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Antimicrobial susceptibility of mastitis pathogens of dairy cows in Switzerland

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Summary

This study evaluates the antimicrobial resistance of pathogens cultured from 3'954 quarter milk samples from dairy cows in Switzerland. A total of 1'228 *Streptococcus (Strep.) uberis*, 1'107 *Staphylococcus (Staph.)* spp. other than *Staph. aureus*, 598 coliform, 490 *Staph. aureus*, 270 *Enterococcus* spp. and 213 *Strep. dysgalactiae* isolates were tested for susceptibility to 9 antimicrobial drugs using agar diffusion. *Streptococcus uberis, Strep. dysgalactiae* and *Staph. aureus* had the highest antimicrobial sensitivities to amoxicillin clavulanic acid (99.6 %, 100 % and 98.8 %, respectively). Of all isolated pathogens, only 2.6 % were resistant to amoxicillin clavulanic acid and 8.0 % to cefoperazone. The overall resistance level to gentamicin was 45.5 %, to penicillin 39.2 %, and to ampicillin 26.7 %. The highest resistance levels occurred with polymyxin (86.0 %), oxacillin (64.7 %) and lincomycin (53.7 %). Our results showed that at least one resistance exists to one antimicrobial agent examined in this study. Ideally the selection of the antibacterial drug for the treatment of intramammary infection should be based on antibiotic susceptibility testing.

Keywords: cattle, mastitis, antimicrobial resistance, agar diffusion, Switzerland

Antimikrobielle Empfindlichkeit von Mastitiserregern bei der Milchkuh in der Schweiz

In der vorliegenden Studie wurden die Resultate der Antibiotikaresistenztests von 3'954 Milchproben aus der Schweiz ausgewertet. Insgesamt 1'228 *Streptococcus (Str.) uberis*-Stämme, 1'107 *Staphylococcus sp.*-Stämme, 598 coliforme Keime, 490 *Staphylococcus (S.) aureus*-Stämme, 270 *Enterococcus*-Stämme und 213 *Str. dysgalactiae*-Stämme wurden mittels Agardiffusionstest auf ihre Empfindlichkeit gegenüber 9 unterschiedlichen antibiotischen Wirkstoffen untersucht. Die höchste Empfindlichkeit zeigten die *Str. uberis*-, *Str. dysgalactiae*- und *S. aureus*-Isolate gegenüber Amoxicillin-Clavulansäure (99.6 %, 100 % bzw. 98.8 % empfindliche Isolate). Bezüglich aller isolierter Erreger wiesen Amoxicillin-Clavulansäure mit 2.6 % und Cefoperazon mit 8 % am wenigsten Resistenzen auf. Im Mittelfeld lagen Gentamicin (45.5 % Resistenzen), Penicillin (39.2 %) und Ampicillin (26.7 %). Am meisten Resistenzen wurden bei Polymyxin (86 % Resistenzen), Oxacillin (64.7 %) und Lincomycin (53.7 %) gefunden. Aufgrund der Resultate der vorliegenden Untersuchung muss davon ausgegangen werden, dass Resistenzen gegenüber jedem der geprüften Wirkstoffe vorkommen. Idealerweise erfolgt die Auswahl des Wirkstoffes zur Therapie von intramammären Infektionen aufgrund vorheriger antimikrobieller Resistenztestung.

Schlüsselwörter: Rind, Mastitis, Antibiotikaresistenz, Agardiffusion, Schweiz

Introduction

Bovine mastitis is an important disease with a significant impact on the dairy industry worldwide. It causes great economic losses and welfare issues in affected cows (Leslie and Petersson-Wolfe, 2012). Intramammary antimicrobial therapy is the most common method of controlling bovine mastitis. Ideally, treatment of subclinical mastitis and mild to moderately severe acute clinical mastitis should be based on culture results of a milk sample from the affected quarter to ensure that the most appropriate antimicrobial drug is selected for treatment. Delaying antimicrobial treatment until bacteriological results are available is associated with a considerable reduction in antimicrobial drug costs (Neeser et al., 2006; Lago et al., 2011). Cows with mastitis accompanied by severe signs of illness should be treated immediately. The range of antibiotics used to treat intramammary infections in cows is broad and includes second-line antimicrobial drugs such as cephalosporins. The use of cephalosporins, particu-

larly 3rd and 4th generation cephalosporins, has increased strongly over the last few years in Switzerland (Büttner et al., 2011). These drugs are also used in human medicine, and their widespread use in veterinary medicine poses a potential problem. There is a concern that the veterinary use of these drugs leads to the development of resistant bacteria, which could have an adverse effect on human health. Indeed, dairy herds in England that used 3rd and 4th generation cephalosporins were almost four times as likely to have extended spectrum β-lactamase *E. coli* than herds that did not use these drugs (Snow et al., 2012). Furthermore, herds that used ceftiofur had an increased occurrence of *E. coli* with reduced susceptibility to ceftriaxone, a cephalosporin used in human medicine, in fecal samples (Tragesser et al., 2006). Third and 4th generation cephalosporins are grouped by the World Health Organisation into one of the top 3 drug classes of "critically-important antimicrobials" for use in human medicine (WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance AGISAR, 2009). Thus, 3rd and 4th generation cephalosporins should only be used as a last resort, and first-line antimicrobial drugs should be the treatment of choice. To this end, bacteriological culture of a milk sample should be carried out before treatment is started to guide the clinician in selection of the most appropriate antimicrobial drug. Exceptions to this recommendation are severe acute cases that require immediate treatment. In addition to the identity of the bacterial pathogen, its resistance pattern to the available antimicrobial drugs must be considered. Antimicrobial susceptibility has been shown to vary regionally in different parts of the world (De Oliveira et al., 2000; Hendriksen et al., 2008). The goal of the present study was to investigate the current resistance patterns of the most common mastitis pathogens in Switzerland. Culture of milk samples from different regions of Switzerland was carried out between July 2011 and July 2013 at a certified laboratory (Idexx Diavet Labor AG, Bäch SZ, Switzerland) and mastitis pathogens from 3'954 positive milk samples underwent antimicrobial sensitivity testing to 9 antimicrobial agents.

Animals, Material and Methods

A total of 3'954 quarter milk samples from cows with subclinical or clinical mastitis submitted by veterinarians from different regions of Switzerland between July 1st, 2011 and July 6th, 2013 underwent bacterial culture at a commercial laboratory (Idexx Diavet Labor AG, Bäch, Switzerland). Information pertaining to lactation number and stage of lactation, days in milk, breed, milk yield, location of herd of origin and type of mastitis for the affected cows was not available. Milk was streaked onto esculin agar plates (Laborgemeinschaft 1, Zurich, Switzerland; Holt, 1984) and incubated at 37 °C for 24 hours adding 5 % carbon dioxide.

From each plate the most prominent Colony Forming Unit (CFU) was regarded as the leading pathogen. A state certified laboratory assistant differentiated the CFU visually and by standard biochemical tests (Holt, 1984). The leading pathogen was tested for susceptibility to 9 antimicrobial agents including amoxicillin clavulanic acid, ampicillin, cefoperazone, gentamicin, lincomycin, oxacillin, penicillin, polymyxin and spiramycin using the agar diffusion test according to guidelines of the Clinical and Laboratory Standards Institute (CLSI). The CLSI standards M31-A3E, 2008 and CLSI M100-S 19 Vol. 29 No. 3, 2009, and in some instances manufacturer's copy right restricted recommendations were used to determine whether an isolate was susceptible, intermediate or resistant to an antimicrobial agent. Natural occurring resistance is also reported. In addition, 100 isolates each of *Staphylococcus* ssp. other than *Staph. aureus* and *coliforms* were tested for susceptibility to neomycin using the same technique.
The program Stata (StataCorp., 2011; Stata Statistical Software: Release 12; College Station, TX, USA: StataCorp LP) was used for frequency distribution.

Results

For agar plates with mixed bacterial cultures, the principal pathogen was determined and used for analysis. The frequency distribution of the 3'954 isolated pathogens was as follows: *Streptococcus uberis* (1'228/3'954, 31 %) and *Staphylococcus* spp. other than *Staph. aureus* (1'107/3'954, 28 %) were the most frequent, followed by coliforms (598/3'954, 15 %) and *Staph. aureus* (490/3'954, 12 %). *Enterococcus* spp. (270/3'954, 7 %) and *Strep. dysgalactiae* (213/3'954, 5 %) were less frequent. Isolates with prevalences smaller than 1 % were *Mannheimia haemolytica, Pasteurella* spp., *Proteus* spp., *Pseudomonas aeruginosa, Serratia* spp., *Streptococcus agalactiae* and *other streptococci.*
The results of the antibiotic sensitivity testing are shown in Figure 1 and listed in Table 1. In Figure 2 the results of the sensitivity testing of the *Strep. uberis* isolates are shown.
Staphylococcus aureus isolates had the highest prevalence of susceptibility to amoxicillin clavulanic acid with only 1.2 % of isolates being resistant. On the other hand, 35 % of the *Staph. aureus* isolates were resistant to penicillin. Even higher resistances to penicillin were seen in isolates of *Staphylococcus spp*. other than *Staph. aureus* (54.2 %).
Of the 598 coliform isolates, 29 (4.9 %) were resistant to gentamicin, 270 (45.1 %) were resistant to ampicillin and 66 (11 %) to amoxicillin clavulanic acid.
Of all 3'954 isolated mastitis pathogens, 86 % were resistant to polymyxin, 64.7 % to oxacillin, 53.7 % to lincomycin, 45.5 % to gentamicin, 39.2 % to penicillin, 27.0 % to spiramycin and 26.7 % to ampicillin. The highest sensitivities were seen with cefoperazone (8.0 % resistant) and amoxicillin clavulanic acid (2.6 % resistant).

Discussion

Strep. uberis was the most frequently isolated mastitis pathogen in this study, which was in agreement with the notion that this organism, together with other environmental streptococci, currently constitutes the principal cause of intramammary infections in many herds (Kalmus et al., 2011). For this agent as well as other gram-positive microorganisms, amoxicillin clavulanic acid was the most appropriate antimicrobial agent with in-vitro sensitivities from 98.5 % to 100 %. This was higher than the sensitivity of 88.2 % of *Staph. aureus* isolates to cefoperazone, a 3rd generation cephalosporin. It is possible that the decrease in sensitivity of *Strep. uberis* to cefoperazone is a result of the increase in the use of this later-generation

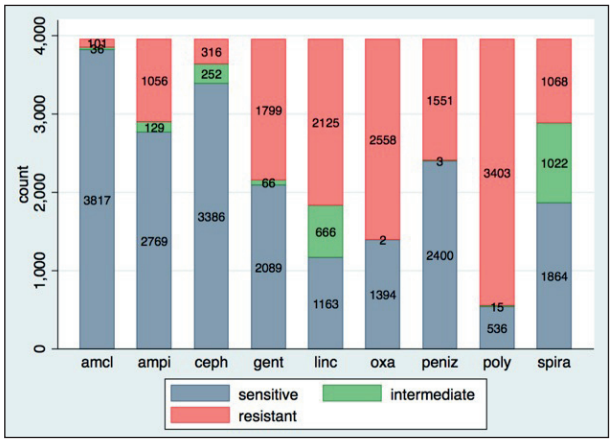

Figure 1: Antimicrobial susceptibility of bacterial isolates from mastitic milk samples (n = 3'954).
amcl = amoxicillin clavulanic acid, ampi = ampicillin, ceph = cefoperazone, gent = gentamicin, linc = lincomycin, oxa = oxacillin, peniz = penicillin, poly = polymyxin, spira = spiramycin.

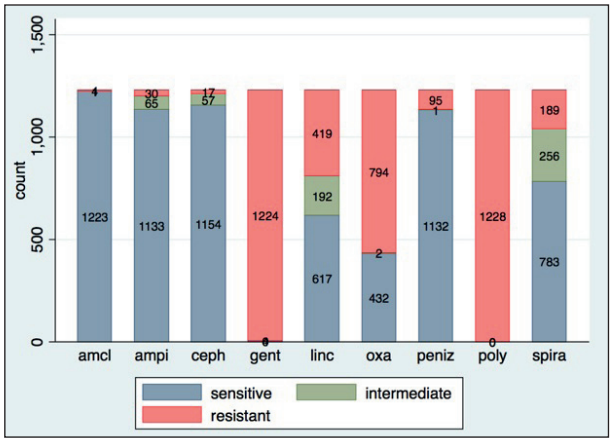

Figure 2: Antimicrobial susceptibility of *Streptococcus uberis* (n = 1'228).
amcl = amoxicillin clavulanic acid, ampi = ampicillin, ceph = cefoperazone, gent = gentamicin, linc = lincomycin, oxa = oxacillin, peniz = penicillin, poly = polymyxin, spira = spiramycin.

Table 1: Antimicrobial susceptibility (%) of bacterial isolates from mastitic milk samples.

Isolate Antimicrobial agent	*Strep. uberis* (n = 1'228) %	*Strep. dysgalactiae* (n = 213) %	*Staph. aureus* (n = 490) %	*Staph. spp.* (n = 1'107) %	*Enterococcus spp.* (n = 270) %	*coliforms* (n = 598) %	*Pseudomonas aeruginosa* (n = 12) %	*Pasteurella spp.* (n = 15) %
Amoxicillin clavulanic acid	99.6	100	98.8	99	98.5	83.3	25	93.3
Ampicillin	92.3	94.8	67.3	51.8	93	45.5	0	6.7
Cefoperazone	94	95.8	88.2	91.7	4.4	89	41.7	73.3
Gentamicin	0.3	0.5	94.3	94.1	2.2	90.3	91.7	93.3
Lincomycin	50.3	62.4	38.2	19.1	1.5	0.8	0	0
Oxacillin	35.2	73.2	72.9	40.1	0.7	0	0	0
Penicillin	92.2	93	64.9	45.7	88.1	0.2	0	0
Polymyxin	0	0	0	0.1	1.1	83.8	91.7	100
Spiramycin	63.8	60.1	51.2	53	39.6	0.2	0	0
Neomycin	NA	NA-	NA	90 (n = 100)	NA	78 (n = 100)	NA	NA

NA, not applicable

cephalosporins in the last few years. Cefquinome, a widely used 4th generation cephalosporin, was not tested in this study but the comparison with cefoperazone showed that 3rd and 4th generation cephalosporins may be replaced in many cases with other suitable antimicrobials.

The susceptibility of *Strep. uberis* and *Strep. dysgalactiae* to penicillin was greater than 90 %. This was in agreement with other reports that streptococci have at least some level of resistance to penicillin (Guérin-Faublée et al., 2002; Overesch et al., 2013), whereas others studies reported a complete lack of resistance (Pitkäl et al., 2008; Minst et al., 2012). Based on our findings, the use of penicillin can still be recommended for the treatment of bovine streptococcal mastitis. In contrast, 35.1 % of *Staph. aureus* and 54.2 % of other *Staphylococcus* spp. isolates were resistant to penicillin. This was surprising because ten years earlier, the prevalence of penicillin-resistant *Staph. aureus* isolates was only 9 % in Switzerland (Corti et al., 2003), and 16.4 % (Overesch et al., 2013) and 20 % (Kretzschmar et al., 2013) in more recent studies. The prevalence of penicillin-resistant *Staphylococcus spp*. was 31 % (Corti et al., 2003), 38 % (Kretzschmar et al., 2013), and 23.3 % (Frey et al., 2013). However, because of methodological differences in resistance testing, direct comparison of these studies is difficult. Nevertheless, our results suggest a marked increase in the prevalence of penicillin-resistant *Staph. aureus* and *Staphylococcus* spp. during the last ten years. Our results suggest that Penicillin is no longer the antimicrobial of choice for staphylococcal mastitis.

Although coliform pathogens had good overall susceptibility to gentamicin, cefoperazone and amoxicillin clavulanic acid ranging from 83.3 to 90.3 %, a considerable proportion of the isolates were resistant. This signals a change from earlier studies in which all investigated coliforms were sensitive to these drugs (Corti et al., 2003) or resistances were seen in less than 3 % of isolates (Moser et al., 2013).

The susceptibility prevalences calculated in this study were subject to a selection bias and are not in consistence with a recently published study (Moser et al., 2013). Only milk samples from quarters with a positive California mastitis test from cows with clinical or subclinical mastitis were used in this study, and sensitivity testing was only carried out on request. For those reasons, the prevalence of intramammary infection as well as the calculated susceptibility prevalences would be expected to be higher than the true prevalences in the general population. Due to the sampling procedure no further information on the kind of mastitis and additional information about the cow was available. In Switzerland, by legal rights, it is not allowed, to use additional information when the owner of the samples is not asked for in advance. The loss of this information does not alter the results presented.

Overall resistance data as shown in Figure 1 are important to help the practitioner in situations where he has to treat a case of mastitis without having any further or a priori information on the causative bacteria. The susceptibility is depending on time and location. Therefore, the results presented in this study are only representative for Switzerland and the respective time of investigation.

Conclusions

The findings of the present study show that complete susceptibility of a class of pathogenic bacteria to a specific antimicrobial agent is rare. It is therefore not always possible to make a reliable recommendation regarding the use of the most appropriate antimicrobial drug based merely on the bacterial species recovered. Ideally the selection of antibacterial drug for the treatment of intramammary infection should be based on antibiotic susceptibility testing.

Competing interests

AT is employed by Zoetis Switzerland GmbH, which markets antimicrobial drugs for the treatment of bovine mastitis. YS and MR are employees of Idexx Diavet Labor AG, Bäch, Switzerland.

Sensibilité aux antimicrobiens des germes responsables de mammites chez les vaches laitières en Suisse

On analyse dans la présente étude les résultats des tests de résistance aux antibiotiques de 3'954 échantillons de laits prélevés en Suisse. Au total, ce sont 1'228 souches de *Streptococcus (Str.) uberis*, 1'107 souches de *Staphylococcus sp*., 598 coliformes, 490 souches de *Staphylococcus (S.) aureus*, 270 souches d' *Enterococcus* et 213 de *Str. dysgalactiae* qui ont été testées par difffusion sur gel d'agar quant à leur sensibilité vis-à-vis de 9 substances antibiotiques. Ce sont les isolats de *Str. uberis*, *Str. dysgalactiae* et *S. aureus* qui ont montré la plus grande sen-

Sensibilità antimicrobica agli agenti patogeni della mastite nelle vacche da latte in Svizzera

Nel presente studio, sono stati valutati i risultati dei test di resistenza agli antibiotici di 3'954 campioni di latte provenienti dalla Svizzera. Un totale di 1'228 ceppi di *Streptococcus (Str) uberis*, 1'107 ceppi di *Staphylococcus sp*, 598 germi coliformi, 490 ceppi di *Staphylococcus (S. aureus)*, 270 ceppi di *Enterococcus* e 213 ceppi di *Str dysgalactiae* sono stati ritrovati dai test di diffusione in agar per la loro sensibilità a 9 diversi antibiotici. La sensibilità massima si è rilevata negli isolati di *Str. uberis*, *Str. dysgalactiae* e *S. aureus* resistenti all'acido amoxicillina-clavulanico (99.6 %, 100 % risp. 98.8 %

sibilité face à l'association amoxicilline/acide clavulanique (99.6 %, 100 % resp. 98.8 % de souches sensibles). Par rapport à l'ensemble des germes isolés, ce sont l'association amoxicilline/acide clavulanique (2.6 %) et le céfoperazon (8 %) auxquels les germes ont été le moins souvent réséstants. Ils étaient suivis par la gentamycine (45.5 % de résistances), la pénicilline (39.2 %) et l'ampicilline (26.7 %). Les plus hauts taux de résistances ont été observés face à la polymyxine, (86 %), l'oxacilline (64.7 %) et la lincomycine (53.7 %). Sur la base des résultats de cette étude, on peut partir de l'idée qu'il existe des résistances contre toutes les substances testées. Le choix de l'antibiotique pour le traitement d'infections intra mammaires doit donc se faire idéalement sur la base d'un test de résistance préalable.

di ceppi sensibili). Per quanto riguarda tutti gli agenti patogeni isolati, l'acido amoxicillina-clavulanico con il 2.6 % e il cefoperazone all'8 % hanno espresso la minima resistenza. Al centro si trovano invece, gentamicina (resistenza 45.5 %), penicillina (39.2 %) e ampicillina (26.7 %). La maggior parte delle resistenze si sono riscontrate per polimixina (resistenze 86 %), oxacillina (64.7 %) e lincomicina (53.7 %). Sulla base dei risultati del presente studio si deduce che le resistenze a ciascuna delle sostanze testate si è verificata. Idealmente, la scelta del principio attivo per il trattamento di infezioni intramammarie dovrebbe essere effettuata sulla base del test di resistenza antimicrobica.

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