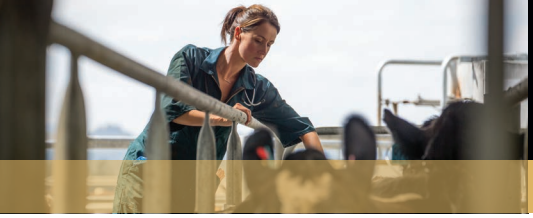

NEW TRENDS FROM ANALYSIS OF NZ MASTITIS MILK SAMPLES

Introduction

Mastatest was developed in New Zealand and is different from conventional milk culture as it uses a colour change reaction. Mastatest is fast and simple to operate – a milk sample is added to a cartridge that slots into the Mastatest lapbox and results are reported within 24 hours. Two cartridges are available:

1) Clinical cartridge is used for clinical mastitis cases and reports the bacteria isolated and the antibiotic sensitivity based on minimum inhibitory concentration (MIC) for 3 common antibiotics used to treat NZ mastitis cases.

Farmers are recommended to take a sample from a clinical mastitis case, treat with an anti-inflammatory and wait 24 hours for the result. Treatment is then targeted using the antibiotic with the lowest MIC while no growths and *E. coli*/gram -ve cases are identified and can be left untreated (without antibiotics).

2) HiSCC cartridge is used for subclinical mastitis cases and reports the bacteria isolated.

Farmers are recommended to sample RMT positive or high SCC cows based on herd test, to identify the bacteria for better management decisions. Both the clinical and HiSCC cartridges have been validated against traditional laboratory bacterial culture and no significant differences were found between the tests in the detection of the major mastitis-causing microorganisms: *Strep. uberis*, *Staph. aureus*, and coliforms for the clinical cartridge[2] or *Staph. aureus*[3] for the HiSCC cartridge.

Bacteria identifed from clinical mastitis cases

198,737 mastitis milk samples were evaluated using the NZP2 clinical cartridges through a Mastatest lapbox during the 6 years ending Dec 2024. Figure 1 below shows total percentage of each bacteria isolated. Bacteria were identified in a total of 179,802 samples, including 126,323 single bacteria species.

No bacteria were grown in 18,935 (10%) of samples. This proportion is lower than NZ commercial laboratories using conventional culture which typically report 20-25% of milk samples with 'no growth' results (from both clinical and subclinical mastitis cases). It is likely there are fewer no growth results with Mastatest because the test procedure recommends fresh milk samples, and a larger sample volume is analysed.

Staphylococcus aureus infections are seen in clinical mastitis cases throughout lactation.

E. coli* / *Klebsiella*/ *Serratia and other Gram -ve bacteria account for 12% of bacteria isolated which is in line with other studies.[5]

Coagulase negative Staphylococcus (CNS, or non-aureus Staph) are also commonly isolated from clinical mastitis cases. There are thought to be over 50 strains of CNS that may cause mastitis in NZ dairy cows.

Streptococcus uberis was the most common isolate. *Strep. uberis* has traditionally been considered an environmental pathogen with the highest incidence coinciding with the wetter months of seasonal calving in NZ.

McDougall[4] cultured milk from 1,561 glands with clinical mastitis and found that the most common bacterial species isolated was *Strep. uberis.* The incidence of *Strep. uberis* declined rapidly in the second and subsequent months of lactation, when *Staph. aureus* and other bacteria were generally more prevalent.

Mastatest results indicate that *Strep. uberis* is significant all year round on NZ dairy farms. This difference in findings may be due to an increase in environmental challenge as dairy farming has intensified over the last 16 years.

Mastitis pathogens isolated each month from Mastatest samples across New Zealand are included in Figure 2 below.

BACTERIA IDENTIFIED FROM CLINICAL MASTITIS CASES

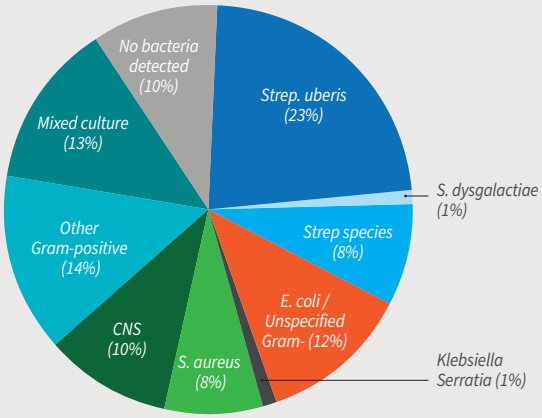

Figure 1: Results from 198,737 Mastatest clinical cartridge samples.

MASTITIS BACTERIA PREVALENCE BY MONTH

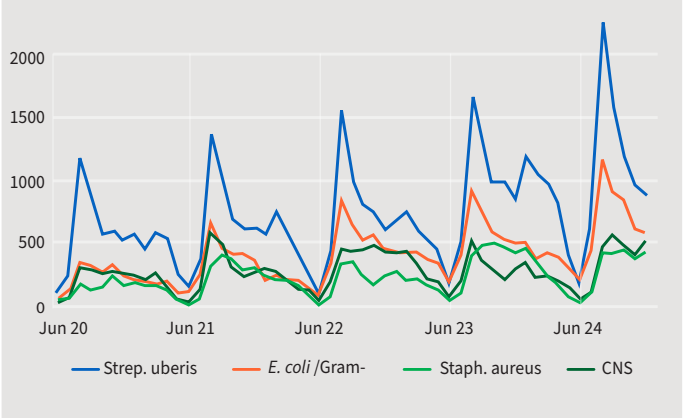

Figure 2: Prevalence of bacterial species isolated from Mastatest clinical cartridge results by month from June 2020 to June 2024.

Antibiotic sensitivity and MIC trends

Mastatest derives the minimum inhibitory concentration (MIC) through serial dilutions of the three antibiotics included in NZP2: Benzylpenicillin, Cloxacillin, Lincomycin/Neomycin (Albiotic).

There are no bovine validated clinical cut-points for the MICs for any of the antibiotics/ bacterial combinations tested. The clinical treatment recommendation is to use the antibiotic with the lowest MIC to obtain the best chance of a cure.[6]

The MIC results for each bacterial type are shown in Figures 3 to 6 to the right. Only single bacterial species are included in the MIC analysis e.g. for *Strep. uberis* there were 46,672 milk samples that grew only *Strep. uberis*. The overall antibiotic sensitivity (MIC) results are shown in Figure 7 below.

Strep. uberis: The MIC results for cloxacillin against *Strep. uberis* are higher than those for penicillin or lincomycin/neomycin (Albiotic). This supports other studies which indicate that *Strep. uberis* is becoming less sensitive to cloxacillin[7].

Staph. aureus: Based on Dairy Antibiogram data McDougall[7] observed a biphasic distribution for the MICs for ampicillin and penicillin for *Staph. aureus*, suggesting a change away from the traditional sensitive wild population of *Staph. aureus* in New Zealand. The author also reports approximately 30% of MICs for penicillin treatment against *Staph. aureus* were 0.06 (the penicillin sensitive population). This is supported by Mastatest results which shows 49% of penicillin MIC results ≤ 0.05.

Coagulase-negative *Staphylococci*: The Mastatest results show that penicillin has a similar MIC distribution for CNS as it does with *Staph. aureus* reflecting the variation in CNS strains that cause clinical mastitis in NZ.

***E. coli* / Gram negative bacteria**: The combined results for all Gram-negative bacteria show that these bacteria demonstrate limited sensitivity to the common antibiotics used to treat mastitis, with 99% of bacteria having MIC >4.0 for all of the antibiotics tested.

Clinical mastitis treatment recommendations

Mastatest treatment recommendations following identification of bacteria and clinical antibiotic sensitivity are shown in Figure 7.

Penicillin was the recommended treatment for 93% of the *Strep. uberis* infections, and was frequently recommended for other mastitis cases. However, penicillin was not deemed the most appropriate treatment in 40% of cases.

	Strep. uberis	*Staph. aureus*	CNS	All results
Penicillin	93%	59%	68%	60%
Cloxacillin	1%	29%	19%	7%
Lincomycin/Neomycin	6%	12%	13%	10%
No antibiotics indicated	0%	0%	0%	23%

Figure 7: Percentage of times each antibiotic was ranked as the recommended treatment for each bacterial species, and overall, from Mastatest clinical cartridges.

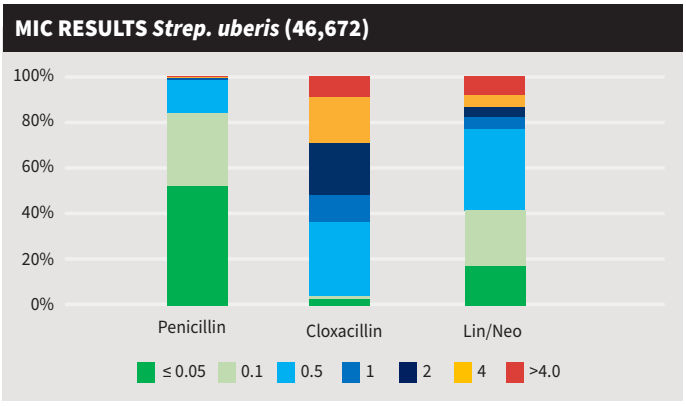

Figure 3: MIC results from Mastatest clinical cartridge samples, for Strep. uberis with three antibiotics

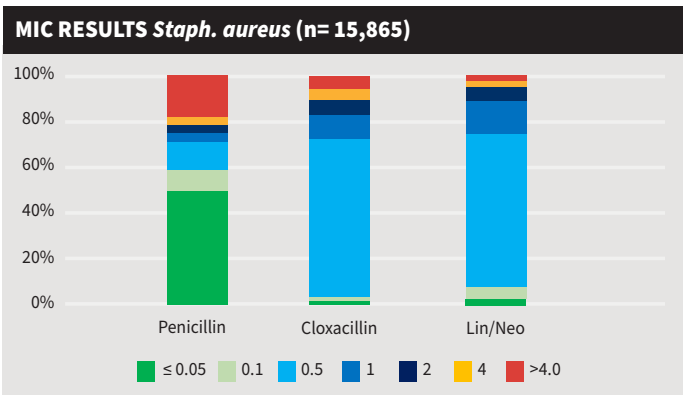

Figure 4: MIC results from Mastatest clinical cartridge samples, for Staph. aureus with three antibiotics

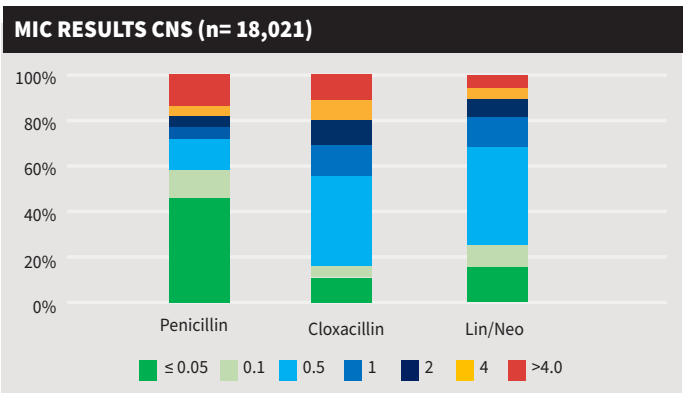

Figure 5: MIC results from Mastatest clinical cartridge samples, for CNS with three antibiotics

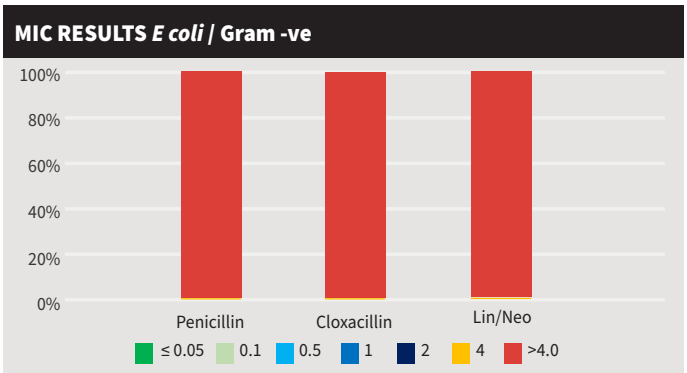

Figure 6: MIC results from Mastatest clinical cartridge samples, for E. coli / gram negative bacteria with three antibiotics

Reducing antibiotic use and AMR

Two key principles for the NZ dairy industry antimicrobial resistance (AMR) goals to be achieved are:

- Only using antibiotics in cows that are truly infected with bacteria
- Use an antibiotic that has a high chance of curing the infection

Based on these criteria, clinical cases that grow no bacteria or Gram-ve bacteria should not be treated with antibiotics.
Fig 8 shows that from 2010 to 2024, 45,351 cases did not require antibiotic treatment. Based on an estimated cost of $200 per clinical mastitis case this equates to a potential saving of $9,070,200 for NZ dairy farmers.

	Number
Total clinical cases tested	198,737
Clinical cases not requiring antibiotics	45,351 (23%)
E. coli / Unspecified Gram-	24,551 (12%)
Klebsiella / Serratia	1,865 (1%)
No bacteria detected	18,935 (10%)
Estimated cost of a clinical mastitis case	$200
GROSS SAVINGS from reducing antibiotic usage	**$9,070,200**

Figure 8: Estimated savings from clinical mastitis cases tested with Mastatest where antibiotics were not indicated.

Bacteria identified from subclinical mastitis cases

Results of 47,221 HiSCC cartridge tests showed that *Staph. aureus* was isolated 24% of the time. This provides opportunity to manage these cows by milking last or culling early to reduce cow to cow transfer of *Staph. aureus* (Figure 9).

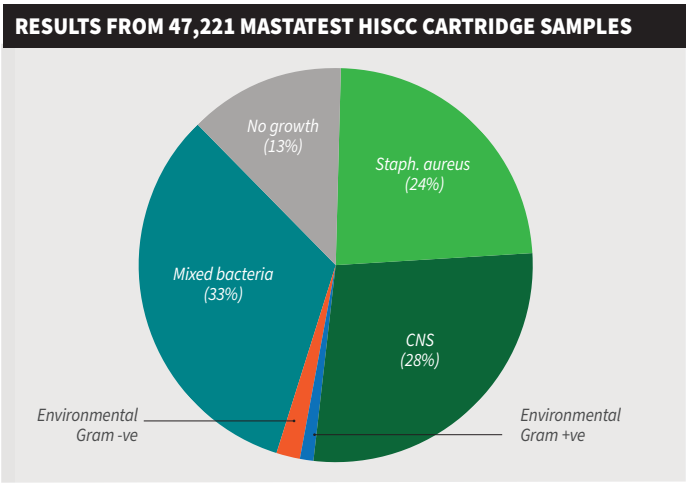

Figure 9: Results from 47,221 subclinical Mastatest cartridge samples.

Conclusions

Analysis of nearly 200,000 Mastatest results shows:

1. The incidence of no growth results is lower with Mastatest compared with typical NZ conventional laboratory testing.
2. *Strep. uberis* is the most commonly isolated bacterial species from clinical mastitis cases on NZ dairy farms throughout the year.
3. *Strep. uberis* appears to be becoming less sensitive to cloxacillin. This finding is supported by other NZ studies.
4. Gram-ve bacteria and coliforms account for approximately 12% of clinical mastitis cases in New Zealand and demonstrate low sensitivity to all antibiotics tested. In most cases for Gram-negative bacteria, rather than authorising antibiotics, it is recommended to treat cows with a nil milk withhold NSAID such as KetoMax 15% and regularly recheck cows at milking.
5. For 45% of *Staph. aureus* mastitis cases the MIC is ≤ 0.05 for penicillin, and 23% had MIC >4, reinforcing that sensitivity to penicillin varies widely in NZ strains.

Clinical Relevance

- Using Mastatest for all clinical mastitis cases reduces antibiotic use, as 'no growth' and Gram-negative cases are better managed using non-steroidal anti-inflammatory drugs (NSAIDs) rather than antibiotics.
- Penicillin is recommended most commonly as the antibiotic treatment for cases of clinical mastitis. However 40% of the time penicillin is not indicated, highlighting the value of testing every clinical case of mastitis.
- Treating with NSAID pain relief while waiting 24 hours for Mastatest test results improves animal wellbeing.
- Higher farmer uptake of mastitis testing ensures tailored treatment programs, and will help achieve NZ dairy industry antimicrobial resistance (AMR) goals.
- RMT positive and HiSCC cows throughout lactation can be tested with the HiSCC cartridge to identify *Staph. aureus* cows so they can be managed to reduce spread of infection.

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