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Short communication

Susceptibility to cephalosporins of bacteria causing intramammary infections in dairy cows with a high somatic cell count in Germany

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ABSTRACT

The objective of this cross-sectional study was to determine the minimal inhibitory concentrations of cephalosporins of the first (cefalonium and cefapirin) and fourth generation (cefquinome) against bacteria isolated from intramammary infections in dairy cows with elevated somatic cell counts in Germany. Additionally, possible regional differences of the minimal inhibitory concentrations within Germany should be evaluated. In total, 6936 quarter milk samples from cows with a somatic cell count >200,000 cells/ml were taken in 43 herds.

The concentrations of the first generation cephalosporins inhibiting at least 90% of the isolates of a pathogen (MIC_{90}) were ≥64 μg/ml against Gram-negative bacteria and enterococci whereas the respective MIC_{90} against the other Gram-positive bacteria were ≤4 μg/ml. The MIC_{90} of cefquinome were ≥16 μg/ml against Gram-negative bacteria, bacilli and enterococci, and ≤2 μg/ml against the other Gram-positive bacteria.

Only the minimal inhibitory concentrations against coagulase-negative staphylococci differed significantly between regions in parametric survival models with shared frailties for the herds. However, the minimal inhibitory concentrations of cefquinome against staphylococci were higher than the minimal inhibitory concentrations of the tested cephalosporins of the first generation. Therefore, cefquinome should not be the first choice to treat staphylococcal mastitis in dairy cows.

1. Introduction

Mastitis is probably the most common cause for the antibiotic treatment of lactating dairy cows in Germany. Clinical and subclinical mastitis during lactation were treated with antibiotics on over 90% and on 40% of the farms contributing to a study in 2010 in northern Germany, respectively (Kreausukon, 2011). Cephalosporins and other β-lactams share the first two positions amongst the antimicrobial drugs used to treat bovine mastitis in northern Germany (Tenhagen et al., 2006; Kreausukon, 2011). Studies in Pennsylvania and Canada showed similar results (Sawant et al., 2005; Saini et al., 2012).

The first objective of the present study was to investigate the *in vitro* susceptibility of bacteria isolated from milk samples from dairy cows with elevated somatic cell count (**SCC**) to first (cefalonium, cefapirin) and fourth (cefquinome) generation cephalosporins in Germany. The second objective was to evaluate regional differences of the susceptibility of mastitis pathogens to cephalosporins. The main milk producing regions in Germany were the North-West and the South. However, dairy farms in the South were hardly comparable to dairy farms in the North-West, as they differed e.g. in the predominant breed, herd size and housing (Table 1). In the Eastern federal states of Germany, dairy farms had been cooperatives or companies with employees and bigger herds for decades. The regions of Germany differed significantly in animal and farm densities (Merle et al., 2012).

2. Materials and methods

2.1. Herds and animals

Farms in the federal states of Lower Saxony and Schleswig-Holstein were considered as examples of dairy farms in the North-West, in Saxony-Anhalt as examples in the East, and in Bavaria as examples of farms in the South of Germany. Only farms that participated in the dairy herd improvement tests, that had a

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Table 1
Characteristics of dairy farms in some federal states in 2009/10 as examples of the differences in dairy farming structures in Germany.

Region State	Farms[a] (*n*)	Avg. herd size[a] (*n*)	Avg. milk yield[b] (kg/cow-year)	Predominant breed[a] (% cows)	Tie-stalls[c] (% farms)	Pasture[c] (% farms)
North-West						
Lower Saxony	13,754	56	7470	HF[d] (87)	47	77
Schleswig-Holstein	5260	69	6994	HF[d] (58)	27	90
East						
Saxony-Anhalt	742	167	8334	HF[d] (84)	17	50
South						
Bavaria	42,810	29	6238	Simmental (78)	79	20

[a] Statistisches Bundesamt (2011a).
[b] Bundesamt für Verbraucherschutz und Ernährung (2011).
[c] Statistisches Bundesamt (2011b).
[d] Holstein-Friesian.

conventional milking system, and with an average bulk milk SCC >150,000 cells/ml were eligible for the study. The study farms also had to have herd sizes and average 305 days milk yields above the mean of the dairy herds in the respective dairy herd improvement association in 2010. Of these, farms were randomly selected per region proportionally to the square root of the percentage of the dairy farms in the respective region in all farms in Germany, that matched the inclusion criteria. In the North-West 18 farms were chosen and asked to participate, in the East and South, 6 and 36 farms, respectively. All lactating cows in the participating herds with an individual SCC >200,000 cells/ml in the recent dairy herd improvement test, and that were free of signs of clinical mastitis at the sampling day, were included in the study.

2.2. Sampling and isolation of bacteria

2.2.1. Collection of the milk samples

The participating farms were visited by trained researchers between November 2011 and April 2012 within a maximum of one week after the recent dairy herd improvement test. Quarter foremilk samples were collected from all lactating quarters of the cows that were included in the study. The samples were taken aseptically into sterile plastic tubes with the preservative Ly20 that contains boric acid according to the guidelines of the German Veterinary Medical Association (2009). The samples were analysed within two days in the microbiological laboratory of the University of Applied Sciences and Arts (Hannover, Germany).

2.2.2. Identification of the isolated bacteria

Culturing of the milk samples and identification of the bacterial isolates were carried out following the guidelines of the German Veterinary Medical Association (2009). On one quadrant of an esculin blood agar plate (Oxoid, Wesel, Germany), 10 μl of a well mixed quarter milk sample were plated with a sterile inoculation loop. The plates were incubated at 37 °C under aerobic conditions and examined after 24 and 48 h. The grown colonies were initially differentiated by their morphology, hemolysis patterns, esculin hydrolysis, Gram staining, and cell morphology. Furthermore, the activities of catalase (3% H_2O_2, Merck, Darmstadt, Germany) and of cytochrome oxidase C (Bactident Oxidase, Merck) were tested. The catabolism of glucose under aerobic and anaerobic conditions were investigated using oxidation–fermentation test medium (OF basal medium with addition of D(+)-glucose monohydrate, Merck).

Presumptive *Staphylococcus aureus* were verified with the clumping factor test (DiaMondiaL Staph Plus Kit, Sekisui Virotech, Rüsselsheim, Germany). Esculin positive streptococci were subcultivated on modified Rambach agar according to Watts et al. (1993) to differentiate between *Streptococcus uberis* and *Enterococcus* species. Esculin negative streptococci were further characterised via Lancefield serotyping (DiaMondiaL Streptococcal Extraction Kit, Sekisui Virotech). Streptococci of group B, C, and G were referred to as *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus canis*, respectively. Asporogenic, Gram-positive irregular rods with V- or Y-shaped cell configuration would be identified as *Trueperella pyogenes*, if they were β-hemolytic and catalase and esculin negative and as coryneforms else. *Bacillus* species are Gram-positive, catalase-positive, form large colonies on blood agar, and are capable of forming endospores.

Gram-negative and cytochrome oxidase C negative rods fermenting glucose were referred to as coliform bacteria. They were subcultured on ChromoCult coliform agar (Merck) to distinguish *Escherichia coli* from other coliforms. *Pseudomonas* species were identified as Gram-negative, cytochrome oxidase C positive bacteria metabolising glucose oxidatively.

The isolated bacteria would be considered indicative of an intramammary infection if at least one colony of a contagious pathogen (*S. aureus*, *S. agalactiae*, *S. dysgalactiae*, or *Trueperella pyogenes*) was identified, and if more than 10 colonies of another bacteria species were found, respectively (German Veterinary Medical Association, 2009). Samples were considered to be contaminated if more than two different types of colonies were detected. However, if any colony from a contaminated sample seemed to be a contagious mastitis pathogen according to colony morphology and growth characteristics, and the suspicion could be confirmed by the additional diagnostics, the minimal inhibitory concentrations (**MIC**) of cephalosporins against these isolates from contaminated samples would be determined as well.

2.3. Determination of the minimal inhibitory concentrations

The minimal inhibitory concentrations of cefalonium, cefapirin, and cefquinome were determined according to the DIN 58940/ISO 20776-1:2006 protocol for the respective microorganisms via microdilution method. Wells of polystyrene sterile microtiter plates (Greiner Bio One, Frickenhausen, Germany) were filled with 50 μl of solutions containing 0.125 μg/ml to 64 μg/ml of the examined antimicrobial agent in Müller-Hinton broth (Merck). To test the susceptibility of streptococcal isolates Müller-Hinton broth was supplemented with 5% defibrinated horse blood (Oxoid). The microtiter plates were inoculated with 50 μl suspension of one of the isolated pathogens and incubated at 37 °C for 24 h. The MIC was defined as the minimum concentration of the tested cephalosporin that inhibited visible bacterial growth. *S. aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 (Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) were used as reference strains. The MIC_{50} and MIC_{90} for a bacteria category were the concentrations identified as MIC