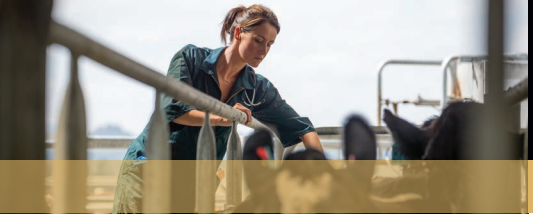

NEW TRENDS FROM ANALYSIS OF MASTITIS MILK SAMPLES

Introduction

Mastatest is a novel way to test clinical mastitis milk samples on the farm or in a veterinary practice. Mastatest is fast and simple to operate – a clinical mastitis milk sample is put into the cartridge that is inserted into the Mastatest lapbox. Within 24 hours the farmer and the vet receive an email reporting the bacteria isolated and a ranking of the MIC (minimum inhibitory concentration) for 3 common antibiotics used to treat mastitis in New Zealand. Having accurate test results in under 24 hours instead of 3 to 5 days means farmers can treat with the most effective drug, to help reduce antibiotic use and improve cure rates by targeting treatment once the bacterial pathogen has been identified[1].

Mastatest was developed in New Zealand and is different from conventional milk culture as it uses a colour change reaction. Mastatest has 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: *S. uberis*, *S. aureus*, and coliforms[2].

Analysis of Mastatest results from 3,060 samples

Mastatest results were collated from 3,060 mastitis milk samples submitted to veterinary practices throughout New Zealand between 1 June 2018 and 1 June 2019 and analysed in Mastatest lapboxes. 446 samples returned a result of no growth (14.5%) leaving 2,614 samples where one or more bacteria were isolated. Commercial laboratories using conventional culture typically report 20—25% of samples returning "no growth" (this includes samples from both clinical and subclinical mastitis cases). There are fewer no growth results with Mastatest likely because the test procedure recommends fresh milk samples and a larger sample size is analysed. Of the 2,614 samples where bacteria were isolated 2,070 isolated a single bacterial species and these results are used in the final analysis below (i.e. samples where more than one bacterial type was isolated are excluded from the discussion).

Bacteria isolated over time

The number of bacteria and percentage of bacteria isolated from Mastatest for each month are included in Figures 1 and 2. Figure 3 shows the total percentage of each bacteria isolated in the 2,070 tests.

a) ***Streptococcus uberis* (SU)** was the most common bacteria isolated. *S. uberis* has traditionally been considered an environmental pathogen with highest incidence coinciding with the wet months of the seasonal calving season (spring). McDougall[3] cultured milk from 1,561 glands with clinical mastitis and found that the most common bacterial isolate was *S. uberis* and that the incidence of *S. uberis* declined rapidly in the second and subsequent months of lactation. The Mastatest results indicate that this pattern may be changing and that *S. uberis* is a significant bacterium all year round on New Zealand dairy farms. This change may be due to an increase in environmental challenge (as farming has intensified over the last 12 years) or as a result of *S. uberis* acting contagiously (spreading from cow to cow).

b) ***Staphylococcus aureus* (SA)** is prevalent throughout the lactation.

c) **Gram positive bacteria:** Mastatest uses a colour change reaction and unlike traditional milk culture this procedure cannot differentiate between low colony numbers of Gram-positive bacteria, so the number of samples reported as "Gram positive" is higher than traditional culture.

d) **Coliform / Gram negative:** In line with other studies[4], Coliforms and Gram negative bacteria account for approximately 10% of bacteria isolated.

NUMBER OF BACTERIA ISOLATED BY MONTH 1ST JUNE 2018 TO 1ST JUNE 2019. MASTATEST DATA FROM 2,070 SAMPLES

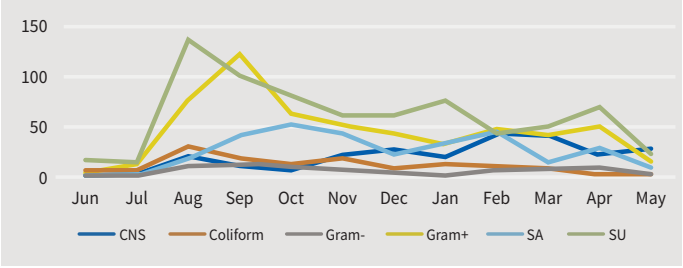

Figure 1

PERCENTAGE OF BACTERIA ISOLATED BY MONTH 1ST JUNE 2018 TO 1ST JUNE 2019 USING MASTATEST

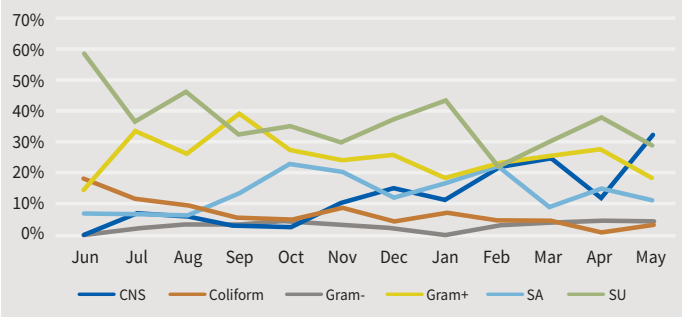

Figure 2

Mastatest

ANNUAL PREVALENCE OF PATHOGENS (ALL TESTS)

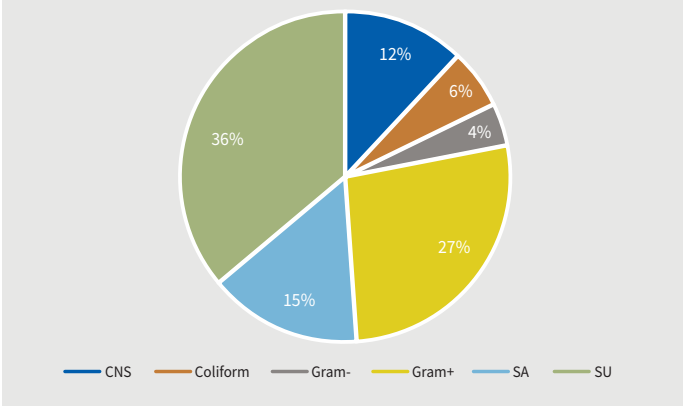

Figure 3

MIC trends

Mastatest derives the MIC through serial dilutions of three antibiotics included in each of the two commercially available cartridges:

NZP2: Benzylpenicillin, Cloxacillin, Lincomycin/Neomycin (Albiotic)

NZP4: Benzylpenicillin, Cloxacillin, Tylosin

As there are no bovine validated clinical cut-points for the MICs for any of the antibiotics/bacterial combinations tested, the clinical treatment recommendation is generally to use the antibiotic with the lowest MIC for the best chance of a cure[5]. The MIC results for each bacterial type are shown in Figures 4 to 7. Only pure growths of each bacteria are included in the MIC analysis (e.g. for *S. uberis* there were 738 milk samples that grew only *S. uberis*). The overall bacteria results are shown in Figure 8.

a) ***S. uberis*** (Figure 4): The MIC results for cloxacillin against *S. uberis* are higher than for penicillin or lincomycin/neomycin (Albiotic). This supports other studies that indicate that *S. uberis* is becoming less sensitive to cloxacillin[6].

MASTATEST MIC RESULTS FOR 738 MASTITIS SAMPLES REPORTED AS *S. uberis*

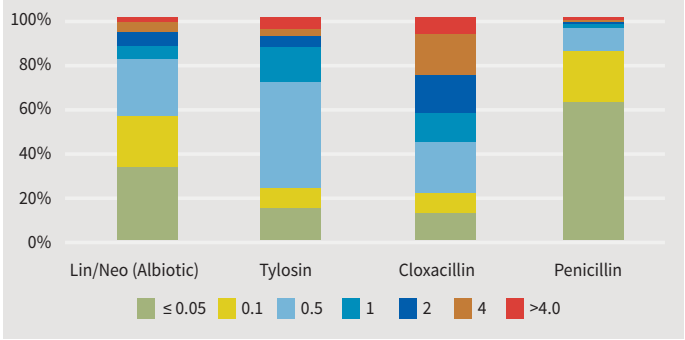

Figure 4

b) ***S. aureus*** (Figure 5): Based on Dairy Antibiogram data McDougall[6] observed a biphasic distribution for the MICs for ampicillin and penicillin for both *S. aureus* and *S. uberis*, suggesting a change away from the traditional sensitive wild population of *S. aureus* in New Zealand. The author also reports approximately 30% of MICs for penicillin treatment against *S. aureus* were ≤0.05 (the penicillin-sensitive population) and this is reflected in the Mastatest results which also shows 30% of penicillin results ≤ 0.05.

MASTATEST MIC RESULTS FOR 309 MASTITIS SAMPLES REPORTED AS *S. aureus*

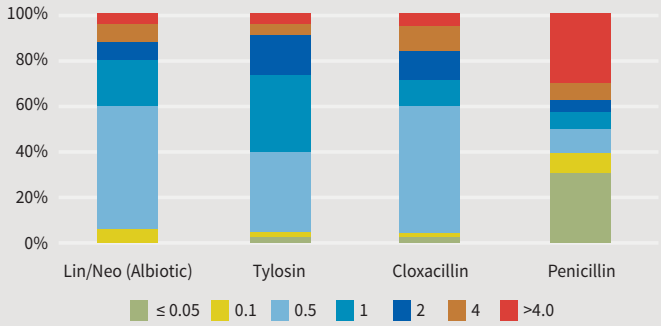

Figure 5

c) Coagulase-negative *Staphylococci* (CNS) (Figure 6): The Mastatest results show that penicillin has a similar MIC distribution to CNS as it does with *S. aureus* (MIC ≤0.05 = 33% and MIC >4 = 23%).

MASTATEST MIC RESULTS FOR 245 MASTITIS SAMPLES REPORTED AS CNS

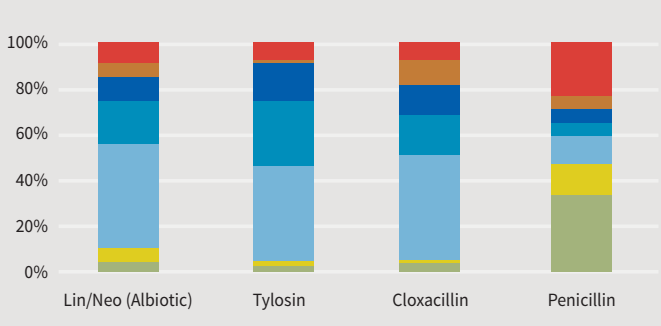

Figure 6

d) **Coliforms plus Gram-negative bacteria** (Figure 7): The combined results for all Gram-negative bacteria show that most of these bacteria demonstrate limited sensitivity to the common antibiotics used to treat mastitis, with 92% of bacteria having MIC >4.0 for all of the antibiotics tested.

MASTATEST MIC RESULTS FOR 210 MASTITIS SAMPLES REPORTED AS EITHER COLIFORM OR GRAM -ve

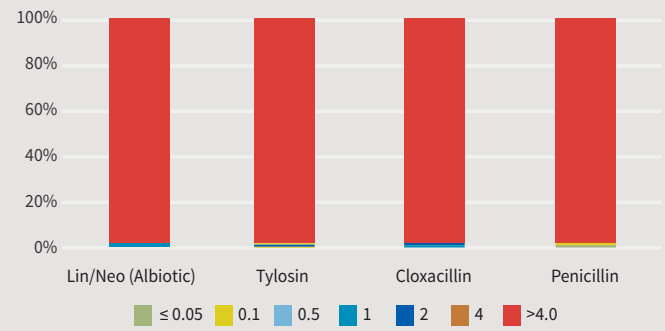

Figure 7

e) **All Bacteria** (Figure 8): Penicillin has the lowest MIC results overall for 2,070 bacteria tested that returned a single bacterial result (excludes mixed growths, no growths and contaminated samples). For penicillin 36% of the MIC results were ≤ 0.05 and 13% were 0.1. The next lowest MICs were for lincomycin/neomycin (Albiotic) with 15.5% of the MIC results ≤ 0.05 and 11.6% were 0.1.

MASTATEST MIC RESULTS FOR 2,070 MASTITIS SAMPLES THAT REPORTED ANY SINGLE BACTERIA

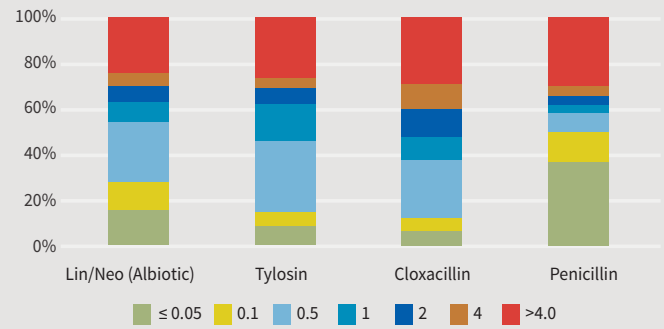

Figure 8

Intramammary penicillin available in New Zealand is registered for use in cows milked twice a day while lincomycin/neomycin (Albiotic) is registered for cows milked both twice a day and once a day. In the absence of any testing, mastitis treatment with penicillin is appropriate for cows with mastitis milked twice a day followed by lincomycin /neomycin (Albiotic). For cows with mastitis milked once a day lincomcin/neomycin (Albiotic) is the most appropriate.

Mastatest

Conclusions

Analysis of the results from 3,060 cases of mastitis analysed through Mastatest has clinical relevance for veterinarians prescribing mastitis treatments.

1. The incidence of no growth results is lower through Mastatest than typical in NZ conventional laboratory culture testing.
2. *Streptococcus uberis* is the most commonly isolated bacteria from clinical cases of mastitis on New Zealand dairy farms, and is isolated from mastitis cases throughout the year.
3. Gram negative bacteria and coliforms account for approximately 10% of mastitis cases in New Zealand.
4. This analysis supports other studies showing that *Streptococcus uberis* appears to be becoming less sensitive to cloxacillin.
5. For 30% of *Staphylococcus aureus* mastitis cases the MIC is ≤ 0.05 for penicillin, and a similar proportion tested MIC >4, reinforcing that sensitivity to penicillin varies widely in NZ strains. Promptly identifying penicillin sensitivity to *S. aureus* cases will influence antibiotic treatment recommendations and will likely improve cure rates, reducing the need for culling.
6. Coliforms and Gram negative bacteria demonstrate low sensitivity to all four antibiotics tested. In most cases for Gram negative bacteria, rather than prescribing antibiotics, it is recommended to treat cows with a nil milk withhold NSAID such as KetoMax15% and regularly recheck cows at milking.
7. In the absence of any milk culture information, treatment with penicillin is appropriate for cows with mastitis milked twice a day, followed by lincomycin/neomycin (Albiotic). For cows with mastitis milked once a day, Albiotic is an appropriate treatment.

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[1] Bates A, Laven R, Bork O, Hay M, McDowell J, Saldias B. 2019. Selective and deferred treatment of clinical mastitis. Conference Proceedings of The Society of Dairy Cattle Veterinarians of the NZVA 2019, p47-52.

[2] Jones G, Bork O, Ferguson SA, Bates A. 2019. Comparison of an on-farm point of-care diagnostic with conventional culture in analysing bovine mastitis samples. Journal of Dairy Research. https://doi.org/10.1017/S0022029919000177

[3] McDougall S, Arthur DG, Bryan MA, Vermunt JJ, Weir AM. 2007. Clinical and bacteriological response to treatment of clinical mastitis with one of three intramammary antibiotics. New Zealand Veterinary Journal, 55:4, 161-170.

[4] Lacy-Hulbert J, Williamson J, Kolver E, Doohan H, Shelgren J.2012. Is coliform mastitis an emerging issue? Proceedings of the New Zealand Milk Quality Conference, 2012, p7.06.1 – 7.06.7.

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[6] McDougall S, Castle R, Macpherson Y, Karkaba A, Graham L. 2018. The Dairy Antibiogram; a novel way to monitor antimicrobial sensitivities of mastitis pathogens in dairy herds. Proceedings of the Society of Dairy Cattle Veterinarians Annual Conference 2018, pp 29-38.

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