

Prevalence of Mastitis Pathogens and Their Resistance Against Antimicrobial Agents in Dairy Cows in Brandenburg, Germany

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ABSTRACT

The primary objective of this study was to determine management practices concerning mastitis in Brandenburg, Germany, the prevalence of mastitis pathogens in dairy cows, and their resistance to selected antimicrobial agents. A further objective was to study the potential effect of parity and stage of lactation on the resistance of *Staphylococcus aureus* isolates against ampicillin. Milk samples for microbiological culture were collected from 4 groups of clinically healthy cows (first lactation, >1 lactation, >50 d in milk, and >250 d in milk; 8 cows/group) in 80 dairy herds. Resistance of gram-positive pathogens against 6 antimicrobial agents was tested using the broth microdilution method. Mastitis pathogens were isolated from 26.4% of the milk samples. Coagulase-negative staphylococci (CNS, 9.1% of quarters) and *Corynebacterium bovis* (7.3%) were the pathogens most frequently isolated. Among the major pathogens, *Staph. aureus* (5.7%) and *Streptococcus uberis* (1.0%) had the highest prevalence. *Streptococcus agalactiae* was isolated in samples from 29% of the herds. Although the prevalence of most pathogens was higher in older cows, the prevalence of CNS was higher in primiparous cows. Results of the mastitis control questionnaire showed that cows with clinical mastitis were transferred to a sick cow pen in 70% of the herds. Cephalosporins were the drug of first choice for treatment of clinical mastitis cases followed by fixed combinations of antimicrobial agents, β -lactamase-resistant penicillins, and penicillin. Most farmers treated cows 3 to 4 times per case. Cloxacillin, alone or in combination, and penicillin were most often used for dry-cow therapy. Antimicrobial resistance of the pathogens was within the range of other reports. Resistance of *Staph. aureus* to ampicillin increased significantly during the first lactation. Further research is required to determine the factors that lead to the selection of *Staph. aureus* strains that are resistant to ampicillin during the first lactation.

Key words: dairy cow, mastitis, antimicrobial resistance

INTRODUCTION

Mastitis is one of the major causes of antibiotic use in dairy cows (Mitchell et al., 1998; DANMAP, 2003). Over 135 different microorganisms have been isolated from bovine intramammary infections (IMI), but the majority of infections are caused by staphylococci, streptococci, and gram-negative bacteria (Bradley, 2002). The contribution of various strains of bacteria to mastitis has shifted over time. Measures to control mastitis such as improved milking hygiene have reduced the prevalence of contagious major pathogens such as *Streptococcus agalactiae* (Hillerton et al., 1995; Myllys et al., 1998; Makovec and Ruegg, 2003a; Pitkala et al., 2004). Somatic cell counts have decreased in many countries (Myllys et al., 1998). However, other bacteria such as environmental streptococci have become more important, and the reduction in subclinical mastitis has not been accompanied by a reduction in clinical mastitis (Myllys et al., 1998).

Resistance of mastitis pathogens to antimicrobial agents is a well-documented challenge in dairy cows (Owens et al., 1997; Lotthammer and Klarmann, 1999; Trolldenier, 1999; Erskine et al., 2002; Makovec and Ruegg, 2003b; Pitkala et al., 2004). The World Health Organization (WHO) has stated that any use of antimicrobial agents is associated with the risk of inducing resistance to antimicrobial agents among bacteria (WHO, 1997). This has called for more investigations into the use of antimicrobial agents in food-producing animals and the determination of potential factors that influence the level of resistance in mastitis pathogens (Lotthammer and Klarmann, 1999; Osteras et al., 1999; Trolldenier, 1999; Aarestrup, 2005). Regional differences in resistance patterns of pathogens exist in Germany and worldwide (Salmon et al., 1998; Lotthammer and Klarmann, 1999; de Oliveira et al., 2000). However, a relationship between the resistance patterns of mastitis pathogens and the intensity of food animal husbandry in the respective regions could not be estab-

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lished (Lotthammer and Klarmann, 1999; Schröter, 2003).

Differences in resistance patterns between reports may be caused to some extent by the variation in methods used for the determination of resistance against antimicrobial agents. Early reports on resistance, but also some recent ones (Erskine et al., 2002; Makovec and Ruegg, 2003b) were based on the disk diffusion method, which has been shown not to correlate well with the minimum inhibitory concentrations (MIC) determined by dilution methods (Kibsey et al., 1994; Kelly et al., 1999). Presently, dilution methods are recommended by expert groups as the methods of choice (Erskine et al., 2004; Luhofer et al., 2004).

Resistance of *Staphylococcus aureus* to penicillin or ampicillin has been extensively studied (Erskine et al., 2004). Although the MIC of penicillin against *Staph. aureus* did not differ between strains isolated from heifers and cows in one study (Watts et al., 1995), a recent report has demonstrated numerically higher proportions of penicillin-resistant CNS in older cows compared with isolates from primiparous cows (Rajala-Schultz et al., 2004). Treatment with penicillin at dry off has been proposed to exert selection pressure toward penicillin-resistant *Staph. aureus* strains (Osteras et al., 1999).

Resistance to antimicrobial agents in mastitis pathogens has 2 relevant aspects: The first is a reduction in cure rates after treatment of clinical mastitis cases (Owens et al., 1997; Sol et al., 2000). The second issue is the potential impact of transmission of resistant bacteria to humans via the food chain (Ungemach, 1999). This is not likely to occur with milk from clinical cases of mastitis, because this milk is banned from human consumption. However, clinical cases may turn into subclinical cases or latent infections. Resistant bacteria from these infections are present in the bulk tank milk and may therefore be transmitted to humans via raw milk products.

In eastern German provinces, herd sizes are bigger than in most other parts of the European Union. Limited literature exists on the contribution of different mastitis pathogens to the mastitis problem of these dairy herds and on their management practices concerning mastitis problems.

Therefore, the objectives of this study were to determine management practices concerning mastitis, and the prevalence of mastitis pathogens in clinically healthy quarters of dairy cows in Brandenburg, Germany. A further objective was to determine the susceptibility of these bacteria to 6 antimicrobial agents that are or have been commonly used in dairy cows. Finally, we investigated whether the prevalence of ampicillin resistance of *Staph. aureus* was influenced by parity or stage of lactation.

MATERIALS AND METHODS

The study was conducted on 80 dairy farms selected from the respondents to a mail survey on dairy cow management practices with particular respect to mastitis. A standardized questionnaire had been sent to 845 farms that were members of the Brandenburg Dairy Herd Improvement Association (Landeskontrollverband Brandenburg, Waldsiedersdorf, Germany). The German province of Brandenburg is located around the German capital (Berlin). Two hundred farms (23.7%) responded to the questionnaire and indicated willingness for further cooperation. A convenience sample comprising 96 of the respondents was chosen to participate in the study. The selection of herds was carried out to achieve representative distribution across the province concerning size and location of the farms. Of the selected farms, 16 were removed from the analysis for reasons of poor data quality or limited data access.

All farms were visited once between July 2001 and October 2002 by the same investigator. Management practices, housing conditions, and milking routines were evaluated and documented on a standardized data capture form. Milking routines were recorded by observation of routine milking over one milking period. Management of cows with mastitis was recorded as observed during the visit. For events that could not be observed during the visit (e.g., dry-cow routines, treatment protocols), management was recorded as indicated by the farm manager in a questionnaire covering 20 items.

Aseptic quarter foremilk samples were collected from 4 groups of animals: 1) Primiparous cows at the beginning of the lactation (≤ 50 DIM); 2) primiparous cows at the end of lactation (≥ 250 DIM); 3) older cows ($\geq$ second lactation) at the beginning of the lactation (≤ 50 DIM); and 4) older cows ($\geq$ second lactation) at the end of lactation (≥ 250 DIM).

Eight clinically healthy cows of each of the 4 groups were sampled at milking time before cluster attachment on each farm. Cows were selected in order of their appearance in the milking parlor during the visit. Cows with blind quarters were included, but they only contributed 3 samples per cow (Table 1). Samples were cooled and shipped to the laboratory at the same day.

Microbiological Analyses

Milk samples (0.01 mL) were plated on blood agar (blood agar base no. 2, Oxoid, Wesel, Germany, with 5% sheep blood) that contained 0.1% esculin. Preliminary identification was by colony morphology, hemolysis, and Gram staining. Samples were regarded as positive for environmental pathogens if a minimum of 1,000 cfu of the respective pathogen was determined. For *Strep. agalactiae* and *Staph. aureus*, a minimum of 100 cfu

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