

On-farm and laboratory testing of bovine mastitis pathogens for penicillin susceptibility

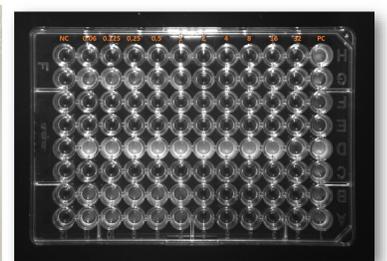
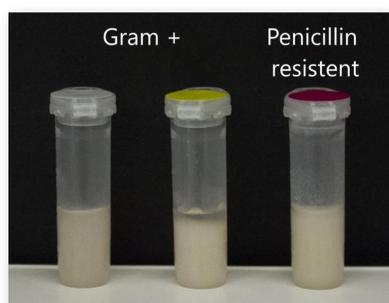
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Introduction

- The aim of this field study was to compare on-farm and laboratory susceptibility testing of bovine mastitis pathogens towards Penicillin.
- On-farm tests, designed to classify mastitic milk samples into Gram-positive and Gram-negative and no growth cases (having low and high spontaneous cure rates respectively), have long been known to be an effective tool in ensuring prudent use of antibiotics without compromising cure rates (Lago et al. 2011). To incorporate antibiotic susceptibility testing into the on-farm concept is a desirable next step, although limited knowledge on clinical breakpoints is available (Barlow, 2011).
- Penicillin is a non-critical antibiotic recommendable as first choice for treatment of mastitis caused by gram-positive bacteria. It is thus a good candidate to investigate the potential of on-farm susceptibility testing compared to the laboratory reference method of determining minimal inhibitory concentration (MIC).

Material & methods



➤ Five dairy farms in Denmark and Germany were provided with stepwise manuals for the collection of milk samples, incubation and reading of on-farm tests for clinical mastitis.

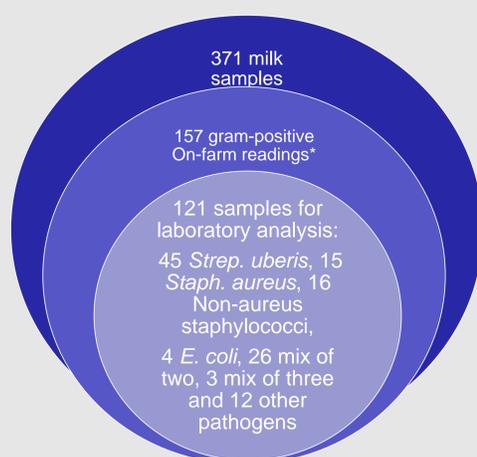
➤ The Mastdecide®plus on-farm test by Quidee was used, where growth changes colour from pink to white (Leimbach & Krömker, 2018). The first two tubes indicate pathogen Gram-status. The third tube contains 0.125 µg/mL Benzyl penicillin.

➤ Milk samples were frozen at -18 °C until they were sent to the milk laboratory at the University of Applied Sciences and Arts (Hanover, Germany).

➤ Based on on-farm test results, the assumed Gram-positive cases were further processed by bacteriological culturing and confirmed by MALDI TOF analysis.

➤ The MIC for penicillin was determined by standardized laboratory procedure using stepwise broth dilution and visual examination.

Samples and Pathogen distribution



*some samples were not analysed further due to contamination or lack of growth in the laboratory.

Results

- 16 of the 121 included samples were positive in the on-farm penicillin test indicating the ability to grow in the presence of Benzyl penicillin.
- MIC values are grouped based on the 0.125 µg/mL dilution + 1 step into a high and low category in order to compare it to the On-farm method in a 2x2 table.
- Within the included samples, the laboratory analysis revealed a total of 5 gram-negative isolates (all E.Coli) which all appeared positive in the on-farm penicillin test and were in the high MIC group.

Agreement between laboratory and on-farm penicillin susceptibility testing

	High MIC in min. 1 isolate** (>0.25 µg/mL)	Low MIC (≤0.25 µg/mL)	Total
On-farm penicillin test+	<u>8</u>	<u>8</u>	16
On-farm penicillin test-	<u>4</u>	<u>101</u>	105
Total	12	109	121

Calculated test parameters with 95% confidence interval

Sensitivity: 0.67 (0.35-0.90)	Specificity: 0.93 (0.86-0.97)
Positive predictive value: 0.50 (0.25-0.70)	Negative predictive value: 0.96 (0.91-0.99)

**Due to mixed cultures, the total of MIC analysed isolates is higher than the number of samples.

Conclusion

- Overall, the agreement between the on-farm penicillin test and high MIC in this trial shows potential for further investigation. The on-farm test was good in excluding true negatives while moderate in the detection and classification of positives.
- Some cases were identified as resistant by the on-farm penicillin test, although they had a low MIC. Mixed cultures or a subjective element in the interpretation of test results might be likely reasons for this incoherency. Both issues should be addressed in future studies
- There was a rather high occurrence of gram-negative E.Coli in the group of on-farm penicillin test positives, which were not in agreement with the on-farm gram-positive classification. However, for evaluating the penicillin sensitivity test on its own, this finding supports the test's capability, as we do not expect E.coli to be sensitive to penicillin.

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Literature

Lago, A., S. M. Godden, R. Bey, P. L. Ruegg and K. Leslie (2011). "The selective treatment of clinical mastitis based on on-farm culture results: I. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes." *Journal of Dairy Science* 94(9): 4441-4456.

Barlow, J. (2011). "Mastitis therapy and antimicrobial susceptibility: a multispecies review with a focus on antibiotic treatment of mastitis in dairy cattle." *Journal of Mammary Gland Biology and Neoplasia* 16(4): 383-407.

Leimbach, S. and V. Kromker (2018). "Laboratory evaluation of a novel rapid tube test system for differentiation of mastitis-causing pathogen groups." *Journal of Dairy Science* 101(7): 6357-6365.

Pictures: All pictures were provided by the team of authors