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AN306

Selective Antibiotic Treatment for Dairy Cow Mastitis[1]

Kathryn Merriman, Fiona Maunsell, Corwin Nelson, and Albert De Vries[2]

Introduction

Mastitis is the most common disease in dairy cattle and continues to result in one of the largest economic losses for the dairy industry. Mastitis results in milk loss and treatment-associated costs for the farmer of $179 per case. Of this $179 total, $50 consists of treatment-associated costs alone (Bar et al. 2008).

When a clinical mastitis case is detected, immediate antibiotic action is usually taken by the farmer; however, it has been reported that 10% to 40% of cultures from clinical mastitis cases yield no bacterial growth and therefore do not need antimicrobial treatment (Roberson 2003). Additionally, mastitis caused by coliform bacteria, a common environmental mastitis pathogen, frequently resolves without treatment. Lastly, most of the intramammary antimicrobials approved for use in dairy cattle have primarily gram-positive spectrum of action and are less likely to be effective in coliform mastitis cases. It is therefore reasonable to ask if a selective treatment approach can be more effective. A selective treatment approach for clinical mastitis implies a two-step strategy with identification of the pathogen first, followed by a treatment decision based on that result. It is expected that a selective treatment approach would decrease the use of antimicrobials as well as treatment-associated costs for the farmer. With selective treatment, more milk will be withheld from the cows that are treated due to the delay in their treatment, but, in aggregate for the entire herd, total milk withheld may be less because not every cow with clinical mastitis will be treated. Selective antimicrobial use for mastitis cases allows farmers to have effective mastitis treatment with reduced treatment costs (Makovec and Ruegg 2003; Schukken et al. 2011). Selective treatment strategy can be implemented by the farmer using a simple decision (yes/no) based on information from on-farm culture systems or from laboratory-based real-time polymerase chain reaction pathogen detection methods.

Diagnosis

For detecting mastitis-causing pathogens, the current gold-standard method is microbiological culture for bacterial identification (National Mastitis Council 1996). Laboratory culture can identify the pathogen in 24 to 48 hours (or more) after taking the sample, depending on how soon the sample is sent into the laboratory for diagnosis. Although it takes 24 to 48 hours to get results, a laboratory culture allows identification of whether the pathogen is gram-negative or gram-positive for selective antibiotic treatment. Currently, there are three methods of diagnosis for dairy farmers that decrease time for pathogen identification when compared with traditional laboratory culture: (1) the Minnesota Easy Culture System II, (2) the Petrifilm system, and (3) real-time polymerase chain reaction (PCR).

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2. Kathryn Merriman, MS thesis candidate, Department of Animal Sciences; Fiona Maunsell, BVSc, PhD, assistant research professor, Department of Large Animal Clinical Sciences, Department of Infectious Diseases and Pathology, Food Animal Reproduction and Medicine Services; Corwin Nelson, PhD, assistant professor, Department of Animal Sciences; and Albert De Vries, PhD, associate professor, Department of Animal Sciences; UF/IFAS Extension, Gainesville, FL 32611.

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On-Farm Culture Systems

Two on-farm culture systems exist to help prevent the delay of submission and time to results by laboratory culture. The Minnesota Easy Culture System II (University of Minnesota, St. Paul, MN) and the Petrifilm system (3M Microbiology, St Paul, MN) are on-farm culture systems. Farmers can use the systems on their farms, making an external laboratory unnecessary for mastitis pathogen diagnosis.

MINNESOTA EASY CULTURE SYSTEM II

The Minnesota Easy Culture System II is a bi-plate system with one side containing MacConkey agar for growing gram-negative organisms and the other side containing Factor agar for growing gram-positive organisms (Figure 1).

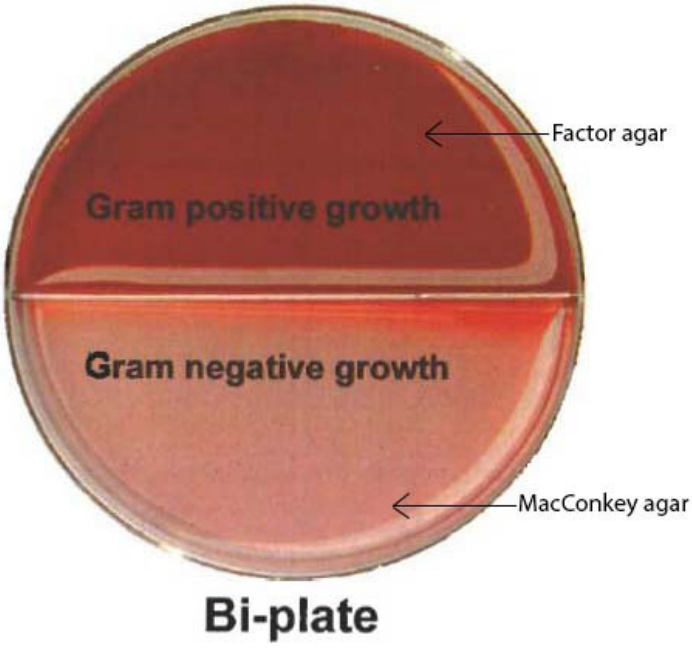

Figure 1. Bi-plate of Minnesota Easy Culture System II. (Top: Factor agar. Bottom: MacConkey agar.)
Credits: University of Minnesota Laboratory for Udder Health (2004)

In order to use the bi-plate on the farm, an incubator and clean lab space are needed for proper culturing and diagnosis of bacteria. A sterilely collected milk sample is spread over the agar, and the plate is placed into an incubator at 37°C for 18 to 24 hours in order to grow the bacteria from the infected quarter. Depending on bacteria growth, the pathogen can be classified as gram-positive or gram-negative within 24 to 48 hours on farm. This system was found effective in classifying common gram-positive and gram-negative mastitis pathogens but is limited if the sample has a low concentration of bacteria or if the pathogen does not grow on the plate (Lago et al. 2014; Sears et al. 1990). The system can also classify specific bacterial species by using the tri-plate version that identifies streptococci or staphylococci species.

PETRIFILM SYSTEM

The Petrifilm system results can be determined in only 24 h of incubation (Graber et al. 2007). These plates are designed to detect specific pathogens like *Staphylococcus* species (gram-positive bacteria) or coliform species (gram-negative bacteria). Milk collected sterilely from the infected quarter can be pipetted onto the center of the plate and incubated for 24 hours at 37°C. After 24 hours, bacterial colonies can be seen on the plate. The Petrifilm Staph Express Count

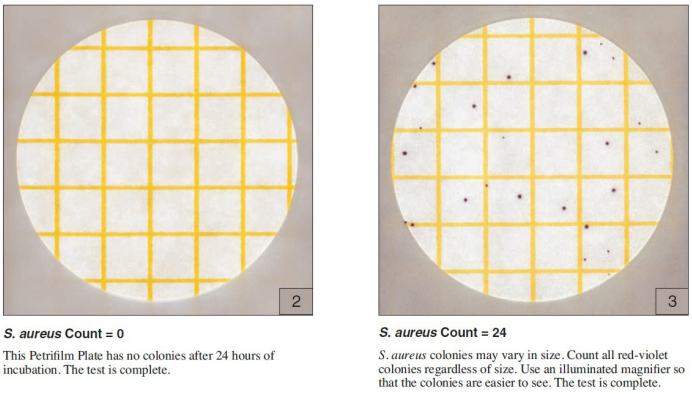

Figure 2. 3M Petrifilm Staph Express Count Plate.
Credits: Pinzón-Sánchez, Cabrera, and Ruegg (2011)

Plate (STX) uses variations of colony color to identify a specific *Staph.* bacteria; for example, the colony is a red-violet for *Staph. aureus* (Figure 2).

Proper application of on-farm culture systems requires a designated culture area on the farm in order to grow the bacteria safely as well as someone who is trained in reading the plates for diagnosis of the pathogen. Training by a veterinarian on sterile collection of milk samples is recommended in order to avoid contaminating the samples needing diagnosis. Using on-farm culture systems has been shown to result in significant reductions in discarded milk and a 50% reduction in antimicrobial use by using selective treatment versus treating all cases (Lago et al. 2011). The two on-farm culture systems can help farmers efficiently and effectively diagnose and selectively treat mastitis infections.

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Real-Time PCR

Real-time polymerase chain reaction (PCR)-based detection methods are another rapid and sensitive method of bacterial identification. They are capable of detecting specific pathogens in just a few hours. PCR-based methods detect DNA of specific bacteria in milk samples through amplification of bacterial DNA.

The PCR-based methods require more technical capabilities, but they are more sensitive, more specific, and faster than culture-based methods. A study by Phuektes, Mansell, and Browning (2001) found that a PCR assay had significantly higher sensitivity when compared with culturing for the detection of *Staph. aureus* and *Strep. uberis.* PCR-based methods can also detect pathogens in milk samples that originally would have had no growth when cultured (3M Microbiology 2010). PCR-based diagnosis is capable of detecting from one to several mastitis-causing organisms (Gillespie and Oliver 2005; Koskinen et al. 2010). PathoProof Mastitis PCR Assay is one current diagnostic PCR technology that can identify eleven mastitis-causing pathogens. The PathoProof Mastitis assay can be performed with milk directly from the infected quarter and provides results in four hours using DNA extracted from the sample (Koskinen et al. 2010). The milk sample must be collected sterilely to prevent contamination of the sample. If contamination of the sample occurs, the PCR technology may detect multiple pathogens that make it difficult to suggest an effective treatment.

PCR detection is usually performed in a diagnostic laboratory. The PCR detection of the pathogen can be performed on the farm only if there is a clean lab area for DNA isolation of the milk sample and the PCR equipment needed to perform the assay. The PCR equipment for the assay is more costly than culture equipment. It also requires technical expertise and training to process the milk sample so that it can be run in the PCR machine. Alternatively, the milk samples are often sent out to a commercial laboratory for PCR-based diagnosis due to the high overhead costs and the need for trained personnel. Altogether, PCR-based detection methods can diagnose milk samples in just a few hours and do not have the problems associated with the bacterial culturing detection methods like no growth or ineffective reading of the plates.

Each pathogen detection method is useful in classifying the infection-causing pathogen. Each system has individual characteristics allowing the user to decide which system they can manage best on their farm (Table 1).

Treatment

Once the pathogen is identified as gram-positive or gram-negative using one of the culture system or PCR-based methods, a treatment decision can be made based on the pathogen type. New cases of mastitis caused by gram-positive pathogens should be treated with antimicrobials, while cases caused by gram-negative pathogens should be left untreated because they will cure on their own (Lago et al. 2014). Identifying the pathogen can be worth the effort in modern US dairies where there is good control of contagious mastitis pathogens and culture-negative or gram-negative mastitis often account for more than half the clinical cases; for example, in one report 27% of clinical cases of mastitis yielded gram-negative pathogens, and 32% had no bacterial growth (Lago et al. 2014).

When the pathogen is identified as gram-positive, further culturing or real-time PCR can be done to determine the bacterial species. Refer to Table 2 for the current antimicrobials on the market that can be used on specific gram-positive pathogens. In order to avoid further culturing of every gram-positive pathogen, routine bulk tank cultures can also determine the types of gram-positive pathogens present in the herd. Gram-positive treatment decisions can then be made based on the spectrum of pathogens in the bulk tank. Treatments should be reassessed regularly by monitoring mastitis cure rates to determine if treatment decisions are effective.

Table 1. Characteristics of three bacterial identification systems.

Characteristics	Minnesota Easy Culture System II	Petrifilm	Real-time PCR
Cost per sample	++[a]	++	+[b]
Time to results	18–24 h	24–48 h	4 h
Ease of use	+	+	++
Identifies gram +/–	+	+	+
Identifies individual pathogens	+	+	++

[a] ++ indicates the method is better for that characteristic
[b] + indicates this method is good for that characteristic

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Table 2. Selective antibiotics for bacterial species for mastitis treatment of dairy cows.

Type	Bacterial species	Antimicrobial	Product Name	Drug Type
Gram-positive	*Staphylococcus aureus, Streptococcus agalactiae*	Amoxicillin trihydrate	Amoxi-Mast (Merk)	Rx[a]
Gram-positive and gram-negative	*coagulase-negative staphylococci, Streptococcus dysgalactiae,* and *Escherichia coli*	Ceftiofur hydrochloride	Spectramast LC (Zoetis)	Rx
Gram-positive	*Streptococcus agalactiae* and *Staphylococcus aureus*	Cephapirin sodium	Today (Boehringer-Ing.)	OTC[b]
Gram-positive	*Streptococcus agalactiae* and *Staphylococcus aureus*	Cloxacillin sodium	Dariclox (Merck)	Rx
Gram-positive and gram-negative	*Streptococcus agalactiae, S. dysgalactiae, Staphylococcus aureus,* and *Escherichia coli.*	Hetacillin (potassium)	Hetacin K (Boehringer-Ing.)	Rx
Gram-positive	*Staphylococcus species*	Pirlimycin	Pirsue (Zoetis)	Rx

Source: FARAD's *VetGram* (2014)
[a] Rx drugs are available only by veterinary prescription.
[b] OTC drugs are available over the counter.

Conclusion

In conclusion, on-farm culturing allows farmers to more efficiently diagnose and treat their cows based on the type of pathogen present. Pathogen-based treatment will result in decreased use of antimicrobials, because mastitis cases that are culture-negative or that are caused by gram-negative bacteria can selectively go untreated. Treatment is reserved for cases of mastitis where it is most likely to be most effective—new mastitis cases caused by gram-positive pathogens. Real-time PCR-based methods offer faster and more sensitive detection of mastitis bacterial pathogens compared to on-farm culturing, but PCR-based methods have higher cost and are typically performed in a commercial laboratory away from the farm.

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