Technote 4

Detecting and managing clinical mastitis at calving

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1 What is clinical mastitis?

Clinical mastitis is an inflammation of the mammary gland, usually caused by bacterial infection, with associated changes to milk composition such as wateriness, clots and/or discolouration that persist for more than three squirts of milk, with or without heat, swelling, or pain in the mammary gland.

Mastitis may be categorised as:

- **mild** (i.e., only changes in milk),
- **moderate** (i.e., changes in milk and the mammary gland), and
- **severe** (i.e., changes in the milk, mammary gland and systemic signs of disease such as fever, anorexia, depression, dehydration).

Clinical mastitis remains common in dairy herds with the median lactation case rate in New Zealand being ~15 per 100 cows (McDougall *et al* 2007b). It has significant negative welfare impacts, is costly, and disrupts milking routines. Cases that are missed may markedly increase the Bulk Milk Somatic Cell Count (**BMSCC**) because they produce very high numbers of somatic cells in their milk.

The incidence of clinical mastitis is commonly highest in early lactation due to an increased risk of new infections across the dry period and immunosuppression around calving (LeBlanc 2020).

➜ **Technote 10** details how to rapidly detect and treat clinical cases in lactating cows.

Detection of clinical mastitis is improved with increased frequency of fore-stripping or use of in-line diagnostic tools. Therefore, advisers need to consider the processes used to detect mastitis when diagnosing a herd situation. Early detection and treatment of clinical mastitis may reduce the risk of severe and intractable cases developing and the transmission of infections.

The **"trigger for action"** is 5 clinical cases per 100 cows (all ages) calved within 14 days of calving. The DairyNZ **Mastitis Focus report** provides a comparison of a herd's clinical mastitis performance with triggers for action.

Note that validation of in-line and cow-side diagnostic technologies in the absence of accepted gold standards is still required in the NZ context to identify the most appropriate intervention point for antibiotics and other treatments, in cases detected by foremilk stripping, in-line tests, or other diagnostic methods.

② The cost of a clinical case of mastitis

Many studies have been performed around the world to estimate the costs associated with mastitis (Hogeveen *et al* 2011). Factors that contribute to these costs include:

- antibiotics and other supportive treatments,
- discarded milk,
- ongoing production losses including losses due to cows or quarters that have to be dried off early,
- penalties from exceeding regulatory BMSCC limits or inhibitory substance violations,
- labour costs for management of infected cows,
- costs associated with ongoing diagnoses and control,
- costs associated with reduced fertility, increased culling, unexpected deaths, and replacement of infected cows.

The DairyNZ **Mastitis Gap Calculator** allows calculation of the potential economic benefits associated with reducing BMSCC, clinical mastitis incidence and culling for individual herds.

The basic calculator assumes an average treatment period 1.5 days (i.e. three tubes at 12 hours), a three day milk withhold, average daily milk solids production of 1.3 kg cow per day, a production loss of 4% across the remaining lactation, a 3% increase in risk of culling, and a total labour cost of 15 minutes to treat the cow.

③ How to find clinical mastitis

Cows in the colostrum group (first 8 milkings) should have their teats and udders palpated, and foremilk checked at each milking, ideally at cups on and cups off. Later in lactation, visual assessment for swollen quarters, and signs of heat and pain, should occur at least daily. Identification of asymmetry amongst quarters should be followed by visual inspection of milk and palpation of the gland and teats.

The glandular tissue of the udder can be palpated superficially and deeply with the flat of the hand and fingers (Donovan *et al* 1992). Acute cases may be hot, swollen or painful but clinical mastitis cases with less obvious changes will require a more thorough examination to assess the consistency of udder tissue. Chronic changes usually manifest as fibrosis, which can be felt as firmness that is local (from pea to fist size) or diffuse (giving the quarter a firmer feel than its opposite number and usually a more nodular surface). Long-standing infections can ultimately result in atrophy (shrinking) of the mammary tissue as it becomes non-functional.

The teat can be palpated with the fingertips by gently rolling it between the thumb and first two fingers. Teat theilitis (inflammation of the internal tissues of the teat) is defined as a hardening or thickening of the internal surface of the teat sinus.

Foremilk inspections are used to detect abnormalities in the milk, such as wateriness, clots or flecks, or more obvious abnormalities such as flakes, discolouration and blood. 'Strings' of material hanging from teat-ends may also be detected; these are inflammatory debris expressed during milking and may be more obvious to the 'cups-off' operator.

Cows should be defined as having clinical mastitis (and require treatment) when there is an observable abnormality of the milk (e.g. wateriness, clots, and/or discolouration) that persists for more than three squirts of milk. To improve sensitivity of detection, strip onto a black or dark surface e.g. strip cup, dark RMT paddle, black plastic.

➜ The DairyNZ **Healthy Udder Guide** is a practical resource for farmers with step by step instructions for:

- Foremilk stripping cows
- Performing a Rapid Mastitis Test (RMT)
- Preparing teats aseptically before sampling a gland or administering treatments.

Note that:

- for cows with abnormalities in the first squirt of milk, followed by milk that is visibly normal, these should be marked and checked again for the next few milkings.
- for cows that received internal teat sealant (**ITS**) at dry off, flecks of teat sealant can be mistaken for clots. The withholding period of ITS requires that each quarter is stripped for 10-12 strips at the first milking after calving, to help reduce residues.

Before cows are moved out of the colostrum mob, every cow should also be tested with a Rapid Mastitis Test (**RMT**) to check for subclinical mastitis. This test should only be used at the last 1-2 milkings in the colostrum period as falsely high results can occur, due to presence of colostrum at earlier milkings. In the absence of clinical signs, RMT positive cows should be withheld from supply and rechecked daily.

As lactation progresses, cows at high risk of mastitis should be stripped regularly (at a minimum, weekly). These cows can be identified by leg bands or electronic records. These include cows that:

- have not milked out,
- have had a clinical episode of mastitis within the last month,
- have positive RMT quarters,
- have been identified as suspect cows using in-line detection systems, or
- have had high Individual Cow Somatic Cell Counts (**ICSCC**) recently.

➜ **Technote 5.3** describes routines for regular foremilk-stripping of the whole herd.

Mastitis Detection Systems

Commercially available in-line mastitis detection systems use technologies to detect changes in the milk, and include:

- electrical conductivity
- automated RMT
- colorimetric detection or
- algorithms based on sudden reductions in milk yield.

These systems vary widely in their sensitivity, and there is an inverse relationship with specificity (Kamphuis *et al* 2016; Bausewein *et al* 2022), meaning higher sensitivity often results in more false positives. Validation information should be sought prior to investment.

The validation of these systems is influenced by the gold standard used. Kamphuis *et al* (2016) defined clinical mastitis as the presence of clots using in-line mesh filters at two out of three sequential milkings, while Bausewein *et al* (2022) used single-time-point examinations by trained technicians. The sensitivity and reliability of these gold standards themselves are not well established, which limits the interpretation of validation results. Therefore, before investing in sensor-based mastitis detection systems, it is essential that farmers are encouraged to critically evaluate the validation methodology and performance data to ensure suitability for herd-level decision-making.

Antibiotic therapy should only be considered for cows with visible clinical signs of mastitis. All other cases should be monitored for clinical signs of mastitis over the next few days.

④ Use bacteriology tests to identify mastitis pathogens

Milk cultures and other bacteriological tests may be used to:

- define the likely aetiology of mastitis cases on a routine basis i.e., when using pathogen-based treatment protocols,
- assess likely pathogens at herd-level during mastitis investigations,
- support veterinarians when authorising antimicrobial use on-farm.

Virtually all mastitis is caused by bacterial infection. Nearly 300 different bacterial species have been isolated from milk but less than 100 are associated with clinical mastitis (Kurban *et al* 2022). Internationally the five most common clinical mastitis isolates are:

- *Escherichia coli*
- *Streptococcus uberis*
- *Streptococcus dysgalactiae*
- *Staphylococcus aureus*, and
- *Klebsiella pneumoniae.*

In pasture-based New Zealand production systems, *Strep. uberis* is the most common isolate from clinical mastitis over the dry period (McDougall and Castle 2021) and across lactation (McDougall*et al* 2007b). However, there is variation amongst herds as to the predominant pathogen so appropriate testing should be undertaken.

Testing of milk samples from clinical cases

It is not possible to determine the organisms responsible for a case of mastitis without bacteriological testing. Samples from cases of clinical mastitis can provide useful information on:

- Pathogen identification for epidemiological assessment to optimise control and therapy advice for a farm,
- Antibiotic sensitivity testing to optimise therapy.

It is good insurance to collect samples from all quarters with clinical mastitis and store them in the freezer. These can be submitted for testing if:

- a cow fails to respond to treatment,
- there is concern about the type of bacteria causing the mastitis, or
- there are a higher number of mastitis cases than expected.

Sampling strategy

The selection of cows for milk sampling depends on the purpose of the mastitis investigation and the diagnostic method used. For outbreaks of clinical mastitis in newly calved cows, samples should be taken from those clinical cases, as isolates from high SCC cows or recurrent cases may not reflect the current aetiology.

The number of samples required varies with the herd size and investigation goals. For general herd-level insights, a minimum of 20 samples is recommended. However, to confidently declare a herd free of a specific pathogen (e.g. at <5% prevalence), up to 155 cows may need to be sampled in a 500-cow herd, assuming test sensitivity of 95% and specificity of 99%.

Mastitis aetiology and antimicrobial sensitivity patterns can shift over time, so sampling should be repeated each lactation. Sampling recurrent clinical cases may help identify the original pathogen or reveal poor treatment hygiene, especially when unusual organisms like *Nocardia*, *Prototheca*, or *Pseudomonas* are involved.

Sample collection

Aseptic technique and proper sample handling are critical for accurate mastitis diagnostics. Contamination or bacterial overgrowth can compromise culture-related results, making interpretation difficult.

> A sample is typically considered contaminated if three or more colony types are isolated from a single quarter.

Common sources of contamination include water, mud, faeces, and skin contact - either from the cow or the milker. To minimise these risks, milk samples should be collected aseptically and either cooled and delivered for testing within 24 hours or frozen immediately after collection for later submission or analysis.

It is essential for advisers to ensure the milkers' sampling technique is satisfactory – a physical demonstration is often very helpful, supported by the **DairyNZ Healthy Udder guide**, which provides step-by-step instructions.

Storage and handling of milk samples

Most bacteria that cause mastitis survive refrigeration for several days or freezing for several weeks. *Nocardia* species are an exception to this general rule, as storage of samples for only a few hours or freezing can reduce the likelihood of isolating these organisms.

The survival of *Staph. aureus*, *Strep. agalactiae, Strep. dysgalactiae* and *Strep. uberis* was not impaired in milk samples that were stored in a commercial freezer at -20˚C for 6 weeks (Murdough *et al* 1996) or 16 weeks (Schukken *et al* 1989). Other studies have found a variable effect on streptococci, especially *Strep. dysgalactiae* (Luedecke *et al* 1972).

The survival of *Escherichia coli* and *Trueperalla pyogenes* can also decrease during freezing, with recovery rates for both pathogens decreasing by about 20% in samples frozen for four weeks (Schukken *et al* 1989).

Freezing may increase the detection of non-aureus staphylococci (Schukken *et al* 1989) and possibly *Staph. aureus*, possibly due to the release of intracellular bacteria after the destruction of leucocytes during the freeze-thaw process. Samples found to have no bacterial growth when cultured fresh may become positive after freezing.

Point-of-care or on-farm culture systems

Multiple culture-based point-of-care (POC) diagnostic systems have been developed to identify mastitis pathogens (Malcata *et al* 2020). These systems have supported increased farmer uptake through offering faster turnaround times, convenience, and cost-effectiveness. Importantly, their use has been associated with a 20-75% reduction in antimicrobial use (de Jong *et al* 2023). In New Zealand, two such systems have been evaluated and shown to reduce antimicrobial use by approximately 20% when used alongside defined treatment criteria (McDougall *et al* 2018b; Bates *et al* 2020).

No diagnostic test is 100% accurate. Results must be interpreted in the context of test performance, disease likelihood, and clinical findings. As Laven (2023) notes, veterinarians must avoid over-reliance on test results alone and instead integrate them with herd history, clinical examination, and knowledge of local disease patterns to make informed decisions.

Laboratory techniques

The *Laboratory Handbook on Bovine Mastitis* (Middleton *et al* 2017), published by the National Mastitis Council (NMC - The Global Milk Quality Organisation), provides detailed microbiological protocols for differentiating common mastitis pathogens and is available at **www.nmconline.org.**

Mastitis pathogen identification can be performed inexpensively through commercial veterinary pathology labs, in-house veterinary practice labs or on-farm point-of-care diagnostic systems. Care is required in interpreting data from different sources due to variation in laboratory techniques and the limitations of some tests.

Factors affecting the sensitivity and specificity of bacteriological diagnoses include:

- Methods for sample preparation, especially after freezing
- Choice of culture media
- Inoculation methods to ensure use of suitable combinations of inoculum volume and plate surface area
- Incubation temperature and times
- Tests used to differentiate isolates and laboratory definitions of bacterial genus or species
- Use of additional confirmatory tests such as MALDI-TOF or PCR (polymerase chain reaction).

Increased sensitivity of detection of bacteria where the number of colony forming units in milk is low may be achieved by increasing the inoculum volume onto the plate (e.g. streaking 25 µL or 50 µL of milk, rather than the standard 10 µL) and/

or reducing the number of colonies at which the sample is defined as test positive.

The sensitivity of conventional culture for detecting common mastitis pathogens varies among pathogens, by case definition and by sampling strategy (e.g. single vs. multiple samples across time, cow composite vs. quarter level samples)

For example, using isolation of a single colony as a definition of infection following plating of a 10 µL of milk, the sensitivity and specificity for *Staph. aureus* was 90.4 and 99.8% respectively (Dohoo *et al* 2011b), but sensitivity declined to 86.8 and 72.0% when the case definition was increased to >2 or >10 colony forming units respectively (Dohoo *et al* 2011b).

In another study, collection of two samples and interpretation in parallel (i.e. a positive being defined where the pathogen was isolated from either of the samples) resulted in a higher sensitivity but lower specificity than interpreting in series (i.e. isolate had to be present in both samples for the gland to be defined as test positive) (Dohoo *et al* 2011a).

Given these complexities, veterinarians should critically assess diagnostic results in the context of test performance, herd history, and clinical findings to guide effective mastitis management.

Reasons for 'no growth' after culture

Clinical cases of mastitis from which no growth is obtained are common. Between 10-50% of clinical mastitis milk samples have no pathogen isolated (de Jong *et al* 2023). Possible reasons can include:

- Low numbers of viable bacteria in the sample due to poor sample storage and handling. Use of a PCR assay, which does not require presence of viable bacteria, demonstrated that almost half of the samples that were culture negative contained bacterial DNA (Taponen *et al* 2009).
- Elimination of the infection by host defence mechanisms by the time that the milk sample is collected or during sample transport as milk contains many antibiotic antibacterial compounds such as lactoferrin, lysozyme, lactoperoxidase, complement and immunoglobulins. This is often suggested in the case of coliform infections.
- The number of bacteria present are below the limit of detection of the culture technique used.
- Intermittent shedding into the milk of the infective organisms e.g. *Staph. aureus.*
- Antibiotic treatment of the quarter prior to sample collection interfering with the ability to culture the infective organism. When submitting milk samples from cows that are not responding to treatment or are repeat cases, it should be noted if they have received antibiotics within 14 days of sampling.
- Contamination of the sample with disinfectant at the time of collection interfering with the ability to culture the infective bacteria.
- The pathogen may not grow under normal culture conditions. For example, standard bacterial culture conditions are unsuitable for the detection of obligate anaerobes, mycoplasma and fungi.
- The clinical signs of mastitis are due to non-bacterial causes such as toxic substances.
- Isolated bacteria may not be reported because they are not considered to be major mastitis pathogens. For example, non-aureus staphylococci are traditionally considered minor pathogens although they have been reported to cause clinical mastitis (Timms & Schultz 1987).

Antibiotic susceptibility testing

Antibiotic sensitivity tests show which drugs are not likely to be effective. Just because an antibiotic is effective at killing bacteria on agar plates in the laboratory does not mean it will have the same success in the cow.

The disc-diffusion method (Kirby-Bauer method) is the most commonly used antibiotic sensitivity test (AST) in veterinary laboratories. This involves applying antibiotic-impregnated discs to agar plates inoculated with a standardised bacterial suspension, followed by incubation for 18 hours and measurement of inhibition zones. Isolates are reported as susceptible, intermediate or resistant to the antibiotics that were tested. However, many of these clinical breakpoints are based on human isolates and human medicine treatment regimens.

Care is required extrapolating clinical breakpoints from human to veterinary medicine.

Broth microdilution testing provides specific minimum inhibitory concentrations (MIC) of antimicrobials that inhibit bacterial growth *in vitro*. This approach may provide more precise data to guide treatment decisions in the absence of clinical breakpoints relevant to a particular bacteria/antimicrobial combination.

A broth microdilution test for pooled *Staph. aureus* and *Strep. uberis* isolates from bulk tank milk is commercially available in New Zealand (McDougall *et al* 2018a) and a point-of-care diagnostic test also makes use of a similar approach (Jones *et al* 2019)

PCR testing for resistance genes involves testing isolates for the presence of genes associated with known resistance factors or enzymes. A commercially available test for the blaZ gene, associated with production of beta-lactamase by *Staph. aureus*, is currently available in New Zealand.

The correlation between antibiotic susceptibility test results and bacteriological cure varies amongst bacteria/antimicrobial combinations. For example:

- Presence of beta-lactamases, indicated by the presence of blaZ in *Staph. aureus*, was associated with poorer bacteriological cure rates with narrow-spectrum penicillins (Sol *et al* 2000; Steele & McDougall 2014).
- Bacteriological cure rates were higher for Gram-positive bacteria that were sensitive to cephapirin when treated with that compound, but across all isolates (i.e. Gram-positive and Gram-negative) there was no difference in cure rates at 14 and 28 d between sensitive and resistant isolates (Constable & Morin 2003).
- *Escherichia coli* isolates sensitive to trimethoprim/sulphonamide had a higher clinical cure than isolates not sensitive (89% vs. 75%) (Shpigel *et al* 1998).
- There was no difference in bacteriological cure rate for clinical cases treated with pirlimycin for Gram-positive isolates that were sensitive or resistant to pirlimycin (Hoe & Ruegg 2005).

Thus, AST results should be interpreted with caution, as *in vitro* inhibition does not guarantee *in vivo* efficacy.

For *Strep. uberis* there is evidence for increasing MIC for beta lactams due to mutations in the penicillin-binding proteins (McDougall *et al* 2020), but there is no evidence of reduced clinical or bacteriological cure rate following beta-lactam treatment. Hence, routine AST for individual clinical isolates may not be justified.

For *Staph. aureus*, penicillin resistance is relatively common and knowledge of penicillin sensitivity at herd level has utility for veterinary antimicrobial authorisation. Sensitivity of Gram-negative bacteria varies widely within and between species, and antimicrobial sensitivity should be tested when antimicrobial therapy is to be used.

⑤ Follow MRS T to clearly mark, record and separate cows to be treated

MRS T means:

- **Mark** – her with the herd's recognized identification system for a new clinical case.
- **Record** – her details in the recognized farm dairy system.
- **Separate** – her from the milking herd, and milk her once the delivery pipe has been removed or shut off from the vat.
- **Treat** – her with the appropriate treatment, according to the farm's Animal Health Treatment Plan.

Marking cows

Marking cows for treatment is the first step MRS T. This system greatly reduces the risk of inhibitory substances reaching the vat milk.

All milking staff should be familiar with the system used on each farm to identify cows that are to receive antibiotic treatment. Some methods for temporary cow identification are described in the Table below.

A separate identification system for marking cows that have received antibiotic Dry Cow Treatment (**DCT**) allows for easy recognition if cows re-join the herd in error and can help relief milkers or casual staff avoid making costly mistakes.

Methods of temporary identification

Method	Visibility	Durability	Ease of use
Velcro strips on legs	Excellent	Good	Easy to apply and remove
Insulation tape on legs or tail	Excellent	Good	Easy to apply and cut off NB. Tightly bound tape can cause impairment of blood circulation, resulting in tail amputation
Plastic hock strap	Excellent	Very good	Easy to apply and remove
Spray paint (non-scourable)	Variable	Good	Very simple
Tailpaint	Good	Excellent	Messy. Can paint over with new colour after treatment to avoid confusion
Paint stick/raddle	Good	Excellent	Simple; use like a crayon
Electronic ID	Variable	Good	Not be relied on as sole means

Record all details

The Ministry for Primary Industries' NZCP1: Design and Operation of Farm Dairies (MPI, 2024) describes the information to be recorded for each clinical case. Supplier contracts provided by milk processors detail their specific requirements for management and reporting of animal health treatments. Generally, these include:

- The unique animal identifier and affected gland(s),
- Condition or symptoms being treated,
- Veterinarian (if consulted)
- Treatment (name of product and dose)
- Date (and milking) treatment started, date of each treatment, and date (and milking) of last treatment,
- Date (and milking) milk returned to vat.
- Milking frequency during treatment.

Permanent records of clinical cases need to be held for up to 4 years. This can be done using paper systems or electronic systems provided by herd improvement organisations or dairy processors.

Advisers should assist farmers in developing a system, or Standard Operating Procedures (SOP), for detecting, identifying, recording, segregating and treating cows with clinical mastitis.

Advisers should also encourage dairy farmers to keep permanent records of clinical mastitis cases so they can manage individual cows and assess herd-level mastitis control. With this information, they can:

- make decisions about cows eligible for antibiotic DCT,
- make decisions about which cows to cull,
- identify 'suspicious' cows (if clots are found on the filter or bulk milk cell counts rise),
- assess the number of mastitis cases and their response to treatment,
- calculate the cost of clinical mastitis in the herd,
- identify risk periods (e.g. stage of lactation) for clinical mastitis,
- determine the main mastitis pathogen(s) in the herd, and
- review effectiveness of mastitis control and udder health on the farm.

Herd improvement databases can link clinical case information with details of a cow's age, production, ICSCC, previous clinical mastitis history, and previous treatment with antibiotic DCT and/or internal teat sealants. Use the DairyNZ **Mastitis Focus report** to analyse clinical records, in association with herd test data.

Separate cows from the milking herd before commencing treatment

Cows should be removed from the milking herd, as soon as clinical signs are detected, and before treatment commences, and at least 1 form of identification applied. Milk from these cases should be withheld from the vat and discarded (see section 10 below) according to the farm protocol.

Gloves should be worn when handling milk from cows with clinical mastitis, and when administering treatments. Gloves are easier than bare hands to rinse and sanitise after becoming contaminated with bacteria or antibiotics.

➜ **Technote 8.1** describes the benefits of hygienic milking practices, including wearing of gloves.

All treated cows should be run in a separate herd and in a secure paddock. These cows must only be brought to the farm dairy when all the milking cows have been milked and have left the dairy.

➜ **Technote 8.3** describes the advantages of segregating infected cows to reduce the spread of infection.

Prior to milking the treated cows, the operator must ensure that the milk delivery line is disconnected from the milk vat. If milk is harvested from cows under treatment or under a withholding period, and stored in a vat in the milk collection area, then this vat needs to be clearly labelled as not 'fit for supply' and the vat outlet must be locked to ensure that the milk cannot be collected by mistake.

⑥ Treat clinical mastitis with veterinarian-authorised treatments

The goal of treatment is to cure the infection (bacteriological cure), return the affected mammary glands to normal milk production (clinical cure), and minimise pain and suffering of the cow. Ideally, the treatment period should be as short as possible and there must be no risk of antibiotic residues entering the milk vat.

Staphylococci or streptococci cause more than 80% of clinical mastitis cases in the majority of New Zealand herds. Antibiotics are the basis of most treatment regimens and are administered by infusion into the affected quarter (intramammary route) or more rarely by intravenous, intramuscular or subcutaneous injection (parenteral or systemic routes).

Given the predominantly Gram-positive identity, and likely sensitivity, beta-lactam antimicrobials delivered by the intramammary route are the preferred first choice for mild to moderate clinical mastitis therapy in New Zealand. Where sensitivities allow, narrow spectrum penicillin should be the first choice. But where beta-lactamase-resistant *Staph. aureus* are likely present, broad-spectrum beta-lactam antimicrobials are required.

There is no evidence that use of highest priority or high priority antimicrobials for mild to moderate clinical mastitis associated with *Staph. aureus,* NAS or streptococci results in better bacteriological cure than 1st or 2nd generation cephalosporins, or beta-lactams (Nobrega *et al* 2020).

Non-steroidal anti-inflammatory drugs (NSAID) should be used for all clinical cases to reduce inflammation and improve cow comfort.

Supportive therapies such as oral or intravenous fluids may be warranted in severe cases. While frequent stripping out and oxytocin may aid milk removal, they have limited impact on clinical or bacteriological cure rates. However, farmers should be encouraged to milk mastitis cows twice a day to remove milk from mastitic quarters, despite the fact that antibiotics have been administered.

If clinical mastitis cases are treated without the benefit of prior bacteriological testing, treatment selection should be based on:

- severity of the case,
- history of treatment for the cow,
- previous bacteriological results for the herd,
- previous antibiotic susceptibility tests, and
- responses to treatment.

Bacteriological testing should be encouraged to establish the farm profile of mastitis-causing organisms and develop appropriate treatment and control protocols.

All treatments must follow label directions and veterinary authorisation, with strict adherence to withholding periods for milk and meat. Only use products registered for administration to food producing animals. Intramammary use of products that are not meant to be administered into the mammary gland should not be practised.

1. Intramammary antibiotics

Intramammary treatment is the preferred route of treatment due to the high concentrations of antimicrobial achieved locally and the reduced total mass of antimicrobials used (relative to parenteral treatment).

For time-dependent antimicrobials such as beta-lactams, extended therapy may be associated with higher bacteriological cure rate, particularly for hard-to-cure infections such as *Staph. aureus.* However, the trade-off is increased milk discard and treatment costs. In most cases extended therapy is not required or cost-effective for streptococcal infections.

Dry Cow Treatment preparations of antibiotics should never be used in lactating cows. Inadvertent use of antibiotic DCT requires milk to be discarded for extended periods of time.

2. Systemic antibiotics

Acute, or severe, mastitis cases may benefit from both intramammary and systemic antibiotics. Peracute cases often require systemic antibiotics and anti-inflammatory preparations, and possibly intravenous fluids. As such cases are often associated with Gram-negative infections, use of 3rd or 4th generation cephalosporins may be justified (Suojala *et al* 2013). The prognosis for peracute cases in cows with severe clinical signs (as indicated by body temperature, dehydration, etc.) is poor with the majority of cows dying or being culled, regardless of treatment (Wilm *et al* 2024).

Systemic antibiotics have the theoretical advantage that drug distribution is more consistent across the udder than intramammary infusions. However, to be effective, systemic antibiotic treatments must be absorbed from the injection site and pass from the blood into the udder. The major difficulty is penetration of the "blood-milk barrier". Experimental studies suggest that the tissue concentrations following parenteral treatment are substantially lower than those achieved by intramammary infusion (Ehinger & Kietzmann 2000; Ehinger *et al* 2006).

Only non-ionised, lipid-soluble, and non-protein-bound drug fractions cross the barrier effectively. Weak acids (e.g. penicillin G) are almost completely ionised in blood and have poor tissue penetration. On the other hand, penethamate hydroiodide achieves very high milk concentrations due to its lipophilic properties and hydrolysis to benzyl penicillin as it crosses into milk. Similarly, lipophilic macrolides like tylosin concentrate in the udder after systemic treatment and have been used for mastitis treatment (McDougall *et al* 2007a). Allowing for antibiotic sensitivity patterns, antibiotics with high milk-to-plasma ratios are most suitable for systemic administration (see Table from Anderson 1989).

A combination of systemic and intramammary therapy was found to increase cure rates amongst beta-lactam sensitive, but not beta-lactam resistant, *Staph. aureus* clinical cases (Taponen *et al* 2003), but no difference in bacteriological cure rate was observed for clinical cases due to Gram-positive infections, treated with parenteral or intramammary products (Kalmus *et al* 2014). Similarly, a randomised controlled study using culture-based therapy demonstrated noninferiority of intramammary treatment alone compared with combined intramammary and parenteral treatment with beta lactams (benzyl-penicillin intramammary and penethamate parenterally) for Gram-positive pathogens (Svennesen *et al* 2023). Given the greater antibiotic mass used in systemic therapy, routine combination treatment is not warranted.

For time-dependent antimicrobials, such as beta lactams, extending treatment duration can increase cure rates (Sol *et al* 2000; Oliver *et al* 2004; Deluyker *et al* 2005; McDougall *et al* 2022). However, extended therapy increases costs and milk discard, and modelling suggests it is only cost-effective for specific pathogens under certain economic conditions (Swinkels *et al* 2005; Steeneveld *et al* 2011).

Milk-to-plasma ratios of some antibiotics used to treat mastitis (Anderson 1989).

Antibiotic	Milk-to-plasma ratio
Trimethoprim	3.7
Lincomycin or Erythromycin	3.0
Tylosin	2.0
Tetracycline	0.7
Streptomycin	0.5
Ampicillin	0.25
Sulphadiazine	0.21
Penicillin G	0.15

3. Published cure rates of antibiotics

In a review of antibiotic treatment of clinical mastitis during lactation, Craven (1987) reported average cure rates for each antibiotic from scientific papers that stated the number of quarters treated and had rigorous bacteriological assessment. From these data it was not possible to draw firm conclusions about the relative effectiveness of different products given the wide range of cure rates for similar antibiotics.

A more recent review (Ruegg 2021) concluded there was insufficient evidence to demonstrate differences in bacteriological cure between antimicrobial products, and limited evidence for any benefit of antimicrobial treatment for mild-to-moderate clinical mastitis cases associated with Gram-negative organisms. Ethical constraints often prevent inclusion of untreated controls, obscuring the true benefit of antimicrobial therapy. Additionally, long-term outcomes such as recurrence, milk yield, and culling risk are rarely reported.

Nobrega *et al* (2020) found no improvement in bacteriological cure rate of *Staph. aureus* when using highest priority or high priority antimicrobials relative to highly important antimicrobials, suggesting no requirement for high priority antimicrobials when treating this pathogen. However, for severe Gram-negative infections, parenteral 3rd or 4th generation cephalosporins remain the preferred treatment, alongside supportive therapies (Suojala *et al* 2013).

In New Zealand clinical cure rates, defined as resolution of clinical signs, typically occur in 80-90% of cases. If more than 20% of cases require retreatment within 4 weeks, further investigation is warranted.

Bacteriological cure rates, defined by absence of the initial pathogen when sampling occurs 2-4 weeks post-treatment, occur in 70-90% of cases, with *Strep. uberis* cure rates in the order of 75-95%, and *Staph. aureus* closer to 25-35% (see Table on following page, McDougall 1998, 2003; McDougall *et al* 2007a, 2007b; Bryan *et al* 2016; McDougall *et al* 2019).

Factors that reduce bacteriological cure rate include:

- isolation of *Staph. aureus* (compared to other pathogens),
- older cow age,
- greater days in milk,
- multiple quarters affected, and
- presence of antibiotic resistance.

Bacteriological cure of clinical mastitis with different antibiotics in New Zealand

Report	Pathogen	Active ingredient	Route	Number of cases	Bacterial cure rate (%)
McDougall 1998	All	Penethamate	Systemic	157	76
		Penicillin/ Dihydrostreptomycin	Intramammary	185	85
	Strep. uberis	Penethamate	Systemic	126	82
		Penicillin/ Dihydrostreptomycin	Intramammary	151	84
McDougall 2003	All	Lincomycin/Neomycin	Intramammary	73	77
		Penicillin/ Dihydrostreptomycin		73	77
	Strep. uberis	Lincomycin/Neomycin		47	75
		Penicillin/ Dihydrostreptomycin		29	74
McDougall *et al* 2007a	All	Penethamate	Systemic	325	82
		Tylosin		334	84
	Strep. uberis	Penethamate		253	88
		Tylosin		235	90
McDougall *et al* 2007b	All	Penicillin	Intramammary	230	75
		Cefurozime		219	70
		Penicillin/ Dihydrostreptomycin		248	76
	Strep. uberis	Penicillin		117	91
		Cefurozime		92	95
		Penicillin/ Dihydrostreptomycin		115	96
Bryan *et al* 2016	All	Penicillin/cloxacillin	Intramammary	178	70
		Oxytetracycline / Oleandomycin / neomycin / prednisolone		187	63
McDougall *et al* 2019	All	Amoxicillin / clavulanic acid (3 x 12 hr)	Intramammary	75	73
		Amoxicillin / clavulanic acid (5 x 12 hr)		103	72

4. Pathogen-specific treatments

The causative bacteria are usually not known at the time of treatment so the choice of treatment is generally based on herd history, clinical judgement, and results of recent bacteriological testing.

When testing has been performed and the pathogen identity is suspected or confirmed, the antibiotic treatment selection can be more specific. Some features of treatment outcomes for specific pathogens are listed below:

Staph. aureus

- Bacteriological cure rate during lactation is low (about 30-60%) because *Staph. aureus* causes micro-abscesses in the udder, survives inside cells, and some forms are resistant to commonly used antibiotics (e.g. strains with the enzyme beta-lactamase are resistant to penicillin).
- The best hope for successful treatment is in young cows with recent infections (of less than two weeks duration) (Barkema *et al* 2006).
- Treatment of clinical mastitis may reduce *Staph. aureus* shedding, and result in milk returning to clinical normality.
- Use of extended therapy to increase cure rates for *Staph. aureus* has shown positive results (Oliver *et al* 2004; Deluyker *et al* 2005). Studies in New Zealand (Spatz Shelgren *et al* 2007), found that doubling the treatment regime from 3 to 6 tubes, administered at 12 hourly intervals, resulted in a 50% cure rate for *Staph. aureus* infections. This was significantly greater than the <30% cure rates observed for cows receiving the standard 3 tube treatment, which was not statistically different from no treatment. The economics of extended therapy in New Zealand has yet to be determined.

➜ **Technote 5** provides information on what should be considered when managing outbreaks of *Staph. aureus* mastitis.

Strep. uberis

- Bacteriological cure rates during lactation are in the order of 70-90%.
- A small proportion of cases are refractory to treatment. Research has found that field strains of *Strep. uberis* are able to invade and live in epithelial cells, which may partially explain why infections are refractory to treatment (Fessia and Odierno 2021).
- Antibiotic resistance is an unlikely explanation for refractory cases. The majority of *Strep. uberis* isolates that have been tested are sensitive to penicillin (>95%), oxacillin (>93%) and cephalothin (99%) (Petrovski *et al* 2011; McDougall *et al* 2014).
- Increases in MIC have been seen in *Strep. uberis* associated with mutations to the penicillin binding proteins resulting in lower affinity of all beta-lactams (McDougall *et al* 2020). The effect of these increasing MIC on clinical and bacteriological cure rate of *Strep. uberis* cases is currently unclear.

➜ **Technote 1** provides information on what should be considered when managing outbreaks of *Strep. uberis* or *E. coli* mastitis.

Escherichia coli

- Most cases due to *E. coli* will self-cure, in the absence of signs of systemic disease. Toxins produced by *E. coli* can lead to systemic involvement. To minimise the effects of toxin, use of anti-inflammatory agents and possibly intravenous fluids, may be helpful
- Prognosis remains poor for severely ill cows despite therapy, with a mean of 64% cows dead or culled in observational studies (Wilm *et al* 2024)

5. Use of anti-inflammatories

There is growing evidence to support use of non-steroidal anti-inflammatory (**NSAID**) products in the treatment of all cases of clinical mastitis, to reduce inflammation, alleviate pain, and improve cow comfort. Note however that NSAID vary in their efficacy, duration of activity, Cox 1/Cox 2 ratios, and milk withholding periods. Thus, care should be taken to not extrapolate likely efficacy derived from a randomised controlled study of one NSAID to another NSAID.

NSAID inhibit production of prostaglandins by inhibition of the cyclooxygenase (COX) enzyme. There are two isoforms of COX (COX-1, COX-2) that are constitutive (i.e. continuously produced) and induced, respectively (Lees *et al* 2004). NSAID reduce the inflammatory response, reduce pyrexia and have analgesic properties (Vangroenweghe *et al* 2005).

NSAIDs may be non-selective (i.e. inhibit both COX-1 and COX-2) or selective, that is inhibit COX-2 more than COX-1 (or vice versa). NSAID belong to different chemical groups including enolic acids (meloxicam), fenemates (flunixin, tolfedine) and proprionic acids (ketoprofen, carprofen) (Arfeen *et al* 2024). Products with label claims for mastitis treatment in New Zealand currently include carprofen, ketoprofen, tolfedine and meloxicam.

Mastitis is associated with an inflammatory response including local release of cytokines and chemokines resulting in prostaglandin release. This results in udder heat, pain and swelling, an elevated SCC, pyrexia, anorexia and reduced milk yield. Additionally, mastitis has been associated with impaired fertility associated with interruption of ovulation or luteolysis (Dolecheck *et al* 2019).

Thus, reducing the inflammatory response associated with mastitis is important.

Key findings include:

- For severe mastitis associated with *E. coli,* NSAID are the drug of choice, as there is stronger evidence for NSAID than for glucocorticoid use (Suojala *et al* 2013).
- For mild to moderate mastitis, including cases associated with Gram-positive bacteria, meloxicam reduced SCC and culling of cows compared with antibiotic treatment alone (McDougall *et al* 2009).
- For culture-negative, mild cases of clinical mastitis, treatment with ketoprofen did not improve clinical or bacteriological outcome compared to no treatment (Latosinski *et al* 2020). Ketoprofen treatment alone of clinical mastitis cases, followed by antimicrobial therapy if clinical improvement did not occur, reduced overall antibiotic usage compared to routine antimicrobial therapy at diagnosis, but resulted in reduced clinical cure at 5 days after diagnosis, lower bacteriological cure and higher risk of recurrent clinical cases within 60 days (Kromker *et al* 2025).

In summary, meloxicam used in conjunction with intramammary antimicrobials resulted in reduced SCC, improved bacteriological cure rate, increased conception rate, and reduced risk of culling amongst cows with mild-moderate clinical mastitis (McDougall *et al* 2009; McDougall *et al* 2016).

6. Use of fluids

Large volumes of isotonic intravenous fluid (25-40 L) can markedly improve the chances of survival of cows suffering from acute toxic mastitis. In the early stages of shock (for example, in cows that had a normal fluid status two hours earlier) small volumes of hypertonic saline have been used as an initial treatment to help restore the circulatory blood volume, provided that they are given with access to water, or oral supplementation of 15-20L of water.

7. Other treatments that have been evaluated

1. Oxytocin

Injection with the milk ejection hormone oxytocin may help remove milk and debris from hard, sore quarters but the value and efficacy of this approach is questionable. Oxytocin is a Prescription Animal Remedy and can only be obtained through veterinarians.

New Zealand research assessed the preventative effect of routine treatment with oxytocin following calving, and found no evidence of prevention of clinical mastitis in heifers (Williamson & Garrett 2010). In a separate study, across 204 cows with clinical mastitis on 4 farms, oxytocin was found to be a poor treatment for clinical mastitis, with a bacteriological cure rate for oxytocin observed for 42% ($p<0.01$), compared to 64% for antibiotic treatment (Williamson & Garrett 2010).

2. Increased milking frequency

A US study of increased frequency of milk out (6 x daily) accompanied by 20 IU of oxytocin found a reduced cure rate of environmental streptococcal infections, compared to antibiotic treatment alone (Roberson *et al* 2004). A combination of oxytocin and frequent milk out resulted in higher milk yield losses (230 kg) and greater economic losses (US$94) than treatment with antibiotics (Shim *et al* 2004).

A German study compared the effect of milking four times a day with twice a day, in support of antibiotic therapy and could find no difference in cure rates between the treatments (Krömker *et al* 2010).

A meta-analysis assessing nonantimicrobial treatment of clinical mastitis concluded that use of oxytocin with or without increased frequency of milking out could not be recommended (Francoz *et al* 2017).

⑦ Administer treatments as recommended

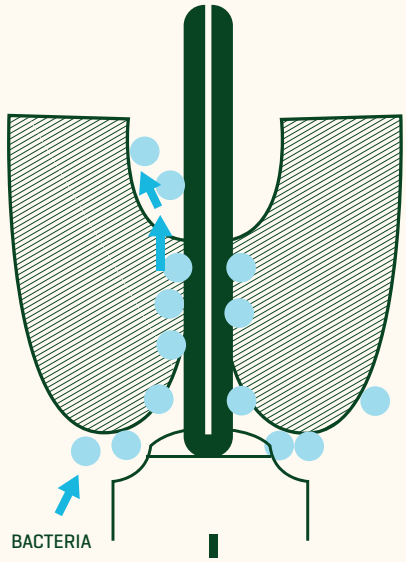
Administration of intramammary preparations

The nozzle of intramammary treatments can introduce bacteria into teats if the teat end is not properly disinfected. The DairyNZ **Healthy Udder Guide** provides step-by-step instructions on the correct way to administer intramammary treatments.

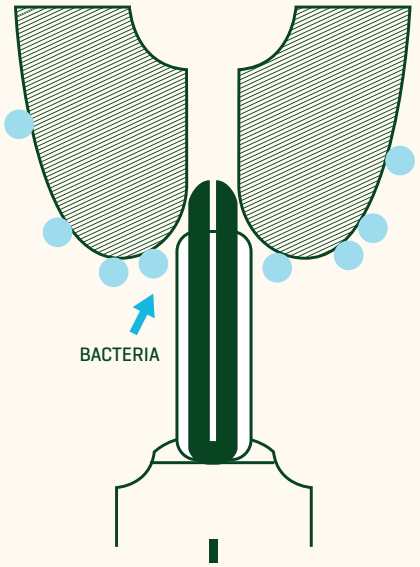
Ideally, antibiotics are given by partial insertion of short nozzle tubes just inside the teat canal (1-2mm). New infection rate following treatment was halved following partial versus full insertion of intramammary tubes (Boddie and Nickerson 1986). This is unlikely to be achieved in cows that are not used to having their teats touched and may therefore not be appropriate for many dairy herds. If an operator is not confident that short nozzle tubes will be used correctly, long nozzle tubes should be used rather than risk damaging the teat canal epithelium.

Use short nozzle tubes if available, or simply insert the tip of the tube just inside the teat canal, to a maximum depth of 3-4 mm.

Full insertion



Partial insertion



Administration of intramuscular antibiotics

Standards adopted for registration under the Agricultural Compounds and Veterinary Medicines (ACVM) Act (ACVM Act, 1997) to prevent carcass downgrades and chemical residue problems are:

- All injections are to be given into the anterior half of the neck
- Some injectable products state maximum volumes that can be injected at any one site. Refer to the label and do not exceed the maximum volume.

This is especially important for dairy cattle that may be culled within 12 months of treatment.

⑧ Use the full course of antibiotics (as specified on the label)

Efficacy and treatment courses for lactating cow formulations have been established through extensive research for registration of the products under the ACVM Act (1997).

Only affected quarters of clinical mastitis cases should be treated. As a significant proportion of cows with clinical mastitis have more than one affected quarter, all quarters should be checked at each milking during the treatment course to enable early detection and treatment of other affected quarters.

Regardless of whether a clinically affected quarter shows rapid improvement, it is important to use the full course of antibiotic treatment specified on the label to reduce the likelihood of infection recurring because of inadequate treatment, and to minimise the development of antibiotic-resistant strains of bacteria.

The development of antibiotic resistance

There is limited information on the risk of bacteria developing resistance to antibiotics commonly used to treat infections in dairy cattle. However, some associations are demonstrated from epidemiological studies such as:

- increasing use of intramammary penicillin/novobiocin DCT products was associated with increased risk of penicillin-resistant *Staph aureus* (Saini *et al* 2011),
- increased ampicillin-resistance in *E. coli* occurred with increasing cloxacillin, penicillin/novobiocin or cephapirin DCT (Saini *et al* 2013),
- there were more ampicillin-resistant *E. coli* following cefquinome or framycetin DCT use (Schubert *et al* 2021), and
- increased resistance was observed amongst faecal coliforms to cephalothin & streptomycin following cephalosporin DCT (Mollenkopf *et al* 2010).

Antimicrobial resistance occurs where a previously susceptible bacteria evolves to survive antimicrobial therapy. Resistance can occur following a *de novo* mutation or more commonly by horizontal gene transfer of an existing resistant gene. Once a resistant gene is in a herd, further antimicrobial use will select for bacteria carrying resistance genes.

The likelihood of antibiotic resistance developing broadly depends on the:

- prevalence of resistant bacteria in the animal population,
- frequency of antibiotic use in the animal population, and
- type of exposure to the antibiotics, e.g. short treatment courses of high doses of antibiotic confer less selective pressure than long-term exposure to low doses of antibiotic.

In addition to these factors, the rate of spread of antibiotic resistance within and between herds (and species) will be influenced by the opportunity for contact between animals and the host specificity of bacterial strains. It is therefore likely to vary significantly with management systems, mix of enterprise types and geographic location.

There are no comprehensive longitudinal studies of antimicrobial resistance of mastitis pathogens in New Zealand. However, analysis of laboratory submissions has found that there is a trend to a reduced prevalence of penicillin resistance among *Staph. aureus* isolates (Carman & Gardner 1997; Petrovski *et al* 2011). Similar findings have been reported internationally (Erskine *et al* 2002; Markovec & Ruegg 2003). One of the few published studies on the change in prevalence of resistant mastitis bacteria is in Finland where there was an increase of 27% in the proportion of *Staph. aureus* strains resistant to at least one antibiotic (mostly due to strains capable of producing beta-lactamase) (Myllys *et al* 1998).

MPI surveillance data from 2022/23 (MPI, 2023) found low levels of amoxicillin/clavulanic acid and tetracycline resistance, but moderate levels of resistance to cephalothin and high levels of

resistance to lincomycin/neomycin, for bovine mastitis *E. coli* isolates. There were high levels of penicillin resistance (29%), but very low levels for all other antimicrobials tested, for *Staph aureus*. A low level of resistance for cloxacillin, and no resistance for other antimicrobials tested, was observed for *Strep. uberis* isolates.

➜ The latest reports from MPI can be found at **www.mpi.govt.nz/animals**, and includes:

- NZ's Antimicrobial Resistance Plan
- Antibiotic Agricultural Compound Sales Analysis
- Antimicrobial Resistance surveys

The Ministry for Primary Industries and Ministry of Health jointly published the New Zealand Antimicrobial Resistance Action Plan (MOH, MPI 2017) and review it periodically. The plan sets out the key objectives of increasing awareness and understanding, surveillance, infection prevention and control, antimicrobial stewardship and governance, collaboration and investment.

Cattle antimicrobial use is the largest consumer of antimicrobials in New Zealand by species, hence the intense focus on reducing use of antibiotic DCT since 2016. While overall sales have been declining, the antimicrobials used for clinical mastitis have been increasing over the past 5 years (Hillerton *et al* 2024).

Veterinarians should continue to practice good prescribing principles including treating only those animals with evidence (clinical or microbiological) for infection, use of narrow spectrum antibiotics and continuing treatment for at least 48 hours beyond the end of clinical signs.

Off-label use

Off-label use refers to an unregistered use of a product. This includes any deviation from the manufacturer's recommendations, such as using:

- a different dose rate than stated on the label;
- a different route of administration;
- a different treatment interval or period; or
- a drug for a different purpose to that stated on the label.

Off-label use can only be authorised by a consulting veterinarian and only where legislation permits (McDougall & Millar 2011). It is done at the vet's discretion, taking knowledge of safety and efficacy into account, and is usually restricted to situations where no suitable registered product is available or where scientific evidence supports off-label use.

In food-producing animals, veterinarians prescribing off-label use of drugs become liable for setting appropriate withholding periods. These must be given to the client in writing. Wherever possible, the proposed treatment should be explained to the owner and informed consent obtained before treatment is started.

⑨ Observe withholding periods for milk and meat

➜ **Technote 3** covers withholding periods and residue management covers withholding periods and residue management for products used at dry off. This section summarises the key principles relevant to treatment of clinical mastitis.

Withholding periods (WHP) refer to the *minimum* time that must elapse after the last administration of a drug before an animal or its products are sold for human consumption.

The product labels provide recommended withholding periods. Antibiotic residues in milk or meat should not exceed the relevant New Zealand Maximum Residue Limit if treatments are used according to the label directions and milk or meat are withheld for the specified withholding periods.

Recommended withholding periods are based on trials that specify the:

- class of livestock, e.g. lactating cows
- dose rate, e.g. milligrams of drug per kilogram liveweight of animal
- dose interval, e.g. given once daily
- duration of treatment course
- frequency of milking, e.g. milked once or twice a day
- route of administration, e.g. intramammary infusion or intramuscular injection
- use of drugs within their expiry date
- use of drugs stored in accordance with label directions, and
- pattern of use for which they are registered, e.g. individual animal treatments.

Non-compliance

For registration purposes, the ACVM Act requires withholding periods to be based on the product sold for consumption. Consequently, withholding periods for intramammary antibiotics for lactating cows refer to cows and calves sold for meat or contributing to vats of milk.

In New Zealand, failure to observe withholding periods after treatment is the most significant cause of residue non-compliance (Beal 2002; Heuer *et al* 2003; McDougall *et al* 2004). In dairy cattle, antibiotic violations in milk are often associated with:

- failure to separate treated cows from the herd in supply
- inadvertent use of antibiotic DCT in lactating cows (note that antibiotic DCT is registered for use only immediately after a cow's last milking for a lactation)
- failure to identify treated cows
- failure to record treatment dates
- cows treated with antibiotic DCT at drying-off inadvertently re-joining the milking herd
- calving of cows treated with antibiotic DCT before expiry of the Minimum Dry Period, and
- 'off-label' drug use.

Any deviation from the registration specifications described above may lead to changes in the withholding periods for a product. Such changes are unlikely to be linear (e.g. doubling the dose cannot be extrapolated to a simple doubling of the required withholding periods) (Whittem 1997; McDougall & Millar 2011).

When giving systemic treatments for mastitis it is important to calculate the correct dose, as withholding periods for milk and meat change markedly when drugs are used at higher dose rates than specified on the label. Weights can be measured on scales or by using girth measurements and height sticks as a guide.

High dose rates constitute an 'off-label' dosage and, for any prescription drug, can only be considered with written permission from a veterinarian. They are a common cause of antibiotic violations.

Withholding periods are determined for a single product and where products are used in

combination (e.g. an antimicrobial and a NSAID), the withholding periods may be altered. However, there is a lack of data for withholding periods and care should be taken when prescribing combined products. At a minimum, an additional milking should be added to the milk withholding period where products are used in combination. Data on off label use (from a US perspective) is available at **www.farad.org/vetgram/search.asp**. Further guidelines are available to New Zealand veterinarians from the NZVA website.

Once daily milking

A number of intramammary antibiotics have specific 'on label' withholding times for use when milking cows once a day. Where herd owners are milking cows under treatment once daily, veterinarians should only prescribe registered products, to remain 'on label'.

Antibiotic residue tests

Traces of antibiotic in milk may cause allergic reactions in people and inhibit some starter cultures used in yoghurt and cheese production. National and international regulations stipulate the maximum levels of antibiotics that may be present in milk and these thresholds are often extremely low. The New Zealand limit is 0.003IU/ml penicillin or equivalent and a full list of allowable limits is available from MPI (2025).

Dairy companies perform regular screening tests to detect antibiotic residues (as inhibitory substances) in the vat milk that they collect. This occurs at several levels:

- Strips or sensors – for rapid screening of tankers before unloading. If the tanker is found to contain detectable levels of inhibitory substances then all suppliers on the tanker will be tested at the laboratory to identify the supplier or suppliers responsible.
- Growth inhibition - Screening of individual supplier milks using a bacterial inhibition assay to detect any substance that is inhibitory to the test bacteria. This is required by the MPI for every farm at least once every 10 days.
- An independent survey of bulk raw milk for antibiotic (and other) residues, called the National Chemical Contaminants Programme (NCCP). This service provides a credible monitoring system that helps the MPI sign off on European Union exports.

All dairy companies can conduct rapid tests on farm if there are concerns that antibiotics may have contaminated the vat. These tests should not be used on individual cows because they have been validated for use on bulk milk, and false positive test results may occur. Non-specific inhibitory substances present in milk of freshly calved cows or clinical mastitis cases have given positive test result on bacterial inhibition assays (Cullor *et al* 1994; Andrew 2000, 2001).

Inhibitory substances and antibiotic residue detected in an individual milk sample may not be excessive once diluted with clean milk in the vat. However, following a violation, on-farm testing of bulk milk is strongly recommended using factory screening tests.

Screening tests are relatively non-specific and vary considerably in their ability to detect all antibiotic families. A more sophisticated and expensive method for quantifying and identifying the type of antibiotic present is Liquid Chromotagraphy Mass Spectroscopy (LCMS). This test is recommended when investigating inhibitory grades where the source of antibiotics is unclear.

⑩ Discard milk from all quarters of cows that receive treatment

Even when a single quarter has been treated with intramammary antibiotic, it is possible that some antibiotic will be absorbed into the bloodstream and pass into the milk of normal quarters. The risk of antibiotic contamination is too great to include milk from treated cows in the vat. Do not use quarter milkers.

Milk that is subject to a withholding time must be harvested and stored in such a way that there is no risk of it mixing with, or cross-contaminating, milk intended for human consumption.

The withheld milk is not to be used for human consumption, and may only be fed to animals when in compliance with the Agricultural Compounds and Veterinary Medicines (Exemptions and Prohibited Substances) Regulations 2011 (MPI 2011).

The accepted best practice is to run all treated cows in a separate herd and in a secure paddock. These cows must only be brought to the farm dairy when all the milking cows have been milked and have left the dairy. Prior to milking the treated cows, the operator must ensure that the milk delivery line is disconnected from the milk vat.

If milk is harvested from cows under treatment or under a withholding period, and stored in a vat in the milk collection area, then this vat needs to be clearly labelled as not 'fit for supply' and the vat outlet must be locked to ensure that the milk cannot be collected by mistake. All operators should know how treated milking animals are identified, and the process to follow if they are accidentally milked into the vat for supply.

Consumables such as gloves, teat wipes and milk filters, must be stored in such a way that there is minimal risk of contamination with veterinary medicines.

11 Treatment failure: causes and management options

The reasons why a clinical quarter may fail to respond to treatment need to be considered when deciding the next course of action. These may include:

Inappropriate choice of active, dose, frequency or duration

For dose-dependent antimicrobials, concentrations in the udder may not reach therapeutic levels, particularly after parenteral use. For time-dependent antimicrobials such as beta-lactams, the course duration needs to be long enough to be effective.

Animal factors

Bacteriological cure is lower amongst older animals and amongst animals with a higher SCC prior to initiation of treatment. Presence of accumulations of inflammatory cells, hyperplasia of alveolar epithelium, fibrosis or formation of micro-abscesses in the udder may reduce likelihood of actives reaching concentrations above the minimum inhibitory concentration. Factors that lead to immune suppression, such as peripartum reductions in PMN numbers and function or trace element deficiencies, may result in poorer bacteriological cure.

Bacterial factors

Innate or acquired antimicrobial resistance is associated with lower bacteriological cure rate. Some bacteria may become dormant, develop into "L" or naked bacterial forms, develop biofilms or survive intracellularly. For example, *Staph. aureus* may locate within the phagolysosomes of macrophages and polymorphonuclear neutrophils. Antibiotic penetration of cells may be poor and even if they gain access to the cell, they may not distribute to the phagolysosomes, resulting in lower bacteriological cure rate.

- If a milk sample was collected and frozen before the initial treatment, it can be cultured to determine the causal bacteria and their antibiotic resistance.
- Pathogens introduced at the time of treatment (due to poor technique when giving intramammary infusions) will only be identified by resampling the quarter.

Options when there is no response to treatment

Options that can be considered when a clinical quarter fails to respond to a full course of treatment include the following:

Repeating the treatment but treating for an extended time with the antibiotic

There is evidence that 'extended' therapy (i.e. 6 to 8 treatments) with time dependent antibiotics such as the beta lactams will result in improved cure rates (Oliver *et al* 2004; Deluyker *et al* 2005; Spatz Shelgren *et al* 2007; Steele & McDougall 2014). However, economic analysis suggests that extended therapy may not be economic in all situations (Steeneveld *et al* 2011)

Some products are registered for extended therapy (generally up to 6 treatments). Individual prescriptions will be required for extending therapy with other products. Note that repeated treatments will extend the required withholding periods.

Trying a different antibiotic treatment

It is generally more effective to extend therapy, rather than change product. However, if new information comes to light (e.g. culture results), an alternative may be more appropriate.

The longer a case of clinical mastitis persists, the greater the degree of fibrosis and abscessation that may occur, and the less likely the quarter is to respond to antibacterial treatment. Some cases just do not respond to treatment, even if the bacteria are sensitive to the product.

Drying-off the infected quarter if it is not hot and swollen

This is an option if the cow is in good general health apart from the infected quarter. The only method of drying-off a quarter is to stop milking the quarter and monitor to ensure that it does not develop into an acute case of mastitis. Cessation of milking should be delayed until the end of the withholding period after administration of the last treatment.

It is important that these quarters are permanently identified to prevent accidental attachment of cups to these teats at the time of milking. Antibiotic DCT must not be used in a quarter when the other quarters are continuing to be milked. Antibiotic DCT products are not registered for use in lactating cows. Some antibiotic will be absorbed into the bloodstream and passed out in the milk from the normal quarters, so there is an unacceptable risk of antibiotic contamination of the vat.

At the end of lactation, it is not appropriate to use antibiotic DCT in a quarter that has been dried off during lactation because intramammary DCT will not be absorbed by dry glands.

Drying-off the affected cow

Drying-off early cows with quarters that fail to respond to treatment removes a source of infection to other cows in the milking herd. These cows may be treated with antibiotic DCT and the affected quarter closely monitored – if the quarter becomes hot and swollen, or the cow becomes systemically ill, treatment with non-steroidal anti-inflammatories and lactating cow antibiotic product in the infected quarter may need to be reinitiated.

Culling chronically infected cows from the herd

Culling chronically infected cows is an important component of any mastitis control programme. The recommended withholding period for meat must be observed if the cow has been treated with antibiotics.

➜ **Technote 15** describes mastitis criteria for culling cows.

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