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The use of bacterial culture for an on-farm method of determining mastitis causing pathogens

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Bacterial culture can be used to determine the species of bacteria and help producers make easier decisions about treatment methods. Depending on the species, Gram-positive bacteria such as *Streptococcus agalactiae* respond to antibiotics and can be treated. Mild to moderate mastitis cases caused by coliform Gram-negative bacteria, such as *E. coli*, have a high spontaneous cure rate, which limits the need for antibiotics on-farm[1]. The use of on-farm bacterial culture can help to quickly determine if the mastitis is caused by a Gram-positive and Gram-negative bacteria and allow producers to treat appropriately.

Environmental Mastitis

Environmental mastitis is mostly caused by unsanitary bedding material that contain bacteria such as *E. coli* and *Streptococcus uberis*[2]. Environmental mastitis can be prevented, at least in part, by applying pre-dip before milking[6] and removing manure from bedding.

Contagious Mastitis

Contagious mastitis is exposed through unfollowed milking protocols and unsanitary milking equipment from an infected cow that was milked previously[5]. Common contagious bacteria include *Staphylococcus aureus* and *Streptococcus agalactiae*, which are Gram-positive species. The best way to prevent contagious mastitis is to apply a teat disinfectant after milking[5] and correctly clean milking equipment.

On-Farm Bacterial Culture

Bacterial culture, the gold standard for identification of mastitis pathogens, is an on-farm method that can provide results in 24 hours.

Materials needed for culturing:

- Incubator set at 37°C (98.6ºF)
- Culture plates (tri-plate, bi-plate, blood agar, MacConkey etc.)
- Disposable gloves
- Milk sample vials
- Sterile cotton swabs
- Cotton swabs soaked in 70% ethyl alcohol for teat disinfection
- Cooler with ice (if transporting milk samples to lab)
- Permanent marker for labeling

Teats should be disinfected with a cotton swab soaked in 70% ethyl alcohol and scrubbed for 10 to 15 seconds[7] to ensure a non-contaminated milk sample. If collecting from all four quarters, make sure to completely disinfect each teat with a different cotton swab to prevent contamination. Make sure milk vials are labeled with cow ID and which quarter the sample is collected from. Then direct milk streams into the vial until 1/3 full[6], while preventing the teat from touching the vial. If applicable, immediately place the sample vial in the refrigerator or samples can be kept frozen up to 60 days[7].

Next, a sterile cotton swab should be submerged in the milk sample until the swab is saturated with milk. Then apply the swab to the culture plate by the pattern shown below (Figure 1), and re-dip the swab in the milk for each of the three sections. After the milk samples are applied to the plate, label plates with cow ID and date. Then place the plates lid down in the incubator at 37°C for 24 hours[7].

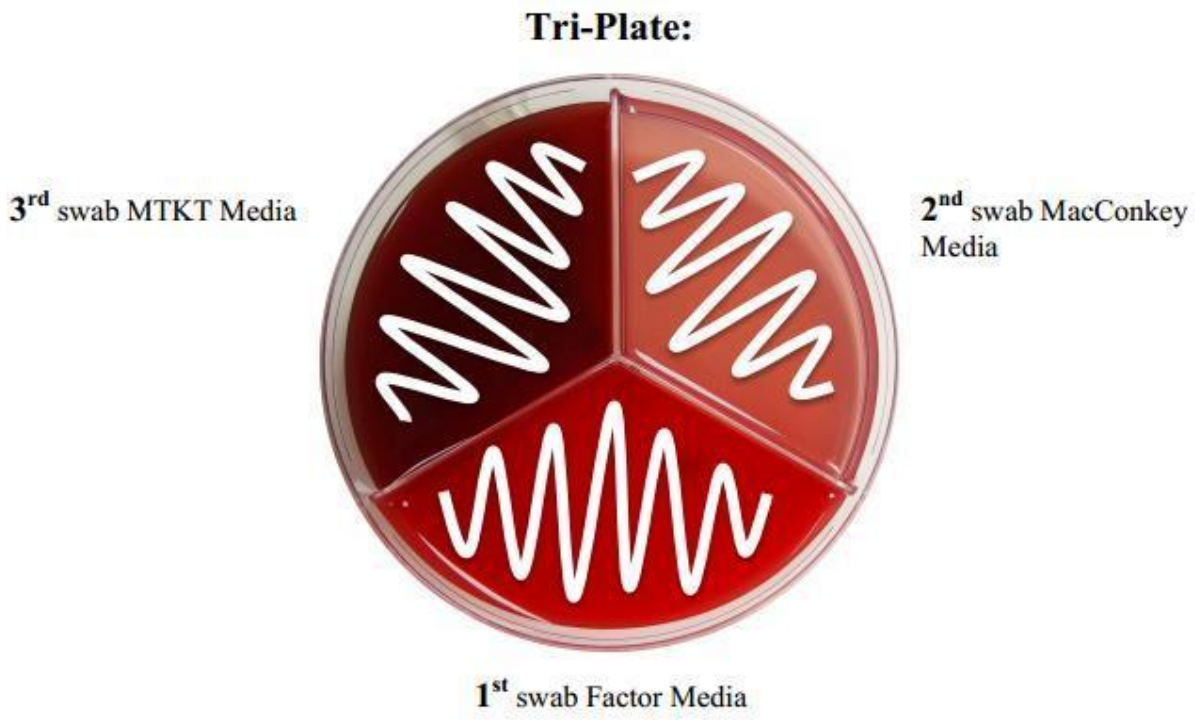

Figure 1 from Minnesota Easy Culture System User's Guide

Interpreting the tri-plates results are different for each agar. Factor agar selectively grows streptococci spp, while Focus agar selectively grow staphylococcus spp[1]. MacConkey agar is designed to grow Gram-negative bacteria. The simple diagnosis can tell producers if treatment with antibiotics is necessary. Figure 2 represents further interpretation of *Staphylococcus aureus* with growth on the Factor agar. Figure 3 represents growth of *E. coli*, which grows on MacConkey agar because this bacterium is Gram-negative. If plates contain three or more bacteria, then it is considered contaminated, and plating should be completed again[6]. If the plate results in no growth, that can be considered a true negative (no infection) or there was an error in the collection[7]. If more than 40% of results are no growth, it is best to consult your veterinarian about diagnosis and treatment protocols[7]. Bacteria such as *Mycoplasma* species cannot be cultured, so further methods are needed for identification such as quantitative polymerase chain reaction (qPCR) or a cell stain.

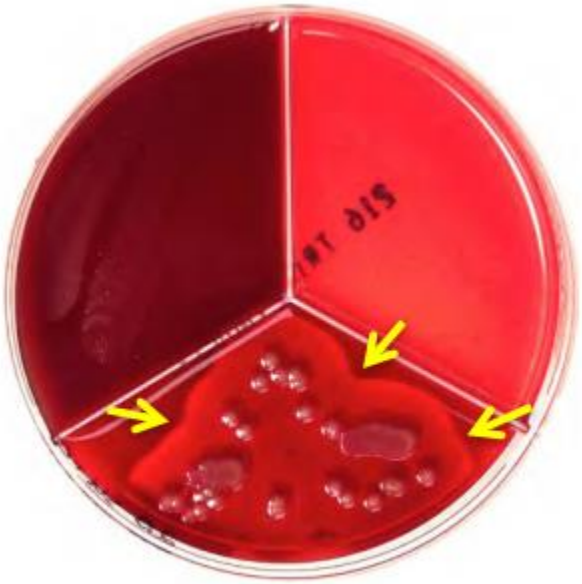

Figure 2 from Minnesota Easy Culture System User's Guide

Figure 3 From Minnesota Easy Culture System User's Guide

Conclusion

On-farm bacterial culture can help the producer to reduce mastitis cases. This is useful to provide the producer with information regarding where the bacteria source is coming from. If a cow's culture contained contagious mastitis, then the producer can separate the cow from the herd to reduce the risk of transmission. If the cow's culture contains environmental bacteria, then the producer can determine if bedding is the source. Bacterial culture overall reduces the on-farm use of antibiotics and reduces cost of treatment. Unnecessary use of antibiotics can lead to antibiotic resistance, making future treatment of mastitis difficult[8]. Using bacteria culture to identify mastitis-causing pathogens can reduce the cost associated with treatment of mastitis cases.

References

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http://texasdairymatters.org March, 2022
