotic therapy is usually less effective against this pathogen (89,103).

Antimicrobial agents can be administered either during lactation or during the dry period. Treatment during lactation will be necessary if clinical mastitis is present, whereas dry cow therapy can be used to treat existing infections and can also be administered in a prophylactic manner to prevent new infections from developing during this period. A cow will usually lactate for a period of approximately 300 days per year and have a dry period of between 50 to 60 days. The most vulnerable period when new mastitis infections occur is at the end of the lactation period and again just before the start of the next lactation period (29). This can be attributed to hormonal and structural changes occurring in the mammary tissue which affects the immune system as the cow prepares for calving or for the drying-off stage (61, 95).

Dry cow therapy

Dry cow therapy is as much a management issue as it is a treatment issue. The manner in which the cows enter this period is important and the way in which the housing conditions and nutrition is handled impacts on the success of the treatment itself. The energy intake of the cows should be lowered to reduce milk production towards the drying-off stage

and then, as soon as drying-off occurs, they need to be treated immediately with either antimicrobial infusions (containing slow release antibiotic preparations) or with internal teat sealant products (60). Antimicrobials will be required if an existing infection is present, whereas an internal teat sealant can be used alone if no infection is present. Commercially available teat sealants such as Orbeseal® (Pfizer Animal Health) are approved for use in North America and Europe.

The teat sealant is composed of an inert salt (bismuth subnitrate) in a paraffin base. The paste is infused into the teat of each quarter using a sterile syringe. After drying-off, the product is stripped out at first milking (64). To ensure that other pathogens are not introduced into the teat along with the teat sealant, trained personnel should perform the administration of the product.

The teat sealant forms an impermeable plug as it lines the teat canal and results in a physical barrier against invading microorganisms through the teat opening, thereby preventing new infections during the dry period. Research has shown that the internal teat sealant (Orbeseal®, Pfizer Animal Health) is effective in reducing the infection rate when compared to untreated cows (4). A recent study also demonstrated the benefit of administering Orbeseal® (Pfizer Animal Health) along with an antibiotic infusion (Orbenin® Extra Dry Cow, Pfizer Animal Health) containing cloxacillin. The use of the teat sealant and the antibiotic infusion performed slightly better in preventing clinical mastitis in the dry period compared with using only the antibiotic infusion (10).

Lactation therapy

The use of antimicrobials during lactation must be carefully considered. Only cases of clinical mastitis and some specific cases of subclinical mastitis, where the quality and production of the milk is severely affected, are treated. Mastitis caused by *S. agalactiae* can be treated most readily during lactation and has a high cure rate (90-95 %). Mastitis caused by S. *aureus* has the lowest cure rate and along with environmental streptococci should be treated during the dry period (65).

An important consideration for treatment during lactation is the presence of antibiotic residues in the milk. A waiting period is required for the duration of the treatment and for a given period after treatment where milk and meat products need to be withheld to ensure that the level of antibiotics present in the product meets the legislative requirements. The withdrawal period and the type of product that is administered vary in different countries (34). The cost of treatment and the loss of milk during the withdrawal period are important in determining the type of product used and the manner in which it is administered. The withdrawal period for milk products marketed during lactation varies between 1 and 4 days (Table 3). A product is considered excellent if it has a high cure rate and a minimum withdrawal period (34).

Efficacy of drug delivery

The administration of drugs can be done either directly into the teat canal, as previously described for dry cow therapy, in the form of intramammary infusions, but can also be given parenterally by intravenous or intramuscular injection (65). The route of choice for subclinical mastitis is usually by intramammary infusion; and in the case of severe acute clinical mastitis, a combination of parenteral and intramammary treatment is usually necessary (104).

To be effective, the drug has to exert specific antimicrobial activity at the site of infection (34) and must have certain characteristics to be an effective agent in the mammary tissue. The pH of blood plasma is 7.4. The pH of milk varies between 6.4 and 6.6, but increases to 7.4 in the case of an infection. Most antibiotics are weak organic acids or bases and exist in both an ionised and non-ionised form in varying proportions in blood and milk, depending on the change in pH of the environment. Drugs that are administered parenterally must pass from the circulatory blood system and into the milk and milk tissue via lipid membranes. The active fraction of the drug must be in a non-ionised, non-protein bound, lipid-soluble form to pass this blood-to-milk barrier (104).

Antibiotics that are administered via the teat opening must reach the site of infection in the teat canal or upper cistern, but

often the distribution is uneven and diffusion through the mammary ducts where severe inflammation and swelling is present may block the movement of the therapeutic agent (24). Added to this, most pathogens have the ability to invade the epithelium tissue. In the case of *S. aureus* infection, interaction with antibiotics is prevented by the formation of fibrous scar tissue. The scar tissue may also have no blood supply, rendering intramuscular or intravenous drug therapy less effective (65). Some bacteria may also evade interactions with antibiotics once engulfed by macrophages, where they remain active within the leukocyte and can cause recurrent infections once the antibiotic has been eliminated from the area (65). The formation of biofilms within the teat canal as bacteria adhere to bacteria on the epithelium surface may also contribute to the ineffectiveness of local intramammary infusions (52).

The type of drug used to treat an infection can be determined once an accurate diagnosis has been made and the pathogens identified. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of a drug that prevents the growth of a specific pathogen (59). Antimicrobial disk diffusion tests are performed on the pathogens isolated from mastitic milk samples to determine the drug sensitivity profile of the pathogens. The veterinarian is then able to select the most effective drug for treatment (65). The ideal drug should have the lowest MIC against the majority of udder pathogens. No single drug can, however, be effective against all pathogens and most need to be used in combinations and in different formulations to increase efficacy and bioavailability within the udder tissue (34,104).

Types of antimicrobial agents

Commonly used remedies available for dry cow and lactation therapy, the recommended withdrawal period and the possible activity spectrum of mastitis pathogens (24) are shown in Table 2 and 3. The antibiotic groups and antimicrobials used in these remedies have different mechanisms of action and many new semi-synthetic compounds have been developed to counter the threat of antimicrobial resistance. The majority of antibiotics used are broad-spectrum antibiotics acting against Gram-positive and Gram-negative bacteria (59).

β-lactam Penicillins (penicillins, ampicillin, cloxacillin, amoxycillin, nafcillin, methicillin) and β-lactam Cephalosporins (cephalexin, cefuroxime, cephapirin) inhibit cell wall synthesis by preventing the formation of cross-links between polysaccharide chains in the cell wall. Many staphylococcal strains produce the enzyme penicillinase, which acts by breaking the β-lactam ring structure of the antibiotic and are therefore resistant. Penicillinase-resistant penicillins such as cloxacillin are specifically used to treat the penicillinase-producing, methicillin-susceptible staphylococci (59).

Clavulanic acid inhibits the activity of penicillinase produced by staphylococcal strains. Combined with β-lactam antibiotics such as amoxicillin it can eliminate β-lactamase activity by pathogens and improve susceptibility to the antibiotic (83).

Tetracyclines such as oxytetracycline inhibit protein synthesis by binding to the 30S ribosomal sub-unit and interfere with amino-acyl-tRNA binding. Tetracycline is bacteriostatic and usually more active against Gram-positive organisms (59). Oxytetracycline is an irritant and should therefore not be administered as an infusion, but rather intravenously (24).

Aminoglycosides (streptomycin, neomycin) inhibit protein synthesis by binding to the 50S ribosomal sub-unit and inhibits peptide chain elongation. Aminoglycosides are mostly active against Gram-negative bacteria and are often formulated together with β-lactam penicillins (59).

Polymixin B is an antimicrobial compound that binds to the cell membrane and disrupts its structure and permeability properties. It is the antimicrobial drug of choice for infections caused by *P. aeruginosa* (24).

Macrolide antibiotics (tylosin, lincomysin, erythromycin) are effective in treating Gram-positive udder infections both by parenteral and intramammary administration (24). They are bacteriostatic and thus act in conjunction with the host immune system to fight infection. The mechanism of action is to inhibit protein synthesis by binding to the 50S ribosomal sub-unit to

prevent peptide elongation (66).

What are the alternatives?

The risks involved in the treatment of mastitis has been discussed in terms of the development of antibiotic resistance, but from a commercial standpoint, milk products containing specific levels of antibiotic residues cannot be sold for human consumption. Processing of milk for cheese and yoghurt manufacture is also affected as bacterial starter cultures are inhibited and the quality of the product produced is generally compromised (54). Completely eliminating the use of antibiotics for the treatment of mastitis is unlikely, as modern intensive farming practices and high demand dictate rapid and intensive treatment strategies, which involve the use of antibiotic therapy in both lactation and dry periods. The ultimate goal would be to reduce the use of antibiotics. This could primarily be achieved through better management and hygiene practices and legislation enforcing a reduction in the indiscriminate use of antibiotics for treatment and for growth promotion, as was done in Nordic countries in 1980's (25). Improving host defences can result in rapid elimination of new infections. Supplementing of selenium and vitamin E and improving general nutrition during high-risk periods such as periparturient and drying-off periods can increase host defence mechanisms (58).

Table 2. Recommended remedies for dry cow treatment, withdrawal period and activity spectrum (24).

Remedy	Milk withdrawal period	Antibiotic Composition	Activity Spectrum (if sensitive)
Bovaclox DC	30 days	Cloxacillin, ampicillin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Cephudder	21 days	Cephapirin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Cepravin DC	4 days	Cephalexin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Curaclox DC	2.5 days	Cloxacillin, ampicillin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Curaclox DC XTRA	4 days	Cloxacillin, ampicillin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Dispolac DC	None specified	Penicillin, dihydrostreptomycin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.), *Clostridium perfringens*, *Bacillus cereus, Arcanobacterium pyogeness*
Dri Cillin	2.5 days	Cloxacillin, ampicillin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Masticillin DC	28 days + 10 milkings after calving	Cloxacillin	*S. aureus*, streptococci
Masticlox DC	2.5 days	Cloxacillin	*S. aureus*, streptococci
Masticlox Plus DC	None specified	Cloxacillin, ampicillin	*S. aureus* streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Masticlox Plus DC EXTRA	4 days	Cloxacillin, ampicillin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Nafpenzal DC	3 milkings	Penicillin, dihydrostreptomycin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.), *Clostridium perfringens*, *Bacillus cereus, Arcanobacterium pyogenes*
Neomastitar DC	5 weeks	Penicillin, neomycin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Noroclox DC	2.5 days	Cloxacillin	*S. aureus*, streptococci
Noroclox DC EXTRA	2.5 days	Cloxacillin	*S. aureus*, streptococci
Orbenin EXTRA DC	4 days	Cloxacillin, blue trace dye	*S. aureus*, streptococci
Pendiclox DC	24 hours after blue colour disappears	Cloxacillin, ampicillin, blue tracer dye	*S. aureus,* streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Penstrep DC	24 hours after blue colour disappears	Penicillin, dihydrostreptomycin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.), *Clostridium perfringens*, *Bacillus cereus, Arcanobacterium pyogenes*
Rilexine 500DC	4 weeks	Cephalexin, neomycin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.)

Table 3. Recommended remedies for lactating cow treatment, withdrawal period and activity spectrum (24, 42).

Remedy	Milk withdrawal period	Antibiotic Composition	Activity Spectrum (if sensitive)
Cloxamast LC	3 days	Cloxacillin, ampicillin	Septic mastitis. *S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Curalox LC	3 days	Cloxacillin, ampicillin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Dispolac RX 4	24 hours after blue colour has disappeared	Penicillin, dihyrostreptomycin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.), *Clostridium perfringens*, *Bacillus cereus*
Lactaclox	2.5 days	Cloxacillin	*S. aureus*, streptococci
Lactaciliin	3 days	Ampicillin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Lincocin Forte	2.5 days	Lincomycin, neomycin	*Staphylococcus aureus,* streptococci
Mastijet Forte	4 days	Oxytetracycline, neomycin, bacitracin, cortisone	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Nafpenzal MC	6 milkings in treatment + 3 milkings after treatment	Penicillin, dyhrostreptomycin, nafcillin	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.), *Clostridium perfringens*, *Bacillus cereus, Arcanobacterium pyogenes*
Noroclox QR	24 hours after blue colour has disappeared	Cloxicillin, blue tracer dye	*S. aureus*, streptococci
Pendiclox Blue	24 hours after blue colour has disappeared	Cloxicillin, ampicillin, blue tracer dye	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.)
Penstrep 300 D	24 hours after blue colour has disappeared	Penicillin, dihydrostreptomycin, blue tracer dye	Acute mastitis. *S. aureus*, streptococci, soliforms (*E. coli* & *Klebsiella* spp.), *Clostridium perfringens*, *Bacillus cereus, Arcanobacterium pyogenes*
Rilexine LC	4 days	Cephalexin, neomycin, cortisone	Acute & chronic mastitis
Spec Form Forte	3 days	Penicillin, dihydrostreptomycin, novobiocin, polymyxin B, cortisone	Acute or chronic mastitis. *S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.), *Clostridium perfringens*, *Bacillus cereus*, *Pseudomonas aeruginosa, Arcanobacterium pyogenes*
Streptocillin	24 hours after blue colour has disappeared	Penicillin, dihyrostreptomycine, blue tracer dye	*S. aureus*, streptococci, coliforms (*E. coli* & *Klebsiella* spp.), *Clostridium perfringens*, *Bacillus cereus*, *Arcanobacterium pyogenes*

BACTERIOCINS – EXPLORING ALTERNATIVES TO ANTIBIOTIC TREATEMNT

INTRODUCTION

The study of the antibacterial properties of peptides that became known as colicins began in 1925 when one strain of *E. coli* produced an antagonistic effect against another *E. coli* culture (33). The antibiotic effect between other enteric bacteria was also reported by Fredericq and Levine (27) and further research into these proteinaceous molecules centred on colicins that were active against *E. coli* and various other

members of the family Enterbacteriaceae.

Colicin-like molecules produced by Gram-positive bacteria have also been studied extensively since the first report of nisin produced by *L. lactis* subsp. *lactis* (71). The term "bacteriocin" was used to describe these antibiotic substances as not all were produced by coliform bacteria (42) and according to Tagg *et al*. (87), were defined as ribosomally synthesized polypeptides that usually possess a narrow spectrum of antibacterial activity against bacteria of the same or closely related species. Jack *et al*. (41) however noted some discrepancies in this definition in that some bacteriocins (or bacteriocin-like substances) have a broader spectrum of activity and some are even active against Gram-negative species.

Klaenhammer (45) classified bacteriocins on the structure and mode of action of the peptide and predominantly included those produced by lactic acid bacteria (LAB). Four distinct classes were identified: class I, small lantibiotics (<5 kDa), that contained the amino acids lanthionine, α-methyllanthionine, dehydroalanine and dehydrobutyrine; class II, small (<10 kDa), heat-stable, non-lanthionine containing peptides; class III, large (>30 kDa), heat-labile proteins and class IV, consisting of complex bacteriocins containing carbohydrate or lipid moieties that were required for bacteriocin activity.

Applications of bacteriocins

The antibacterial activity of bacteriocins has resulted in research into the practical applications thereof and can be broadly divided into two focus areas: food production and preservation, by preventing the growth of unwanted or disease-causing organisms and secondly, medical and veterinary applications. Traditionally, antibiotics have been administered to prevent and treat disease. However, with the widespread development of antibiotic drug-resistant strains, the importance of alternative antimicrobials is becoming increasingly urgent and bacteriocin-producing organisms could be considered as an important source of antimicrobial agents in the medical and veterinary fields. The important role that bacteriocins continue to play in food production and clinical applications will be discussed.

Application in medical and veterinary fields

Bacteriocins, by definition usually only target closely related species; they could offer an advantage over antibiotics in that treatment could be targeted against specific pathogenic organisms. Bacteriocins, identified for potential use as antimicrobials include lantibiotics produced by Gram-positive lactic acid bacteria, and colicins and microcins, produced by Gram-negative bacteria (30). Applications are widespread, ranging from topical applications in the treatment of skin infections to the treatment of inflammation and ulcers. Commercial products are currently available for the treatment of mastitis in dairy cattle and will be discussed in more detail. Table 4 summarises some of the potential applications of some bacteriocins in the medical and veterinary field. Most testing for clinical applications have been carried out in animal models, however the bacteriocin nisin has already undergone human clinical trials for the treatment of peptic ulcers caused by *Helicobacter pylori* (35). Bacteriocins produced by Gram-negative bacteria can be advantageous in that they can be used to target other pathogenic Gram-negative strains. Bacteriocins produced by Gram-positive LAB are not active against Gram-negative strains without pre-treatment strategies to compromise the integrity of the outer membrane (15). For example, nisin, after treatment with ETDA, citrate and lactate, was shown to be effective against *Salmonella typhimurium* and *E. coli* 0157:H7 (18). In contrast, colicins produced by Gram-negative *E. coli* are naturally active against other *E. coli* strains as well as some Salmonella strains (11). Microcins produced by enteric bacteria, usually target strains in the family *Enterobacteriaceae* (55).

Bacteriocins produced by Gram-positive strains can substitute antibiotics such as ionophores routinely applied as feed additives for livestock animals, such as cattle. The ruminal bacterial populations of Gram-positive bacteria that produce excessive fermentation products, such as methane and ammonia, can be inhibited, without the dangers and perceived risks of antibiotics in feed rations (72).

Table 4. Potential medical and veterinary applications of some bacteriocins

Bacteriocin	Producer	Potential use	Reference
Gram- positive bacteria			
Nisin	*L. lactis* subsp. *lactis*	Treat peptic ulcer disease Antimicrobial activity in medical devices such as catheters Treat *S. pneumonia* infections Treat mastitis in cattle Vaginal contraceptive agent	(7,31,35,68,81)
Lacticin 3147	*L. lactis* subsp. *lactis*	Treat mastitis in cattle	(73)
Galliderm	*Staphylococuccus gallinarum*	Treat skin infections such as acne	(44)
Epidermin	*S. epidermidis*	Treat skin infections such as acne	(1)
Mutacin B-Ny266	*Streptococcus mutans*	Bacterial infection caused by methicillin-resistant staphylococci	(57)
Tomicid	*Streptococcus* sp. Thom-1606	Streptococcoal respiratory infections (Scarlet Fever) in children	(12,32)
Gram-negative bacteria			
Microcins J25 and 24	*E. coli*	Treat *E. coli* and salmonella infections in chickens	(75,102)
Colicins E1, E4, E7, E8, K &S4	*E. coli*	Treat haemorrhagic colitis and haemolytic uremic syndrome cause by *E. coli* 0157:H7	(43)

Bacteriocins used in the treatment of mastitis

The most economically costly disease in cattle is mastitis. As a result the dairy industry could benefit greatly from the development of safe antimicrobial agents and bacteriocins could be an attractive alternative to antibiotics. The treatment of mastitis has been a target of research since the inception of scientific research into the applications of bacteriocins (91). To date, only the Lactococcal bacteriocin, nisin, has been developed for commercial application and the lantibiotic, lacticin 3147, has been extensively researched for dry cow therapy. Applications for prevention and treatment using these lactococcal bacteriocins will be discussed in detail below.

Other bacteriocins that are active against mastitis pathogens have also been investigated. Researchers have targeted staphylococci and streptococci isolated from the normal flora of the teat canal and other areas as these could be a source for bacteriocins to treat mastitis pathogens. The potential applications for these bacteriocins will also be discussed.

Lactococcal bacteriocins

Nisin: was the first bacteriocin applied to the preservation of food products and was approved for use in pasteurised processed cheese spreads in 1988 by the FDA (19). Nisin is classified as a class Ia lantibiotic (45) and is a 34 amino acid peptide (3488 Da). Nisin has a dual mode of action, which essentially involves the prevention of cell wall synthesis and pore formation, leading to cell death. The precise mechanism involves binding to lipid II molecules (Undecaprenyl-pyrophosphate-MurNAc(pentapeptide)-GlcNAc) located in the cell membrane of the target cells. Lipid II is the main transporter of peptidoglycan subunits from the cytoplasm to the

cell wall and when nisin binds to lipid II, it prevents the transfer of the peptidoglycan across to the cell wall (15). The process of pore formation is initiated in the membrane of the target cell after docking at lipid II occurs and results in the efflux of cytoplasmic compounds that are required to maintain ion gradients, thereby affecting trans-membrane potential and the pH gradient across the membrane. Biosynthetic processes such as ATP synthesis driven by proton motive force cease and cell death occurs (69,76).

Nisin has a wide spectrum of activity against Gram-positive bacteria, including species of *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Pediococcus* (14). Nisin is also active against *L. monocytogenes* and its efficacy against this food pathogen in raw meat products have been evaluated by Pawar *et al*. (62), as well as in dairy products (5). Nisin has also been applied to cheese products to control the growth of spores produced by *Clostridium tyrobutyricum* (70, 78).

Sears *et al*. (81) investigated the use of a nisin-containing germicidal formulation in preventing mastitis in cattle. Teat sanitisers are routinely used before and after milking cows to prevent the introduction of pathogens into the teat canal, which could lead to intramammary infections. The study compared the nisin-based formulation (Ambicin® N, Applied Microbiology, Inc., New York, NY) with that of conventional chemical treatments such as iodines and chlorohexidines. Initial performance data for a nisin-based teat sanitizer (Amibicin N®) showed a significant reduction in pathogen in experimentally challenged teat surfaces after 1-minute exposure to the germicidal formulation (Table 5). The formulation also showed little potential for skin irritation after repeated exposure in contrast to 1 % iodophore and 5 % chlorohexidine digluconate preparations. Table 6 shows the skin irritation data reported by Sears *et al*. (81). Dermal irritation scores indicated the degree of redness or scab formation, with a score of <1.0 indicating a product with little or no potential for irritation. Products with a score of ranging from 3.0-4.9 would have the potential to cause severe irritation.

Table 5. Performance data for nisin-based germicidal teat sanitizer (81).

Mastitis-causing organisms	**Reduction using Ambicin N®**
S.aureus	61.8 %
S. agalactiae	98.6 %
E. coli	85.5 %
S. uberis	67.1 %
K. pneumonia	76.5 %

Table 6. Comparative skin irritation to rabbit skin after exposure to teat sanitizer.

	Dermal irritation scores	
Teat sanitizer	**Single application (72 hr after application)**	**Multiple application (72 hr after the last of 7 daily applications)**
Amibicin N® (nisin-based sanitizer, 1x concentration)	0.21	0.30
Amibicin N® (nisin-based sanitizer, 12x concentration)	0.09	0.04
1 % Iodophor	0.5	3.34
5 % Clorohexidine digluconate	0.38	2.34

Contamination of milk with a sanitizer chemical based product is a concern if it is not completely removed before milking. Using bacteriocin-based sanitizers or products would be advantageous in that complete removal of the product would not necessarily be required.

In addition to Ambicin®, two other nisin-based products, namely Wipe-Out® Dairy Wipes and Mast Out® were developed by Immucell Corporation (15). Mast Out® was used in January 2004 in initial field trials involving 139 cows with subclinical mastitis. Significant cure rates were reported and the product was subsequently licensed to Pfizer Animal Health for further development and distribution (39). The product has however not been made available by Pfizer Animal Health and no further trial results have been reported.

Lacticin 3147: is produced by *L. lactis* subsp. *lactis* DPC3147 and was first isolated from Irish Keffir grain (74). As with nisin, it is also classified as a Class 1a lantibiotic, but it differs from nisin in that it is a two-peptide lantibiotic, requiring both the LtnA1 and LtnA2 peptides for full activity. The mode of action of lacticin 3147 is similar to that of nisin in that it results in the inhibition of cell synthesis and pore formation in the target cell (98).

The primary structure of the lacticin A1-peptide, LtnA1, consists of 30 amino acids (3306 Da) and has a lanthionine-bridging pattern resulting in a globular structure similar to class Ib lantibiotics such as mersacidin. The LtnA2 peptide consists of 28 amino acids (2847 Da) and is an elongated peptide. Wiedeman *et al.* (98) proposed a three-step model to describe how both peptides are involved for antibacterial activity of lacticin 3147. LtnA1 first binds to lipid II (i), thereby inducing a conformation that facilitates the interaction with LtnA2. This enables the formation of a two-peptide-lipid II complex (ii). When bound to the complex, LtnA2 is able to adopt a transmembrane conformation that results in the formation of a defined pore and the release of ions across the membrane (iii). In an earlier study, McAuliffe *et al*. (53) reported that the pore formation resulted in the efflux of potassium ions and inorganic phosphate, resulting in the dissipation of the membrane potential and hydrolysis of internal ATP, the collapse of the pH gradient and cell death.

Lacticin 3147 has a broad spectrum of antimicrobial activity and inhibits the growth of *Bacillus* sp., *Enterococcus* sp., *Lactobacillus* sp., *Pediococcus pentriceans*, *S. aureus*, *S. thermophilus* and most mastitis-causing streptococci. Food-borne spoilage bacteria, including *L. monocytogenes* and *C. tyrobutyricum*, are sensitive to lacticin 3147 and the peptide could be used to prevent food spoilage and disease (74).

Lacticin 3147 was investigated for use as an antimicrobial agent as it inhibited common mastitis-causing pathogens, including *S. aureus*, *S. dysgalactiae*, *S. uberis* and *S. agalactiae* (73). The producing organism is GRAS and is active at both low and physiological pH and was heat stable (73,74).

Teat seal formulations such as Orbeseal® (64) are recommended for use during the dry period as a prophylactic measure to reduce the number of new mastitis infections (4). The inert property of the teat seal formulation has no antimicrobial effect and therefore relies on good udder hygiene practices for effective treatment. Antibiotics such as cloxacillin have been added to the formulations (Orbenin® Extra Dry Cow, Pfizer Animal Health) to prevent new infections during this period. However, prolonged exposure to antibiotics at low levels could increase the risk of antibiotic resistance by pathogenic bacteria. Bacteriocins, such as lacticin 3147 could replace antibiotics in these formulations (73, 74, 93). Studies to date have shown that resistance by mastitis pathogens *S. dysgalactiae* and *S. aureus* to the bacteriocin lacticin 3147 were not significant (73).

In separate studies, the bismuth subnitrate-based teat seal (Osmonds Teat Seal 2, Cross Vetpharm Group Ltd., Dublin, Ireland) combined with lacticin 3147 was evaluated against the mastitis-causing pathogens *S. dysgalactiae* (73) and *S. aureus* (16,93). Irritancy to the teat area and the somatic cell response were evaluated.

The protection given by the teat seal plus lacticin 3147 and the teat seal only were compared after experimental challenge with *S. dysgalactiae*. The results showed significant improvements in the level of protection afforded by the teat seal containing the bacteriocin 3147 (Table 7). Ninety-one

percent of quarters treated with the teat seal plus lacticin 3147 remained free of new infections compared with only 33.3 % of quarters treated with the teat seal alone (73, 74).

Tissue tolerance studies were done comparing the SCC in the milk from quarters treated with the teat seal alone, teat seal plus lacticin 3147 and with a commercially available antibiotic infusion containing sodium cloxacillin. The SCC over 5 consecutive days after infusion was 7.22 x 10^5 and 5.71 x 10^5 SCC.mL^{-1} for the teat seal and the teat seal plus lacticin 3147 respectively. The highest SCC of 1.01 x 10^6 SCC.mL^{-1} was for the quarter infused with the antibiotic cloxacillin, while the untreated quarter had a SCC of 6.27 x 10^5 SCC.mL^{-1}. This data indicated that the lacticin 3147 was tolerated within the udder tissue and no visible sign of irritation or abnormality was reported (73, 74).

Twomey *et al*. (93) evaluated the effect of the teat seal plus lacticin 3147 with untreated quarters as controls, against experimental challenge by *S. aureus*. The concentration of the bacteriocin and inoculum of the *S. aureus* challenge was varied to optimise treatment conditions. The presence of the teat seal plus lacticin 3147 using a concentration of 32 768 AU/4g of teat seal, resulted in a significant decrease in the number of teats shedding *S. aureus* (Table 8). The antagonistic effect of the bacteriocin at the same concentration was however reduced when the inoculum of the *S. aureus* challenge introduced into the teats was increased. The concentration of the bacteriocin used was found to be significant factor for the teat seal to be effective in reducing *S. aureus* in the teats.

Table 7. Clinical mastitis and recovery of *S. dysgalactiae* in non-clinical mastitis in quarters after treatment with the teat seal only and the teat seal plus lacticin 3147 (73).

Treatment	Total no of quarters treated	New clinical infections by *S. dygalactiae*	New non-clinical isolations of *S. dysgalactiae*
Teat seal	33	16 (48.5 %)	6 (18.2 %)
Teat seal plus lacticin 3147	35	3 (8.6 %)	0 (0 %)

Table 8. The effect of teat seal plus lacticin 3147 in eliminating *S. aureus* in artificially infected cows. Shedding evaluated after 18h (93).

Inoculum	Lacticin 3147 AU/4g of teat seal	Treatment	Total teats inoculated	Teats shedding *S. aureus*	% Teats successfully treated
1.7 x 10^3	32 768	Untreated	29	19	34.5
		Teat seal + lacticin 3147	29	4	86.2
6.8 x 10^3	32 768	Untreated	20	16	20.0
		Teat seal + lacticin 3147	20	11	45.0

The initial evaluation of lactitin 3147 by Ryan *et al*. (73, 74) indicated that bacteriocin produced in a synthetic growth medium was not adequately released from the teat seal formulation without the addition of a surfactant (Tween 80). Later research improved the efficacy of the teat seal formulation by producing lacticin 3147 in milk-based (whey) medium which resulted in an increase in activity from ~320 AU.mL^{-1} to ~500 AU.mL^{-1} in the fermentate after 24 hr incubation. The increase in activity of the bacteriocin preparation resulted in a significant release of the peptide in the teat seal formulation without the addition of Tween 80, thereby providing a cost-effective method of producing larger

quantities of the bacteriocin (16).

The lacticin 3147 produced in the milk-based (whey) medium reduced the number of *S. aureus* recovered after experimental challenge. The average recovery of *S. aureus* from teats infused with teat seal plus lacticin 3147 was 7.3 x 10^2 cfu.mL^{-1} compared with 1.6 x 10^4 cfu.mL^{-1} for those treated with the teat seal alone. The bacteriocin-teat seal preparation also appeared to eliminate *S. aureus* cells already present in the teat canal prior to the infusion of the product compared to the teat seal alone. No viable *S. aureus* cells were recovered from the teats where the bacteriocin was present in the teat seal, compared to four of the teats where only the teat seal was used (n = 8) (16).

The stability of the product for the dry period of 50-60 days would still need to be assessed adequately as the teat seal-bacteriocin product evaluated by Twomey *et al.* (93) and Crispie *et al.* (16) was only infused for a period of 18 hours. Ryan *et al.* (73) however showed that in an 8-day period, lacticin 3147 retained activity in the teat environment.

To summarise, research has shown that the bacteriocin lacticin 3147 has the potential for use in a teat seal preparation to effectively prevent new infections by streptococci and offer some protection to *S. aureus* infection. The bacteriocin could potentially be produced on large scale using a milk-based (whey) medium at concentrations that are active against target organisms. The bacteriocin is also active and insoluble at physiological pH and thus remains effective in the teat canal environment.

Other bacteriocins that could have potential use in mastitis treatment

Staphylococcal bacteriocins: Bacteriocins from Gram-positive bacteria have, to a large extent, been limited to applications in the food industry. Potential applications of other bacteriocins in mastitis treatment have been limited to that of lacticin 3147 (16) and nisin (81).

Growth inhibition studies of mastitis pathogens by normal bovine teat skin flora (20,101) have been attempted to evaluate the antagonistic or other effect that these less pathogenic bacteria could have on major mastitis-causing pathogens such as *S. aureus*, *E.coli* and streptococci. Staphylococcal strains associated with mastitis were investigated and it was found that bacteriocins active against mastitis-causing *Streptococcus agalactiae* isolates were primarily produced by *S. epidermidis*, *S. saprophyticus* and *S. arlettae* (23).

Streptococcal bacteriocins: Many streptococci have been found to produce bacteriocins and the potential applications of these bacteriocins range from those produced by the thermophilic lactic acid bacteria, for their potential application in cheese production to the oral streptococci for use in the treatment of dental carries.

No potential streptococcal bacteriocins have as yet been isolated for use in the treatment of mastitis. However, the natural ecological niche of a particular bacteriocin producer is often the specific area that is targeted for practical application. The mastitis pathogen *S. uberis* is commonly found in the natural environment of dairy cattle and thus could also be competing with other bacteria in this ecological niche. Wirawan *et al.* (99,100) screened more than 200 *S. uberis* strains from their culture collection to determine whether any of these strains produced bacteriocin-like inhibitory substances. Strain 42 was found to produce two bacteriocins, a natural nisin variant, nisin U and a circular peptide, uberlysin (100) The bacteriocin nisin U had activity spectra against *S. agalactiae*, *S. dysgalactiae* and *E. faecalis* that are considered to be potential mastitis-causing pathogens (99). The discovery of this natural nisin variant, which is active against mastitis-causing pathogens, could offer a potential alternative to nisin A, especially in cases where nisin A resistance may occur in pathogenic strains. A combination of antimicrobials, such as a nisin variant with other bacteriocins could potentially be more effective in treatment strategies (100).

Other streptococcal bacteriocin producers occur in the oral cavity where the normal flora such as *S. salivarius*, *S. pyogenes* and *S. mutans* are readily found. These produce bacteriocins or uncharacterised bacteriocin-like inhibitory substances (86, 88). Normal flora of the nasopharynx also consists of bacteriocin producing strains, including *S. salivarius* strains, and has been

investigated for the prevention of streptococcal pharyngitis and otitis media (86, 96). The type of treatment used is known as bacteriotherapy or bacterial interference, where bacteriocin producing, non-pathogenic strains are introduced into the nasopharynx to protect against recurrent streptococcal infections (96). The bacteriocin salvaricin A2 (SalA2), produced by *S. salivarius* K12 has been developed as an oral probiotic (BLIS K12 Throat Guard, BLIS Technologies, New Zealand) to treat streptococcal infections especially by *S. pyogenes* in children (88).

Streptococcal bacteriocins produced by *Streptococcus thermophilus* strains are often investigated for use in yoghurt starter cultures, including thermophilin 81 (40) and thermophilin 13 (50), while thermophilin 580, produced by *S. thermophilus* 580 has been studied for possible application in cheese production as starter cultures with the added benefit of bacteriocin inhibition of *C. tyrobutyricum* in the cheese ripening process (51).

Larger bacteriocins (>10kDa) also produced by some streptococci are characterised as non-lytic inhibitory agents or as bacteriolytic enzymes. Examples include dysgalactin produced by *S. dysgalactiae* subsp. *equisimilis* and streptococcin A-M57 produced by *S. pyogenes* M-57. Stellalysin is an example of a large 29-kDa bacteriocin produced by *S. constellatus* subsp. *constellatus*. The activity spectra of stellalysin includes *S. pyogenes, S. gordonii* and *S. mutans* (37).

The mutacins B-Ny266, J-T8 and B-JH1140, produced by *S. mutacin* have been characterised as belonging to the lantibiotic class of bacteriocins. Potential practical applications of mutacins include the treatment of dental carries (38). Mutacin B-Ny266 has been of particular interest due to its wide-spectrum of activity against many pathogenic Gram-positive and Gram-negative bacteria, including staphylococcal and streptococcal strains resistant to antibiotics. It could therefore find application for therapeutic use (56, 57).

Rumen streptococci have also been investigated as a source of bacteriocins, with *S. bovis* as the predominant strain isolated (97). Bovacin 255 produced by *S. gallolyticus* 255, a class II bacteriocin and bovicin HC5 from *S. bovis* HC5 could find application in cattle farming (49, 97). Bacteriocins that inhibit Gram-positive LAB found in rumen can be advantageous as these bacterial species, through fermentation produce large quantities of methane and ammonia waste products. Bacteriocins could be applied as feed additives to alter ruminal fermentation, and as a substitute to conventional antibiotics, such as monesin (72).

The first report of a bacteriocin, namely macedocin produced by the thermophilic *S. macedonicus* ACA-DC 198, was characterised by Geogalaki *et al*. (28). The bacterium was first isolated from Greek Kasseri cheese from Macedonia in Northern Greece and was subsequently named as *S. macedonicus* (92). Flint *et al*. (26) also isolated *S. waius* from biofilms on stainless steel structures exposed to milk, but *S. waius* was subsequently found to be synonymous to *S. macedonicus* isolated by Tsakalidou *et al*. (92) and reclassified as such (48). The species forms part of the larger *S. bovis / S. equinus* complex but remains as a separate species, as low level of DNA homology (less than 70 %) exists with other closely related species such as *S. gallolyticus* (International Committee on Systematics of Prokaryotes Subcommittee on the taxonomy of staphylococci and streptococci, 2003). More recently, *S. macedonicus* strains isolated from Italian raw milk cheeses were characterised (47).

Macedocin ACA-DC 198 is a bacteriocin that has been assessed as a food grade bacteriocin for use in cheese manufacturing as a starter culture, because it is able to produce the lantibiotic at pH and temperature conditions that prevail during cheese manufacturing, and it also inhibits the food spoilage bacteria *C. tyrobutyricum* (94). It has a molecular mass of 2,794 Da, as determined by electrospray mass spectrometry. Partial N-terminal amino acid sequence analysis revealed some homology to other streptococcal bacteriocins, SA-F22 and SA-M49, both produced by *S. pyogenes* (28). No therapeutic applications have as yet been investigated for macedocin ACA-DC 198 and its activity spectrum has been largely restricted to food spoilage organisms.

CONCLUSION

The economic implication of mastitis as a recurrent disease in dairy farming warrants further research into developing new technologies in antimicrobial therapy. Bacteriocins can be considered as an alternative and does offer some advantages over conventional antibiotic therapy. Increasing concerns for human health, primarily due to the emergence of antibiotic resistance in pathogenic bacteria, also necessitates the development of alternative anti-infective agents.

Bacteriocins are usually active against specific bacterial strains based on target receptors on the surface of sensitive strains. When diagnosing mastitis, the causative bacteria needs to be clearly identified and a targeted approach for specific pathogens should be considered. Bacteriocins can kill susceptible organisms quickly by cell lysis. This rapid action could ensure that resistance is less likely to develop in pathogens. Antibiotics used are usually broad-spectrum, killing all Gram-positive or Gram-negative bacteria to which it is exposed to, not only those causing infection. Bacteriocins offer the advantage of a target-specific action. If a broader spectrum of activity is required, a combination of two or three bacteriocins could be considered to ensure that more than one pathogen be targeted during treatment.

The lowest minimum inhibitory concentration (MIC) of the bacteriocin should be established, as this would reduce the amount of bacteriocin used in the treatment product. The bacteriocin should also remain active and should persist in the target environment for a given period of time in order to come in contact with potential pathogens.

The method of drug delivery in a treatment strategy for mastitis is important and a teal seal offers many advantages. Firstly it acts as a physical barrier and is prophylactic. By combining an antibacterial agent in a teat seal, the inhibitor is localised in the teat canal, targeting pathogens that may be present near the teat opening and thus prevent bacteria from colonising the mammary tissue. Topical preparations can also be used and due to the lack of invasiveness are more easily accepted as a form of drug delivery. The persistence and stability of the bacteriocin on the surface of the teat skin is essential but should not cause irritation or an allergic reaction to further inflame the teat area.

Bacteriocin-based products have been successfully tested in the past. Nisin has been used as a teat disinfectant in the commercial product, Wipe-Out® Dairy Wipes (Immucell Corporation) (15) for use throughout the lactation period, while lacticin 3147 has been evaluated for use as a dry cow therapy in a teat seal formulation (73). Thus the route of administration, considering the teat-canal environment of the cow, as well as the production cycle of the cow are important considerations when determining the type of treatment product produced.

Bacteriocins produced by LAB are considered to be GRAS (generally regarded as safe) and would therefore be more acceptable when compared to antibiotics. Antibiotic therapy during lactation requires a withdrawal period, which results in economic losses due to wastage and loss of production time. Bacteriocin residues in milk are more acceptable as digestive enzymes easily destroy the peptides. Thus, the withholding periods would be significantly reduced if bacteriocin therapy were used instead of antibiotic therapy.

Considering the extensive costs of a disease such as mastitis to the dairy industry, research directed towards viable and safe alternatives should be considered. Bacteriocins can thus be viewed as a real treatment solution to augment other management strategies and reduce the amount of antibiotics used in the treatment of mastitis.

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