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Laboratory evaluation of a novel rapid tube test system for differentiation of mastitis-causing pathogen groups

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ABSTRACT

Because clinical mastitis, one of the most common diseases in dairy cows, is routinely treated with antimicrobial substances, it offers a high potential for future reduction of antimicrobial usage. In fact, intramammary antibiotic administration is not advisable in cases of clinical mastitis caused by coliform bacteria, yeasts, or prototheca or in cases with no detectable mastitis pathogen. To avoid unnecessary treatments with antimicrobials for the benefit of animal health and public welfare, the rapid identification of the mastitis-causing pathogens becomes necessary. Therefore, 4 different incubation time schemes for a newly developed tube test system (MastDecide, Quidee GmbH, Homberg, Germany) were analyzed in terms of sensitivity, specificity, negative and positive predictive values, and apparent and true prevalence compared with the conventional microbiological investigation results for 251 clinical mastitis milk samples from 11 dairy farms located in northern Germany. An aliquot (100 μL) of a quarter foremilk sample was taken in both cases. The evaluation of the tube test result after 14 h of incubation at 37°C resulted in sensitivity values of 83.6, 72.2, and 70.7% and specificity values of 94.1, 83.3, and 90.8% for gram-positive cocci, coliform bacteria, and no growth or further pathogens, respectively. Moreover, for the present pathogen distribution, the overall tube test sensitivity was highest after 14 h of incubation (sensitivity = 80.9%; specificity = 70.7%). The described tube test system has the potential to provide a new option for an evidence-based mastitis therapy, with the aim of reducing the future usage of antimicrobials in dairy cows and a larger goal of decreasing antimicrobial resistance. However, a subsequent on-farm test validation should be performed before implementation in an evidence-based mastitis therapy concept can be recommended.

Key words: on-farm test, antimicrobial usage, dairy cow, treatment decision

INTRODUCTION

The development and spread of antimicrobial resistance have become a major issue for public health, threatening to cause up to 10 million human deaths annually by the year 2050 (O'Neill, 2014). Presently, about 700,000 people worldwide die of infections with multidrug-resistant bacteria every year. Intervention strategies comprise the reduction in the general usage of antimicrobials as well as the restricted use of substances with critical importance for human health (EMA, 2014). Thus, rising concerns and political pressure regarding the use of antimicrobials in veterinary medicine—especially in livestock production—increase the demand for novel tools and therapeutic concepts that can enable a prudent, optimized application of antimicrobials (Trevisi et al., 2014). In dairy production, the major proportion of antimicrobials is applied for the treatment of udder inflammation (68%; Kuipers et al., 2016). A significant reduction of antimicrobial usage in dairy production can be achieved either directly by optimization of antimicrobial usage for clinical mastitis and dry cow treatment or indirectly by general udder health improvement (Krömker and Leimbach, 2017). The evidence-based treatment of clinical mastitis considers individual factors such as the age and lactation number of cows, mastitis history, SCC, clinical mastitis grade (IDF, 2011; GVA, 2012), and the mastitis-causing pathogen (Krömker and Leimbach, 2017).

Antimicrobial therapy can increase bacteriological cure rates above those achieved by self-healing depending on the presence of certain mastitis-causing pathogens. However, in a significant number of mastitis cases, cows do not benefit from antimicrobial treatment. In particular, earlier studies have shown that intramammary antibiotic treatment in mild to moderate clinical mastitis cases caused by gram-negative bacteria, such as *Escherichia coli*, does not significantly improve bacteriological cure rates (Barlow, 2011; Suojala et al., 2013; Persson et al., 2015). Furthermore, IMI caused

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by yeasts, *Prototheca* spp., *Mycoplasma* spp., or cases without bacterial growth in the conventional microbiological investigation do not justify any therapeutic antibiotic treatment (Roberson et al., 2004; Hoe and Ruegg, 2005; Lago et al., 2011a,b; Roberson, 2012). However, antimicrobial treatment of udder inflammations caused by gram-positive bacteria, especially streptococci or staphylococci, can significantly enhance bacteriological cure rates (Roberson, 2012). Thus, intramammary antibiotics can be responsibly used considering the results of a microbiological investigation (Roberson, 2003, 2012; GVA, 2012), but a cytomicrobiological laboratory investigation takes at least 48 to 72 h, making it difficult to arrive at a timely treatment decision (Duarte et al., 2015). In fact, based on empirical results from former therapy, after this time span an antimicrobial treatment has usually at least been started if not concluded. This leaves up to 50% of the antimicrobial applications unnecessary in retrospect (Owens et al., 1997; Roberson, 2003, 2012; Neeser et al., 2006; Barlow, 2011; Lago et al., 2011a,b; Viora et al., 2014; Mansion-de Vries et al., 2015). Validated rapid on-farm culture systems such as the Petrifilm system (Mansion-de Vries et al., 2014) or the Minnesota Easy Culture System (University of Minnesota Laboratory for Udder Health, St. Paul; McCarron et al., 2009; Lago et al., 2011b) are able to produce results within 24 to 48 h, allowing earlier treatment decisions or corrections.

With the help of rapid differentiation of the pathogen group in a time span of 12 to 24 h, a targeted treatment decision can be made without risking animal health and welfare (Guterbock et al., 1993; Neuling et al., 2011; Roberson, 2012). Intramammary antimicrobial treatment is required and recommended if the mastitis-causing pathogen is gram positive; thus, a classification of the pathogen based on the Gram staining reaction (gram-positive, gram-negative, or no growth) is sufficient and necessary for an evidence-based treatment decision (Roberson, 2012; Krömker and Leimbach, 2017). As reported by Mansion-de Vries et al. (2014, 2016), who evaluated a 24 h-Petrifilm-based treatment concept, selective intramammary antibiotic treatment of cows with gram-positive test results not only reduces antibiotic usage significantly in mastitis therapy but also does not have a negative influence on udder health. Nevertheless, further rapid test systems that can enable earlier treatment decisions while delivering easy performance, distinct result interpretation, and high consumer and handling safety need to be developed.

The objective of this study was to evaluate the performance of a novel, rapid on-farm test system (MastDecide, Quidee GmbH, Homberg, Germany) for the identification of gram-positive cocci and coliform mastitis–causing pathogens. The described tube test system is particularly easy and safe to handle and simple to interpret. It has a shelf life of at least 6 mo if stored in a cool, dark place. The system consists of 2 test tubes containing pink test medium that can discriminate between gram-positive cocci, coliform bacteria, and no growth. A visible discoloration of the test medium after its inoculation with a 100-μL sample and incubation at 37°C for a defined time is considered as a positive reaction. A discoloration of both test tubes indicates the growth of gram-positive cocci, a discoloration of only the first tube indicates the growth of coliform bacteria, and no change in color indicates no microbiological growth. Because some microorganisms (e.g., lactic acid bacteria, yeasts, *Prototheca* spp., *Pseudomonas* spp., *Bacillus* spp., *Corynebacterium* spp., or *Trueperella pyogenes*) are not able to grow in the tube system, the test will result in no growth for these microorganisms.

To evaluate the performance of the MastDecide test, the laboratory results of the new test system were compared with conventional cytomicrobiological investigation results as a reference method for 269 clinical bovine mastitis milk samples, wherein both tube tests and microbiological investigation used an inoculum of 100 μL. To identify the most suitable incubation time for the tube test evaluation, 4 different time periods were examined. Additionally, the resulting correct and false treatment decisions were included in the analysis.

MATERIALS AND METHODS

Sample Processing

Between April and September 2017, a total of 267 clinical mastitis samples of Holstein Friesian dairy cows from 11 farms in northern Germany, which arrived at the microbiological laboratory of the University of Applied Sciences and Arts (Hanover) for routine cytomicrobiological investigation, were included in the study. The samples with a transportation time of more than 2 d were excluded from the study. In case of clinical mastitis, milking personnel were instructed to collect a quarter foremilk sample according to the rules of the GVA (2009). The sampling tubes contained a boric acid–based preserving agent (Ly-20; Heeschen et al., 1969) and were stored below 8°C until transported to the laboratory. In the laboratory, 100 μL of the well-mixed quarter foremilk sample was plated onto esculin blood agar (Oxoid, Wesel, Germany), and simultaneously 100 μL was added to each of the MastDecide test tubes. After proper mixing, the test tubes and agar plates were immediately incubated at 37°C under aerobic conditions.