ANTIMICROBIAL RESISTANCE OF THE MASTITIS PATHOGENS IN DAIRY COWS

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ABSTRACT
There has been a rapid awareness about multiresistant pathogens in the past 10 years and some bacteria are now resistant to most antimicrobial agents used in veterinary practice. Occurrence of multiresistant bacteria in milk from both dairy cows with high somatic cell count and clinical mastitis were analyzed. Multiple resistance of bacteria isolated from milk depends on bacteria species and is more prominent for coagulase negative staphylococci and Streptococcus agalactiae.

KEY WORDS: mastitis, antibiotic resistance, dairy cow.

INTRODUCTION
There has been a rapid awareness about multiresistant pathogens in the past 10 years and some bacteria are now resistant to most antimicrobial agents used in veterinary practice. According to knowledge the bacteria rapidly develop resistance to antibiotics if they are used at subterapeutic level or short treatment episodes. Mastitis due to often spread and its treatment undertakes considerable part of all antibiotic amount used in dairy cows, therefore bacteria found in milk could be important source which spreads multiresistant bacteria into dairy products, food chain and environment as well (Chaslus-Dancla, 2000).

Clear species-related differences in the percentage of antibiotic resistance were found, but they did not differ significantly between isolates from cows kept on organic and conventional farms (Roesch, 2006), except for Streptococcus uberis, which exhibited significantly more single resistances (compared with no resistance) when isolated from cows kept on organic farms (6/10 isolates) than on conventional farms (0/5 isolates). Different percentages were found (not statistically significant) in resistance to ceftiofur, erythromycin, clindamycin, enrofloxacin, chloramphenicol, penicillin, oxacillin, gentamicin, tetracycline, and quinupristin-dalfopristin, but, importantly, none of the strains was resistant to amoxicillin-clavulanic acid or vancomycin. Multidrug resistance was rarely encountered. The frequency of antibiotic resistance in organic farms, in which the use of antibiotics must be very restricted, was not different from conventional farms, and was contrary to expectation. The antibiotic resistance status needs to be monitored in organic farms.

The aim of study was to test antimicrobial resistance of mastitis pathogens during years 2008…2010 and find out risks of concern in the future.

MATERIAL AND METHODS
There were performed udder health check visits at 36 dairy farms in Latvia regions, namely, Bauska, Daugavpils, Dobele, Jelgava, Jekabpils, Kraslava, Kuldiga, Ludza, Preiļi, Saldus, Tukums and Ventspils, during the period from January 2008 till December 2010. From the cows with clinical mastitis or high somatic cell count in milk (>400 000 cells ml-1) and positive reaction with subclinical mastitis test, aseptically quarter milk samples were taken for bacteriological investigation. Samples were refrigerated and taken to the laboratory.
of herd health and reproduction problem laboratory at the Faculty of Veterinary Medicine in Jelgava. 10 ml from each sample were inoculated onto the Columbia blood agar plate with 5% sheep blood. Growth on the plates were evaluated according to Nacional Mastitis Council methods and recommendations (NMC, 1999). One, most relevant, mastitis pathogens isolated from every cow was tested on resistance to antimicrobials. Modified Kirby-Bauer disc method was applied and a set of following antibiotics included Penicillin, Amoxicillin, Cephalexin, Cefotaxime, Oxitetracycline, Norfloxacin, Amoxicillin with clavulanic acid (BD BBL Sensi-Disc Antimicrobial Susceptibility Test Discs). Occurrence (%) of resistance against antibiotic substances were registered. Intermediate susceptibility was registered too. Multiple resistance against penicillin and other antibiotics were estimated by correlation coefficients calculated by means of statistical software STATA 9.0.

RESULTS AND DISCUSSION

In total 721 quarter milk samples were cultured from 268 cows. 71 (26.5%) cows did not show significant infection in neither quarter. Among quarters with significant growth most commonly isolated bacteria were following Staphylococcus aureus 29%, coagulase negative staphylococci 43%, Streptococcus agalactiae 14%, Streptococcus uberis 4%, Streptococcus dysgalactiae 1%, Corinebacterium spp. 6%, Klebsiella spp. 2% and Streptococcus fecalis, Pseudomona spp., Actinomyces pyogenes, less than 1%.

Resistance investigations of Staphylococcus aureus isolates (n=53) (Figure 1) showed that more than half of them were resistant to penicillin, closely followed by resistance against amoxicillin in 48% cases. Resistance against cephalosporins was rather low at 5% level.

Less prominent occurrence of resistance against penicillin were observed for coagulase negative staphylococci (n=57) (Figure 2). Resistance against amoxicillin was different from resistance against penicillin. However, there were much often expressed resistance against cephalosporin group antibiotic substances and norfloxacin.

Figure 1. Occurrence (%) of resistant (black column) and intermediately sensitive (white column) Staphylococcus aureus isolates (n=53) from all mastitis cases in dairy cows.

Figure 2. Occurrence (%) of resistant (black column) and intermediately sensitive (white column) Coagulase negative Staphylococcus isolates (n=57) from all mastitis cases in dairy cows.
Resistance against penicillin highly correlates with resistance to amoxicillin. Only Streptococcus agalactiae showed high correlation between all beta-lactam structure antibiotics. Other streptococci showed each very different resistance profile, so not significant correlations were detected.

According to knowledge the bacteria rapidly develop resistance to antibiotics if they are used at subtherapeutic level or short treatment episodes. Mastitis due to often spread, its treatment, which is rather short and unspecific prophylaxis in form of dry of cow treatment undertakes considerable part of all antibiotic amount used in dairy cows, therefore bacteria found in milk could be important source which spreads multiresistant bacteria into milk products and environment as well. Our results showed current mastitis pathogen spectra, this comply with other authors data (Jemeljanovs, Bluzmanis, 1999). Also the level of antibiotic resistance of Staphylococcus aureus was at the same level as reported in recent studies (Hendriksen, 2008). According to some authors (Pyorala, 1999), coagulase negative staphylococci are more resistant to tetracycline, than Staphylococcus aureus. Also their penicillin resistance is becoming more common.

CONCLUSIONS
1. Resistance of bacteria isolated from milk depends on bacteria species.
2. Multiple resistance is more prominent for coagulase negative staphylococci and Streptococcus agalactiae.
3. Transfer of resistance characteristics is more evident between contagious mastitis pathogens, comparing to environmental pathogens.
LITERATURE