ON-FARM MILK CULTURING FOR BOVINE MASTITIS MANAGEMENT

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Abstract

Bovine mastitis is the most common reason for antibiotic usage in dairy cows, its costs being estimated at more than $350 per mastitis case. Accurately identifying the bacterial species at the core of a mastitis infection may help narrow down treatment choices and cut down on the overuse of antibiotics. The aim of the present study was to evaluate the prevalence of etiological agents in milk samples from cows with clinical mastitis using commercially available culture plates (*ClearMilk*, LabMediaServis, Czech Republic). Briefly, milk samples were collected from udder quarters with clinical mastitis and were aerobically cultured using the on-farm culture system. If two prominent mastitis pathogens were identified, the sample was labelled as "mixed growth," and if three or more pathogens were observed, it was labelled as "contaminated". Our results indicate that ClearMilk might be an useful on-farm milk culturing tool for detecting pathogens linked with bovine mastitis.

Key words: mastitis; on-farm culturing; milk; antibiotics

INTRODUCTION

The most common reason for antibiotic usage in dairy cows is mastitis. Clinical mastitis may result in annual costs of more than $350 per cow due to treatment and wasted milk, according to one research. Clinical mastitis is often treated with antibiotics, despite the fact that drugs are often inefficient or unnecessary in treating the condition (Dego, 2020).

The most prevalent mastitis pathogens are found in udder tissues (contagious pathogens such as *Staphylococcus aureus*, *Streptococcus uberis* or *Escherichia coli*) or in the herd's environment (environmental pathogens), such as bedding, manure, and soil (Heikkilä *et al.*, 2018).

Accurately identifying the bacterial species at the core of a mastitis infection may help narrow down treatment choices and cut down on the overuse of antibiotics. Adapting herd management practices may reduce the prevalence of some pathogens. During milking, the parlor might get contaminated with pathogens like Staphylococcus aureus. Milking affected cows last, dipping teats, and using gloves are all strategies to limit the spread of the disease. If the culprit pathogen could be identified, farmers may adapt their practices to lessen the likelihood of infecting any further cows. A farmer's decision to not treat a cow may also be influenced by local norms.

In order to identify the pathogen agent, producers may submit milk samples to nearby labs for culturing. The delay of several days between milk submission and farmers receiving test results is the major drawback of this conventional diagnosis method. Since producers have to wait for test findings, they often make uninformed treatment choices. Thus, timely preventative treatment choices may be implemented with the support of an on-farm culturing system (Ganda *et al*, 2016).

The aim of the present study was to evaluate the prevalence of etiological agents in milk samples from cows with clinical mastitis using commercially available culture plates (ClearMilk, LabMediaServis, Czech Republic).

MATERIAL AND METHOD

Milk sample collection

The study was conducted to evaluate the efficiency of on-farm culture system in identifying bacteria linked with clinical mastitis, using 29 milk samples collected within a four-month interval between July and October of 2022, from the dairy farm within the research center. Milk samples were collected aseptically by qualified research employees from mammary quarters with clinical mastitis using a methodology previously described by Ferreira, *et al*. 2018.

Teats were cleansed and disinfected with a napkin containing 70% ethanol. The first strips

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were discharged before milk samples were collected in sterile 12 mL tubes, without preservatives.

Clinical assessment

A clinical mastitis case was defined as the presence of abnormal milk (i.e., watery secretion, occurrence of flakes or clots) with or without visible signs udder or general inflammation (i.e., swelling, redness, pain, increased local heat, fever, milk yield decrease), which were detected by trained research personnel at each milking.

The severity of the clinical infection was assessed by trained research personnel as described previously by Bradley and Green, 2001. Clinical mastitis score 1 was attributed to cases in which macroscopic modifications in milk were observed, but no other inflammation signs were present. Clinical cases in which both macroscopic modifications in milk and udder inflammation signs were present were considered as score 2. Finally, cases in which the macroscopic modification of milk were present alongside udder inflammation signs and general inflammation sign such as fever were considered as score 3.

The herd samples were taken to the Research and Development Station for Cattle Breeding Dancu, Iasi, Laboratory of Nutrition, Food Quality and Safety where they analyzed withing two hours from sample collection.

On-farm culturing test

The inoculation wand included in the kit was dipped in the sterile plastic tube holding the milk sample, and then wiped over the surface of each Petri dish with selected growth medium in a zig-zag pattern in accordance with manufacturer's specifications. Plates were incubated aerobically at 37°C for 24 hours before being read on-site by a single member of the research team.

Interpretation of the culturing results

If two prominent mastitis pathogens were identified, the sample was labelled as "mixed growth," and if three or more pathogens were observed, it was labelled as "contaminated". The following were the preliminary selection criteria for microorganisms: five identical colonies were found for major pathogens (with the exception of S. aureus and Streptococcus, where the presence of a single colony was deemed positive); 10 identical colonies for the other bacteria.

RESULTS AND DISCUSSIONS

Figure 1 depicts the percentage of mastitis caused by various infections on each farm. The most frequent encountered pathogen in the collected milk sample was *Streptococcus dysgalactia* (24.14 % of the cases). *Streptococcus spp.* are a prominent cause of bovine mastitis across the globe, impacting both milk production and animal welfare. Pathogens *Streptococcus dysgalactia* or *Streptococcus uberis* are often found in manure and organic material, including bedding materials, and may colonize both animals and the environment, potentially leading to both clinical and sub-clinical mastitis in dairy cattle (Wente *et al.*, 2019).

Dairy cows are most typically infected through environmental sources during the dry period, often leading to clinical cases during the subsequent lactation. In our study, 75% of the cows diagnosed with clinical mastitis, in which *Streptococcus uberis* was identified as causative agent, presented both macroscopic modifications in milk as well as local signs of infections, consistent with a mastitis score 2, while 25% additionally presented an overall altered health status, consistent with mastitis score 3. Another major pathogen that generated more severe infection manifestation is *Escherichia coli* (Figure 1, Table 1). Milk modifications and udder inflammation signs are generally accompanied by acute general symptoms such as diarrhoea, and chilled extremities. Because this pathogen may cause serious foodborne illness, its presence is considered a food safety concern and a health risk for both people and animals (Goulart and Mellata, 2022).

0ver 17% of the analyzed milk samples were positive for Coagulase-negative staphylococci (CoNS), *Staphylococcus chromogenes* being the main coagulase-negative staphylococci isolated from the analyzed mastitis samples (Figure 1; Table 1). Coagulase-negative staphylococci (CoNS) are less virulent because they do not generate a high number of harmful enzymes and toxins (Lee and Lee, 2022). Yet, they are becoming more recognized as a significant cause of bovine mastitis due to their capacity to build biofilms which in turn may lead to increased resistance to antimicrobials.

The severity degree for the other pathogens was assessed as score 1, respectively score 2. This difference may be relevant when analyzing a herd's problems and the strategies that may be implemented to minimize or treat mastitis (Lee and Lee, 2022).

Even if the sampling collection protocol is accurately respected, there is still a risk of sample contamination (Figure 1), which is way the interpretation of the results should be made with carefully consideration. If a strongly changed inflammatory secretion is observed, treatment options should be considered even if no growth was observed. Mastitis control has gained increasing attention as a result of the emphasis on the judicious use of antibiotics in dairy herds, especially in terms of prevention of new infections and alternative treatment approaches (Neculai-Valeanu *et al.*, 2021).

Concerns about antibiotic residues in farm-raised meat and milk were usually at the forefront of discussions about antibiotic stewardship in the dairy sector in order to guarantee a secure food supply. However, in the past years, antibiotic stewardship measures, which aim at reducing antibiotic-resistant bacteria to benefit both human and animal health, have become a key point of focus worldwide (More *et al.*, 2022).

While *Streptococcus uberis* requires extended parenteral and/or intramammary antibiotic treatment durations for at least 5 days, mastitis generated by coagulase-negative staphylococci may be treated exclusively with anti-inflammatory therapy (Smulski *et al.*, 2020). Mixed growth samples and no growth cases should be treated taking into consideration the presence or absence of macroscopic modifications in milk.

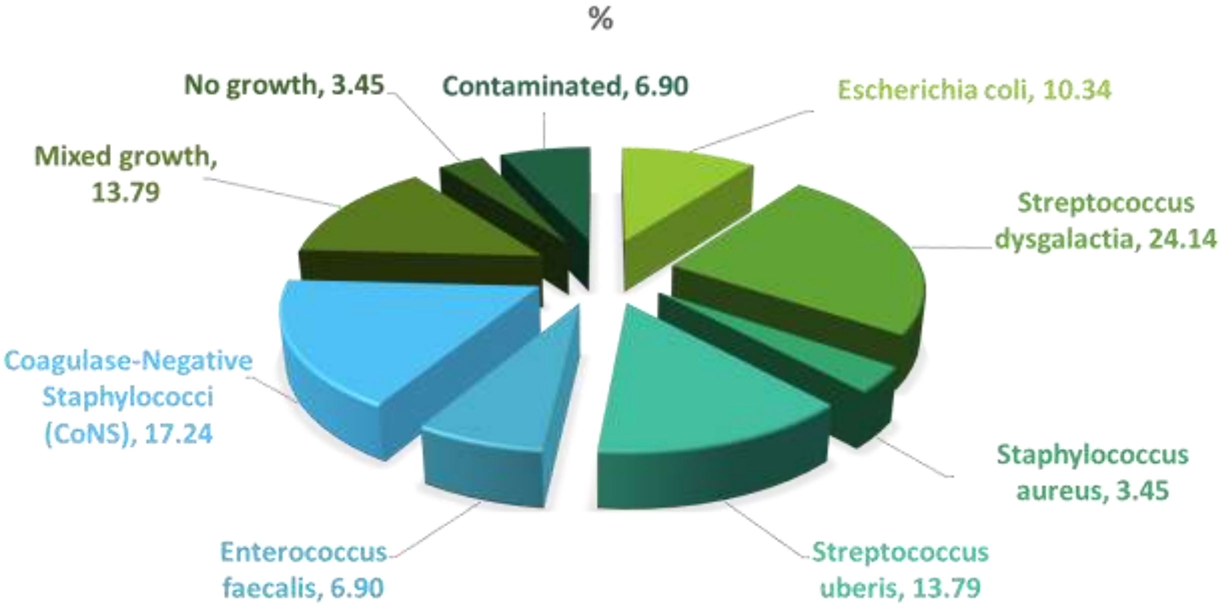

Figure 1 **Percentage distribution of mastitis pathogens in clinical mastitis samples with positive bacteriologic growth**

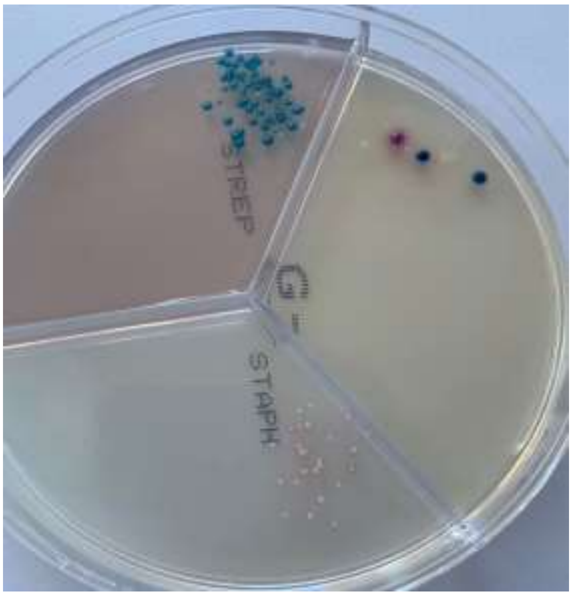

Figure 2 **Contaminated milk samples from cows with mastitis (*Streptococcus dysgalactia*, *coagulase-negative staphylococci* and *E. coli* colonies present in the sample**

Table 1

Percentage cases of mastitis of different grades of severity associated with each pathogen

Pathogen	Mastitis Score		
	1	2	3
Escherichia coli	-	33.33	66.67
Streptococcus uberis	71.43	28.57	-
Staphylococcus aureus	-	-	100
Streptococcus dysgalactiae	-	75.00	25.00
Enterococcus faecalis	50.00	50.00	-
Coagulase-Negative Staphylococci (CoNS)	60.00	40.00	-
Mixed growth	50.00	50.00	-
Contaminated	100.00	-	-

CONCLUSIONS

On farm culturing systems are intended to inform and guide farmers toward preventative mastitis treatment strategies. While antibiotics may not be considered necessary in mild clinical cases, pathogens such as *Streptococcus spp.* and *Staphylococcus aureus* should be handled with intramammary antibiotics to maximize cure and reduce risk of transmission. In fungal mastitis immunomodulators and modification of milking routine, with more milkings per day may improve the outcome. Our results indicate that ClearMilk might be an useful on-farm milk culturing tool for detecting pathogens linked with bovine mastitis in order to adopt an appropriate treatment protocol.

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