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Best practice: Why mastitis treatment is not successful?

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Background

In a practical environment, veterinarians estimate the prevalence of mastitis on a dairy farm using the Equation:

$$\text{Prevalence} = \frac{(\text{duration of each infection} * \text{number of cows infected})}{\text{number of cows on the farm}}$$

Thus, the prevalence of mastitis at any given time depends on the duration of each intramammary infection and the number of infected cows. Effective mastitis control programs rely on decreasing the risk of exposure to mastitis-causing organisms (MCOs) and elimination of existing infections. Cows affected by clinical and/or subclinical mastitis are an obvious source of cross-infection to herd mates (regardless of whether the pathogens were contagious or environmental in origin). Therefore, minimising exposure of healthy cows during the shedding phase of infected cows can reduce the potential risk of new infection, and is one avenue of approaching mastitis control. Shortening or cessation of the shedding phase can be achieved by different means, including temporary or permanent removal of the infected cow/s from residence within the 'healthy' herd, pre-term drying off (extending the dry phase) and lactational or dry cow treatment. Mastitis treatment and its failure, with an emphasis on antimicrobial therapy, are the main focus of this paper. Pharmacokinetic properties of the product formulation used for mastitis treatment are not discussed.

The successful use of antimicrobial drugs for mastitis treatment depends on the same basic principles that apply to all microbial infections:

1. Selecting an antimicrobial agent that is effective against the target MCOs.
2. Attaining and maintaining therapeutic concentrations of the drug at the infection site until the infective MCOs are suppressed and/or inhibited.
3. Minimising local or systemic side effects of therapy, and
4. Coordinated administration of supportive, non-antimicrobial therapies as considered relevant in each case¹.

Despite an appropriate choice of antimicrobial, mastitis treatment often fails. Current treatments used for clinical mastitis during lactation often have a poorer cure rate than predicted by *in vitro* susceptibility, especially in cases of mastitis caused by *Staphylococcus aureus* which, as a chronic infection, is responsible for huge economic losses. Bacteriological cure of *S. aureus* infections during lactation has been estimated at a rate of only 25% - 50%. Antimicrobial resistance of MCOs is commonly not the predominant factor in treatment failure^{2, 3}. The explanation for unsuccessful treatment outcomes must therefore be elucidated from other factors which contribute toward therapy efficacy.

There are four major groups of factors associated with bovine mastitis treatment failure:

1. Management and iatrogenic factors

2. Drug factors
3. Mastitis-causing organism factors, and
4. Mammary gland factors.

Additionally, herd, cow and quarter level factors can also affect the results of bovine mastitis treatment.

Management and iatrogenic factors

Many management and iatrogenic factors can be the reason for mastitis treatment failure. Commonly listed include:

- inaccurate diagnosis
- delayed initial treatment
- improper dose or treatment regime
- improper route of administration,
- depth of insertion of the infusion cannula
- duration of treatment
- inadequate supportive treatment, and
- failure to ensure treatment is performed according to best practice, e.g. in suitably aseptic conditions

Inaccurate diagnosis, delayed initial treatment and assessment of cure

Quality and precision of mastitis diagnostics, and time elapsed between diagnosis and treatment initiation are important factors affecting the cure rate of intramammary infections. In many countries, excluding the Nordic countries of Europe, mastitis diagnosis and antimicrobial treatments are responsibilities assigned to farm personnel. Treatment of mastitis in such cases is often empirically based on a presumptive cause and perceptions of historical efficacy. Inaccurate diagnoses (*i.e.* over-diagnosing, treating sub-clinical mastitis as clinical, and teat canal infections) lead to inappropriate therapy and consequently, to overuse of antimicrobials. Overuse of antimicrobials is associated with an increased risk of development of broad-scale antimicrobial resistance and unnecessary loss of milk/income.

To decrease the risk of failure, treatment of mastitis should commence immediately upon appearance of clinical signs. Prompt treatment inhibits the progression of invasive mastitis (*i.e.* becoming chronic or abscess-forming) and increases treatment success rates⁴. Failure to treat mastitis early increases risk of treatment failure as some MCOs transgress from the gland, into the protected environment within cells including mammary epithelial cells and neutrophils. Additionally, accurately defining a 'cured' status in the field is largely circumstantial and presumptive. Therefore, the perception of farm personnel of 'how well' a particular treatment works on a given farm can be misleading, with antimicrobials frequently used without valid justification.

In many cases, particularly with staphylococcal (e.g. *S. aureus*) and some streptococcal (e.g. *Streptococcus uberis*) infections, mastitis treatment results in a clinical cure but microbiological cure is not achieved. At present, diagnostic methodologies for bovine mastitis are not technically rigorous, and translation from a clinical to a subclinical state is often mistakenly considered as a cure.

In the assessment of cure and repeated cases, the principles of super-infection and re-infection are important. Super-infection can occur during intramammary treatment if the cannula is contaminated or there is poor sanitation of the teat end prior to treatment. Often, the secondary intramammary infection, caused by the newly introduced organisms, results in a worse clinical implication than if no intramammary treatment was administered at all, particularly if the new MCOs are *Pseudomonas aeruginosa*, *Candida* spp, and *Nocardia* spp. Re-infection of treated quarters is a common

explanation for failure of the mastitis treatment in practice, and in studies reporting efficacy of products for treatment of mastitis. The mechanisms promoting re-infection can be summarised as:

1. Treatment or the mammary defence mechanisms were not sufficiently effective. Thus, an inoculum of MCO has remained.
2. Trauma of the teat canal or teat end. This will be discussed later.
3. Advancement into deeper/higher recesses of the gland by the MCOs. The ultimate potential for vertical dispersal and penetration by the antimicrobial in the upper parts of the mammary gland is poor. This is compounded by cytotoxic effects which incite a local inflammatory reaction.
4. Increased susceptibility of the gland to re-infection. This will be discussed later.

Prevention: Bovine practitioners should educate their clients of the:

- importance of accurate diagnosis
- importance of prompt and appropriate treatment
- differentiation of sub-clinical and clinical mastitis
- differentiation of teat canal infection and clinical mastitis
- concepts of super-infection and re-infection, and
- assessment of treatment outcomes

The best approach to improved treatment outcomes and general mastitis management is a combination of maintaining good, ongoing communication with dairy clients about their farms' current udder health status, and regular focus events (usually semi-annually or annually), e.g. discussion groups or farm-based short seminars.

Improper dose and route of administration, depth of insertion of infusion cannula and duration of treatment

The success of antimicrobial therapy depends on early intervention which results in appropriate drug concentration at the infection site. Thus, administering a lower dose than required or parenteral administration in a dehydrated or shocked cow is inadvisable. This scenario can lead to low drug concentration at the site of infection, below the minimum inhibitory concentration (**MIC**). It is worth mentioning that the dose of antimicrobial within currently commercially available intramammary preparations may now need some revision. Many of these products were first registered in the 1970s and 1980s. Dairy cows of that era produced less than half of what they do today. Additionally, the dose of antimicrobial used in intramammary preparations should follow susceptibility changes which influence MICs of mastitis pathogens. Unfortunately, no dosage adjustment in these antimicrobial products has been made, in light of increased milk volume production or changes in the MICs.

Pharmacological treatment of bovine mastitis can be administered intramammary or systemically. Systemic treatment is generally parenteral, to avoid disturbance to the rumen and gastro-intestinal micro flora. Following intramammary administration, the concentration of antimicrobial in the milk compartment of the mammary gland is expected to be higher. These higher concentrations are achieved using smaller amounts of the active substance, as the drug is administered directly to the infection site. The intramammary route of administration is preferential for the treatment of mild cases of mastitis which don't result in 'sick-cow syndrome'⁵). In practice, the intramammary route of administration is used when one or two quarters in the same cow are concurrently affected. Parenteral therapy becomes a recommended addition to the treatment regime when significant oedema or inflammation is a feature of mastitis. The potential swelling-associated occlusion of milk ducts may impede the distribution of intramammary administered antimicrobials^{1, 6}. Additionally, parenteral treatment should be used when the general health of the cow is impaired^{1, 5}. In practice, the parenteral

route of administration is also preferentially used when two or more quarters are affected at the same time.

Pharmacokinetics of the active requires some consideration when selecting the route of antimicrobial therapy. In particular, parenteral therapy requires administration of higher doses of the antimicrobial active due to distribution of the drug throughout the body of the cow, prior to reaching the mammary gland via the bloodstream. A combined treatment approach (concurrent administration of antimicrobials by intramammary and parenteral route) may insure somewhat against achieving only sub-therapeutic concentrations of the active at the infection site. Therefore, it is a commonly used treatment tactic for mastitis that features swollen, hard quarters, or when treating repetitive cases.

Full insertion of the cannula tip through the teat canal has been shown to reduce effectiveness of mastitis treatment. The intramammary cannula tip can transfer microbes from their harbour at the teat end or among keratin which lines the teat canal, riding into the teat cistern. The proportion of new intramammary infections induced this way can potentially be reduced by over 50% by only partially inserting the cannula (e.g. only into the distal 2-3mm of the teat canal). Damaged teat ends or teat canals are easily colonised by MCOs, particularly staphylococci, which can then re-infect the gland at any time. Partial insertion can minimise teat canal injury, and as a result, disturb keratin plug formation during the early dry period. Maintaining the integrity of the teat ends and canals is a crucial component of the defence mechanisms of the udder.

Field trials with commercial antimicrobial products have demonstrated higher rates of mastitis cure when duration of treatment is extended past traditional convention. This is particularly important for the time-dependent antimicrobials. Extended treatment periods promote maintenance of therapeutic concentrations of antimicrobial at the infection site for longer. Better distribution of the antimicrobial throughout the gland results. Higher cure rates associated with extended treatment is likely due to extended contact time between the antimicrobial and MCOs⁷. Negative aspects of prolonged treatment duration include the additional costs associated with the antimicrobial product, extra discarded milk and labour, increased risk of introducing super-infection and risk of breaching withholding periods. The costs and benefits should be carefully considered when deciding if the extended therapy is a worthwhile option.

Prevention: Bovine practitioners should educate their clients of the:

- importance of treating with full dose for sufficient length of time
- importance of proper administration of drugs (including route of administration, hygiene and the optimal depth of insertion of the cannula)
- choice of route of treatment according to presenting signs
- compatible products for combined approach, and
- action in case of treatment failure

The best approach to prevention of improper dose and route of administration is through preparing treatment protocols (e.g. standard operating procedures) made specifically for each farm.

Inadequate supportive treatment

Inflammatory change in the mammary gland can impair the drug distribution. The use of supportive treatment (e.g. anti-inflammatories) can decrease swelling and provide better distribution of the drug. In some cases, such as coliform mastitis, the supportive treatment is a priority to the antimicrobial treatment of mastitis. For success in treating mastitis associated with the 'sick-cow-syndrome', it is essential to have the general condition (e.g. shock, toxæmia, dehydration, septicaemia) of the cow addressed.

Prevention: Bovine practitioners should educate their clients of the:

- importance of supportive treatment for the treatment success and survival of any sick cattle

The best approach to on-farm improvements to supportive treatment and care for acute cases is clear, easily interpreted definitions and instructions of guidelines within the treatment protocols. In the event of any signs of systemic infection, it should be standard protocol to provide parenteral therapy and pain management for promotion of better animal welfare, higher likelihood of therapy success and for faster recovery from mastitis.

Drug factors

Many antimicrobial, vehicle and/or formulation factors are associated with failure of the mastitis treatment and discussing these is out of the scope of this paper. Most of the factors from this group are due to the pharmacokinetic characteristics of the active and the effects of the formulation design on these properties. The common factors include:

- Low bio-availability
- Inadequate local tissue concentration
- Weak or excessive passage of drug across the blood-milk barrier
- High degree of milk and serum protein binding
- Improper antimicrobial selection
- Antagonism between concurrently used antimicrobials
- Extent of drug dissociation in regard to milk pH and composition changes with mastitis
- Short half-life of the drug
- Side effects of the drug
- Other factors that lead to inactivation of the antimicrobial *in vivo* or *in vitro*

Factors related to MCOs

Many factors related to MCOs can be the reason for mastitis treatment failure. Commonly listed include:

- tissue invasive or intracellular-dwelling pathogens
- microbial mechanisms that overcome antimicrobial effects in milk
- drug tolerance and resistance
- short lived mastitis-causing organisms in the mammary gland
- microbial dormancy and metabolic state, and
- 'L' form of MCOs

Unfortunately, many of these factors are difficult to predict and control, and are little known about on dairy farms.

Tissue invaders or intracellular location

Tissue invading organisms, such as coagulase-positive staphylococci, become walled off in the udder parenchyma by creating thick fibrous scar tissue encapsulation within deep-seated abscesses. They can also gain refuge within the acid phagolysosomes of macrophages and neutrophils. Similarly, some strains of *Str. uberis* seek refuge among the epithelial, macrophage, and/or secretory cells of the mammary gland⁸. Additionally, chronic *S. aureus* infections pose therapeutic problems through promotion of localised avascular scar tissue, meaning that parenteral therapy probably provides little benefit⁵. Therapy may kill the organisms that are not walled off, but at a later date, the organisms within the scar tissue can break out, multiply, cause additional damage to the udder secretory tissue and promote further formation of scar tissue. Consequently,

when antimicrobial treatment is administered, antimicrobials cannot reach the MCOs and treatment failure may occur even when the organisms are susceptible to the used antimicrobial.

Microbial mechanisms that overcome antimicrobial effects in milk

Microbes often survive due to their ability to rapidly acclimatise to environmental conditions. Evasion strategies for escaping antimicrobial factors include encapsulation or slime formation, receptor-mediated absorption of host proteins, interference with phagocyte function, production of enzymes capable of digesting antimicrobials and other inherent factors. Other mechanisms include leukocidin production; adherence of bacteria to tissue lining the gland that results in avoidance of the wash-out effect during milking; buoyancy of microbes in cream, and an increase in microbial replication rate. These factors are additional to the drug tolerance and resistance.

Drug tolerance and resistance

Drug tolerance and resistance are usually characteristics of bacterial species or strains. Global, widespread use of antimicrobials in mastitis treatment has raised questions over the prevalence of antimicrobial resistance genes in mastitis-causing organisms flourishing in preference to 'susceptible' genes. Selection of therapy for resistant organisms may necessitate use of a different antimicrobial. Even though widely varying results of the efficacy of antimicrobial treatments have been reported, there is no real evidence that antimicrobial resistance poses an emerging crisis in bovine mastitis pathogenesis and treatment^{9, 10}.

Selecting an ineffective antimicrobial agent, such as penicillin to treat β -lactamase-producing *S. aureus* or *Bacteroides fragilis*⁵ can result in treatment failure. Therefore, it is the opinion of the author that antimicrobial susceptibility testing should be used to make 'herd profiles' regarding resistance. The choice of an inappropriate antimicrobial should not be an excuse for failure of the mastitis treatment, particularly when antimicrobial susceptibility testing is readily available and not financially significant. However, due to the diverse source of MCOs, the 'herd profile' can have limited merit in a herd affected with environmental mastitis.

Short lived mastitis-causing organisms in the mammary gland

Some MCOs, particularly coliforms, are short lived in the mammary gland. Thus, antimicrobial therapy may be of secondary importance in comparison to immediate supportive treatment for systemic conditions (e.g. endotoxic shock). The acute illness that regularly accompanies mastitis caused by a coliform organism can distinguish them as the likely causatives, thereby guiding the actions and therapy decisions of the facilitated stockperson at cow-side.

Microbial dormancy and metabolic state and 'L' forms

Mastitis-causing organisms are most susceptible to antimicrobials during their exponential growth phase. Non-multiplying organisms are not susceptible to most antimicrobials due to their mode of action. All microbial populations contain some organisms that are not in the active growth phase, which therefore survive. Bacteria exposed to antimicrobials may become growth-inhibited, and can remain so for some time after the termination of therapy⁵. Low multiplication rates are also seen in organisms within phagocytes, particularly important for *S. aureus* infections.

Sometimes certain organisms develop an acapsular 'L' form that is contained only in a cell membrane. Such L-forms are not susceptible to antimicrobials such as penicillins and cephalosporins that attack the cell wall, even when the drug concentrations and the contact time are sufficient⁵.

Mammary gland factors

Some factors related to the mammary gland itself can be the reason for mastitis treatment failure. Commonly listed include:

- teat canal infections
- trauma
- adverse effects of treatment products
- uneven distribution and physical obstruction of the drug within the gland
- udder tissue necrosis
- circulatory inhibition due to inflammation

Unfortunately, many of these factors at the current state of knowledge and with cow-side technology are difficult to identify, predict or control, aside from appropriate use of anti-inflammatories.

Teat canal infections

Standard methods of intramammary antimicrobial administration into a mastitis quarter or as dry cow therapy do not necessarily eliminate teat canal infections. MCO's harboured in the teat canal serve as a potential reservoir for later infection of parenchyma of the mammary gland. After dispensing antimicrobial therapy into the gland, the resident teat canal infection may then facilitate a new, super - infection, or be a source for re-infection.

Trauma and adverse effects of treatment products

Trauma or adverse reactions associated with the therapeutic product result in decreased capacity of the mammary gland defences. This predisposes the quarter to infection or re-infection. Trauma of the teat canal and teat end has been discussed previously, within the management and iatrogenic factors associated with failure of mastitis treatment. The tissues of the mammary gland can be irritated by the antimicrobial or additives in the preparation, such as vehicle or thickeners. Irritation exacerbates the inflammatory process, and further weakens potential for distribution of the drug through the intricate structure of the mammary gland. Biological response to the antimicrobial or its vehicle can decrease defence powers of the mammary gland (e.g. altered oxidative 'burst' activity of bovine polymorph-neutrophils, resulting in their use as concealment by intracellular MCOs).

Uneven distribution and physical obstruction

In all cases of mastitis, oedema and inflammatory products obstruct the diffusion of antimicrobials to some extent, by compression or blockage of the milk duct system. Necrosis of the affected area of the gland and abscess formation also results in a similar effect. The diffusion of antimicrobial products throughout the gland is thereby impaired. For this reason, it is often very difficult to ensure that antimicrobials have good contact with MCOs, particularly when administered via the intramammary route⁶. It has been proposed that systemic administration of the antimicrobials may overcome these problems¹.

Udder tissue necrosis

Mastitis which causes udder tissue necrosis leads to a poor blood supply to the affected areas, and consequently, a decreased redox potential that favours anaerobic MCOs. There is no effective passage of drugs into necrotic, avascular udder tissue.

Circulatory inhibition due to inflammation

Oedema throughout the tissues of the mammary gland can impose constriction of vasculature throughout the infected, mastitis udder. While all therapeutics indicated for systemic use against mastitis are able to cross the blood/milk barrier to some extent, the carriage of the drug in blood, as within the gland, becomes compromised where it is needed most. As a result, in the case of advanced swelling/oedema, even the combination of intramammary and systemic antimicrobial therapy can be ineffective.

Herd, cow and quarter factors

An important part of the cumulative bovine mastitis strategy, whereby we seek to minimise the use of antimicrobials, is to refrain from treating cases with a poor prognosis at the outset. As discussed previously, the probability of cure can be estimated, and is significantly influenced by cow and quarter factors, such as: age or parity, number and location of quarters affected, days in milk or lactation stage, number of positive samples and colony-forming units prior to treatment, and somatic cell and dry-cow therapy history before treatment initiation.

In general, higher parity, sustained higher somatic cell level and increased numbers of positive samples or colony-forming units prior to treatment are associated with a lower cure rate. Additionally, cure rates are lower with increased number of quarters, and when the rear quarters are affected.

Significantly lower treatment success in older cows or cows of higher parity is likely due to morphology of the mammary gland, and decreased general resistance to infection. Older cows are more likely to have experienced clinical or subclinical intramammary infections and have changes in morphology of the mammary gland that factor for decreased cure rates. Older cows in general terms have larger tissue volume within their mammary glands compared to heifers. The extent of affected tissue is also greater when more than one quarter is affected, or when rear quarters are affected. The volume and larger mass of mammary gland can influence drug distribution and subsequently, the concentration of drug penetrating to the target/infection site.

Selection of cows for treatment

The probability of a cure can be estimated prior to treatment. There are many factors associated with a successful cure. The following are the most important: age, lactation stage, causative organism, quarter location and level of individual cow somatic cell count (ICSCC) history prior to initiation of the treatment. For example, Schukken *et al.*, (2007) provides the following calculations:

Scenario 1) Older cow, treated at 150 days in milk, infected with *S. aureus* in a hind quarter with a SCC of 2,000,000 cells/mL has approximately 1% chance of cure.

Estimated probability to cure = $(1/1[1 + \exp(-1 \times (0.40-1.25-1.05-1.53-0.95))]) = 1\%$

Scenario 2) Heifer, treated at 220 days in milk, infected with *S. aureus* in a front quarter with a SCC of 500,000 cells/mL has approximately 61% chance of cure.

Estimated probability to cure = $(1/1[1 + \exp(-1 \times (0.40))]) = 61\%$

(Calculations reproduced with permission from the author – 11th April 2007)

A similar predictive model is described by Bradley *et al.*, (2005) in the New Zealand Veterinary Association, Dairy Cattle Veterinarians Proceedings (available on-line for AVA members) to aid in the decision to treat sub-clinical mastitis.

Prediction of the probability of cure is vastly underutilised by pharmaceutical companies, farmers and veterinarians alike in the identification of the most suitable candidate animals for therapy. As a result, appropriate culling decisions regarding cows

with an incidence of lactational infection has been a sub-optimally employed management tool in practice^{4, 10}.

Cow's general condition

Sick or dehydrated cattle are prone to have reduced circulation. This can have an effect on the normal course of systemic distribution of drugs and the rate of elimination of drugs. Thereby, overt illness can result in prolonged duration of detectable levels of the drugs in tissue and milk.

Previous mastitis history

Cows that have suffered previous mastitis episodes are at increased risk of re-occurrence and generally have lower treatment success rates. Reasons for this increased risk include changes to defence mechanisms of the mammary gland. There is increased probability of chronic changes within the udder, resulting in weakened vascularisation and consequently, impaired drug distribution.

Herd level factors

Additional factors associated with bovine mastitis treatment failure include some herd level variables such as hygiene, drug storage conditions, bulk milk somatic cell count level, number of new infections as judged by increase in somatic cell count and observed prevalence of *S. aureus* in the herd. In herds with high prevalence of *S. aureus* infection the risk of new intramammary infections or reinfection is higher.

Prevention: Bovine practitioners should educate clients in regard to:

- Best-practice selection of cows for treatment or culling
- Identifying and avoiding treatment of teat canal infections
- Appropriate storage conditions for drugs
- Role of general management practices in reducing mastitis incidence
- Impacts of high mastitis prevalence, and
- Importance of the incidence of new infections as related to treatment outcomes.

Conclusion

Failure of mastitis treatment is a common problem in clinical practice. It is associated with the following groups of factors: management-, iatrogenic-, drug-, mastitis-causing organism-, and mammary gland-related factors. Strategy gaps associated with any of these factors results in inappropriate therapy via choice of antimicrobial, inadequate concentration of antimicrobial at the site of infection, and/or an inadequate at-concentration duration than required. Additionally, herd, cow and quarter level factors can also affect the reliability of successful treatment of bovine mastitis. Treating cases with a poor prognosis will increase the risk of treatment failure.

Veterinarians should take an active role in the education of farmers in the treatment and management of bovine mastitis. Management and iatrogenic factors can be easily influenced. The value of veterinarians fostering good communication and promoting approachability in this regard cannot be underestimated.

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