

PREVALENCE OF GRAM POSITIVE BACTERIA IN COW MASTITIS AND THEIR SUSCEPTIBILITY TO BETA-LACTAM ANTIBIOTICS

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Summary. The aim of this study was to investigate the prevalence of gram-positive bacteria causing cow mastitis in cows and determine their susceptibility to beta-lactam antibiotics. Eight hundred and seventy one isolated gram-positive bacterial strains belonged to *Staphylococcus* (n=506), *Streptococcus* (n=196) and *Enterococcus* (n=169) genera from sub-clinical and clinical cases of bovine mastitis in Lithuania were investigated. The most common causative agents of udder disease included: *S. epidermidis* (n=176), *S. aureus* (n=166), *S. agalactiae* (n=128), and *E. hirae/durans* complex (n=136). Isolates were analysed for their susceptibility to several beta-lactam antimicrobial agents: penicillin, ampicillin, amoxicillin, cephalothin, cephalexin, amoxicillin + clavulanate. The susceptibility patterns were studied by Agar Disk Diffusion method (ADDM). *S. aureus* showed the highest level of resistance to amoxicillin (81.7%), penicillin (81.6%) and ampicillin (83.2 %). The corresponding values for coagulase-negative staphylococci (CNS) strains were 59.5 %, 52.0 % and 50.8 % against penicillin, ampicillin and amoxicillin, respectively. *Streptococcus* spp. strains mostly were resistant to amoxicillin (31.6%), *Enterococcus* spp.– to penicillin (28.3 %), ampicillin (21.8 %), amoxicillin (37.8 %) and amoxicillin + clavulanate (31.7 %). The susceptibility of our tested mastitis pathogens to ampicillin and penicillin highly correlated $r=0.87$. In comparison with other antibiotics amoxicillin and clavulanic acid combination was the most effective ($p<0.05$) *in vitro* against all tested gram-positive bacteria. However, *S. aureus* strains in 41.5 % of cases demonstrated resistance to this combination.

Keywords: *Staphylococcus*, *Streptococcus*, *Enterococcus*, bovine mastitis, antimicrobial resistance, beta-lactam antibiotics.

GRAMTEIGIAMŲ BAKTERIJŲ, IŠSKIRTŲ IŠ KARVIŲ MASTITO BANDINIŲ, PAPLITIMAS IR JAUTRUMAS BETA LAKTAMINIAMS ANTIBIOTIKAMS

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Santrauka. Darbo tikslas – įvertinti gramteigiamų bakterijų, išskirtų iš karvių mastito bandinių, įvairovę ir nustatyti jų fenotipinį atsparumą beta laktamams. Tyrimais analizuota 871 padermė, priklausanti *Staphylococcus* spp. (n=506), *Streptococcus* spp. (n=196) ir *Enterococcus* spp. (n=169), išskirta iš karvių, sergančių subklinikinio ir klinikinio mastito. Dažniausi mastito etiologiniai faktoriai buvo *S. epidermidis* (n=176), *S. aureus* (n=166), *S. agalactiae* (n=128) ir *E. hirae/durans* (n=136). Išskirtų patogenų jautrumas antimikrobinėms medžiagoms (penicilinui, ampicilinui, amoksicilinui, cefalotinui, cefaleksinui, amoksicilinui ir klavulano rūgščiai) tirtas difuzijos agare metodu. *S. aureus* dažniausiai buvo atsparios amoksicilinui (81,7 proc.), penicilinui (81,6 proc.) ir ampicilinui (83,2 proc.). *S. epidermidis* padermės buvo atitinkamai 59,5 proc., 52,0 proc. ir 50,8 proc. atsparios penicilinui, ampicilinui ir amoksicilinui. Streptokokų padermės dažnai buvo atsparios tik amoksicilinui (31,6 proc.), enterokokai – penicilinui (28,3 proc.), ampicilinui (21,8 proc.), amoksicilinui (37,8 proc.) bei amoksicilino ir klavulano rūgšties deriniui (31,7 proc.). Mastitą sukeliančių patogenų jautrumas ampicilinui ir penicilinui koreliavo tarpusavyje ($r=0,87$). Amoksicilino ir klavulano rūgšties derinys efektyviau ($p<0,05$) nei kiti beta laktamai veikė tirtus gramteigiamus mikroorganizmus, tačiau net 41,5 proc. *S. aureus* padermių buvo atsparios amoksicilino ir klavulano rūgšties deriniui.

Raktažodžiai: *Staphylococcus*, *Streptococcus*, *Enterococcus*, karvių mastitas, antimikrobinis atsparumas, beta laktaminiai antibiotikai.

Introduction. Mastitis is inflammation of the mammary glands, usually caused by microbial infection. It places a heavy economic burden on milk producers all over the world. Different studies on mastitis pathogens were performed in Lithuania before and different causative agents are referred. The most frequently causative agents of mastitis are indicated as streptococci (5.43 – 20.35 %), staphylococci (11.43 – 41.59 %), coagulase-negative staphylococci (CNS) (2.86 – 58.15 %), enterobacteria (8.47 %). Different authors also indicate different frequency of *S. aureus* as mastitis causative agent with the rate of 19.97 – 65.0 % (Ramanauskienė et al., 2008). Diversity of pathogens depended on farm capacity, keeping conditions, level of sanitary, management, qualification of personnel and other factors.

Mastitis is one of the major causes of antibiotic use in dairy cows. There is a variety of antimicrobials that are used for the prevention and treatment of mastitis. Therefore, resistance to antimicrobials is expected. Resistance of mastitis pathogens to antimicrobial agents is a well-documented challenge in dairy cows (Bradley, 2002; Erskine et al., 2002; DANMAP, 2008). According to Ramanauskienė et al., (2008) in Lithuania causative agents of mastitis were the most frequent susceptible to the combination of beta-lactams and clavulanic acid, cephalexin and amoxicillin. Authors indicates, that CNS are more susceptible to penicillin, whereas *S. aureus* – to combination of amoxicillin and clavulanic acid and the most frequent resistance of *S. aureus* was observed to penicillin.

Leger et al. (2003) suggests that penicillin, half synthetic penicillins and cephalosporins are the antibiotics of choice in dairy herds. Within this class, penicillin, amoxicillin, cloxacillin and ampicillin are the mostly used agents. In the Nordic countries penicillin is used as the first-line antibiotic treatment for bovine mastitis because of a low resistance rate and narrow spectrum. According to Tenhagen et al. (2006), Roesch (2006) penicillin was used on 31 – 66% of the farms.

According to the recent studies in the United States and Finland (Rossitto et al., 2002; Pitkälä et al., 2004) the existence of antibiotic resistance of udder pathogens is worldwide. Regional differences in resistance patterns of pathogens are prevalent. In Switzerland there was no increase of antibiotic resistance of mastitis pathogens during the last 20 year. In other countries, for example Norway and Sweden, levels of resistance have remained fairly constant at around 10-15%. Therefore, the rate of narrow-spectrum penicillin resistance varies per country and also over time within countries. The data presented by researches varies. Makovec and Ruegg (2003) indicate that antimicrobial resistance was identified in 24.5% of the analyzed bacterial isolates. For some antibiotics, particularly penicillin, the frequency of resistance appears to be considerably lower in Canadian herds than it is reported by researches in Europe and the United States. According to Vintov et al. (2003) considering the studies of European herds, penicillin resistance is more heterogeneous, ranging from only 2% (Norway) to more

than 70% (Ireland), with an overall average of 32%. In accordance with Smith and Coast (2002) the observed localized differences in the distribution of antibacterial resistance may reflect the use of specific antimicrobials that act as a selective agent for resistance. However, according to De Oliveira et al. (2000), Schröter (2003) the relationship between the resistance patterns of mastitis pathogens and the intensity of food animal husbandry in the respective regions could not be established. There is worth mentioning that the resistance of the microorganisms directly depends not only on the use of antimicrobial drugs in the medicine practice but on its use in farming, animal treatment, prophylaxis and growth stimulation as well. Some antibiotics, which are used in farming, may pass human organism through food products. Despite considerable use and sometimes misuse, some antimicrobials continue to remain effective today however, antimicrobial resistance is worldwide pending problem.

The purpose of this work is to isolate gram-positive bacteria which usually cause cow mastitis in animal farms of Lithuania and determine their susceptibility to antibiotics of beta-lactam group.

Material and Methods. Examinations were carried out using a retrospective research method during 2007-2009 in Lithuania. A survey of quarter milk cultures obtained in 1340 samplings at the cow level from 34 Lithuanian dairy herds was conducted between January, 2007, and December, 2009. The medium age of cows in this study was 6.7 year (2.2 to 10.4 year). 38% of the Lithuanian Red crossbreed and 62% of Lithuanian Black & Whites cows were examined respectively. The diagnosis of mastitis was made on the basis of anamnesis and clinical examination of the udder, macroscopic evaluation of secretion and California Mastitis Test (CMT). A quarter of milk (inflamed secretion) samples from cows with subclinical or clinical forms of mastitis were collected aseptically. The teats were cleaned and dipped in a disinfectant and then teat ends were wiped with alcohol swabs and allowed to dry. The closing streams were 2–4 ml of the secretion and were collected into sterile tubes. The samples were cooled and immediately transported to laboratory. Bacteriological testing was performed according to commonly accepted rules with a standardised methodology (“Laboratory and field handbook on bovine mastitis“, National mastitis council, 1990) with some new knowledge of some species. The milk samples were inoculated on blood agar base enriched with 5% sheep blood („Oxoid“, England). The milk samples were inoculated onto media using loop of 10 µl. Inoculated Petri dishes were incubated at the temperature of 36.0±1.0°C in aerobic conditions for 24 – 48 hours. The morphology of the microorganisms was examined by microscoping after staining by Gram. 3% of hydrogen peroxide was used for detection of catalase production. The staphylococci were further identified based on coagulase activity, DNase activity, and pigment production. Commercial test kit “STAPHYtest (Pliva-Lachema)“ was used for additional identification inside the genus. For *S. agalactiae* identification CAMP test and

aesculin hydrolysis („Oxoid“, England) were performed. For identification of *E. faecalis* and *E. faecium* CAMP test, hydrolysis of aesculin and fermentation tests of some carbohydrates (sorbitol, manitol, arabinose) were used. *S. dysgalactiae* and *S. agalactiae* were further identified using Lancefield grouping (streptococcal grouping kit, „Oxoid“). Supplementary identification of gram-positive bacteria suspected of belonging to the genera *Staphylococcus* or *Streptococcus* was performed using a miniaturized identification method (BBL Crystal, Becton Dickinson Microbiology Systems, Franklin Lakes, NJ). Quality control organisms included *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), in accordance to the recommendations Clinical and Laboratory Standards Institute, 2002.

Antimicrobial susceptibility of tested bacteria was performed by Kirby Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines in Mueller-Hinton Agar II (Oxoid). Optical measurer Liap 2 (Latvia) was used for determination of bacterial suspension density. The following discs of antimicrobials („Oxoid“) were used: penicillin (10 UI), amoxicillin (25 µg), amoxicillin with clavulanic acid (30 µg), ampicillin (10 µg), cephalothin (30 µg), cephalixin (30 µg). Interpretation of the test results sensitive (S), resistant (R) was based on CLSI criteria. Activity of 6 antibiotics to each bacterial species was analysed.

The statistical analysis was performed by programme „Epi Info“ (1996) Centers for Disease Control & Prevention, U. S. A., Version 6.04). Arithmetical means (X), standard deviation (SD) and vary coefficients (Cv) were set by minimal and maximal meanings. The Student multiple comparison method was applied to determine the criterion of significance concerning the difference among groups (p). The difference was considered statistically significant when $p < 0.05$. Pearson correlation factor (r) were calculated as well.

Results. In the testing period 871 strains of bacteria were isolated from the cows sick with mastitis (Table 1). The rates of isolation of different genus were as follows:

Staphylococcus spp. – 506 ($p < 0.05$), *Streptococcus* spp. – 196 strains and *Enterococcus* spp. – 169 strains. While analyzing isolation frequency of staphylococcus species there were indicated that 3 species of staphylococci were divided quiet equal in tested amples: *S. epidermidis* (34.8 %), *S. aureus* (32.8 %) and *S. hyicus* (26.9 %). Whereas *S. intermedius* ($p < 0.05$) was the most unusual and it constituted only 5.5 percent of all staphylococci. *S. agalactiae* ($p < 0.05$) were the leading species among streptococci. *S. agalactiae* constituted 65.3 % of all identified streptococci. *E. hirae/durans* complex enterococci dominated among all enterococci and constituted 80.5 % of all identified enterococci. *E. faecalis* constituted 11.2 %, while *E. faecium* – 8.3 %.

The most frequent resistance to penicillin was detected in *S. aureus*: even 81.6 % of them were resistant (Table No. 2). The lowest resistance demonstrated *E. faecalis* (5.3 %), *S. uberis* (11.5 %), *E. hirae/durans* complex enterococci (15.1 %) and *S. equinus* (16.7 %). *S. intermedius* (87.5 %) and *S. aureus* (83.1 %) in most cases were resistant to ampicillin. Whereas *E. faecalis* (8.4 %), *S. equinus* (14.4 %) and *E. hirae/durans* (16.7 %) had low resistance. The resistance of mastitis pathogens to ampicillin and penicillin highly correlates ($r = 0.87$). Resistance to amoxicillin was the most frequent among *S. aureus* (81.9 %) and *S. epidermidis* (50.6 %). Other species were more frequently susceptible to this antibiotic. The highest susceptibility demonstrated *Enterococcus hirae/durans* complex enterococci (75.5 %) and *S. agalactiae* (75 %). The correlation coefficient of the tested strains susceptible to penicillin and amoxicillin was $r = 0.59$. If comparing with other antibiotics, a combination of amoxicillin and clavulanic acid was more effective ($p < 0.05$) against all tested gram-positive bacteria. However *S. aureus* in 41.5 % cases was resistant to this combination of antimicrobials. Correlation coefficient among strains susceptible to penicillin, amoxicillin and clavulanic acid combination is equal to $r = 0.46$, and the susceptibility to ampicillin and amoxicillin – $r = 0.52$.

Table 1. Bacteria isolated from udder secretions

Organism	Year of isolation				Total	%
	2007	2008	2009			
<i>Streptococcus dysgalactiae</i>	14	11	7		32	3.67
<i>Streptococcus uberis</i>	7	8	9		24	2.75
<i>Streptococcus equinus</i>	6	6			12	1.37
<i>Streptococcus agalactiae</i>	34	52	42		128	14.69
<i>Enterococcus durans</i>	100	14	22		136	15.61
<i>Enterococcus faecium</i>	6	4	4		14	1.60
<i>Enterococcus faecalis</i>	11	3	5		19	2.18
<i>Staphylococcus intermedius</i>	6	9	13		28	3.21
<i>Staphylococcus hyicus</i>	73	44	19		136	15.61
<i>Staphylococcus aureus</i>	91	35	40		166	19.05
<i>Staphylococcus epidermidis</i>	65	54	57		176	20.20
Total	413	240	218		871	
Mean	37.5	21.8	21.8		79.1	
Std.Dev	37.36	20.16	18.39		67.84	

Table 2. Susceptibility to penicillins of different bacterial species isolated from udder secretions

No of tested isolates	Organism	Susceptible, %				Mean	Std.dev
		Penicillin	Ampicillin	Amoxicillin	Amoxicillin and clavulanic acid		
n=32	<i>Streptococcus dysgalactiae</i>	73.3	67.6	66.7	90.9	74.6	11.23
n=24	<i>Streptococcus uberis</i>	88.5	88.3	64.2	92.2	83.3	12.85
n=12	<i>Streptococcus equinus</i>	83.3	85.6	68.3	90.1	81.8	9.44
n=128	<i>Streptococcus agalactiae</i>	66.2	53.9	75.0	83.0	69.5	12.47
n=136	<i>Enterococcus durans</i>	84.9	83.3	75.5	60.3	76.0	11.24
n=19	<i>Enterococcus faecalis</i>	94.1	91.6	50.0	87.5	80.8	20.71
n=14	<i>Enterococcus faecium</i>	36.3	60.0	61.2	57.1	53.6	11.69
n=176	<i>Staphylococcus epidermidis</i>	40.5	48.0	49.2	88.2	56.4	21.49
n=166	<i>Staphylococcus aureus</i>	19.4	16.8	18.3	58.46	28.2	20.17
n=28	<i>Staphylococcus intermedius</i>	45.0	12.5	50.2	80.9	47.1	28.00
n=136	<i>Staphylococcus hyicus</i>	44.3	29.6	68.5	83.8	56.5	24.23
Mean		61.4	57.9	58.8	79.3		
Std.dev		25.28	28.69	16.45	13.74		

Table 3. Susceptibility to cephalosporins of *Streptococcus spp.* and *Staphylococcus spp.* strains isolated from cows with mastitis

No of tested isolate	Organism	Susceptible, %		Mean	Std.dev	Std.err
		Cephalothin	Cephalexin			
n=32	<i>Streptococcus dysgalactiae</i>	91.3	78.1	84.7	9.34	6.60
n=24	<i>Streptococcus uberis</i>	83.3	79.2	81.3	2.89	2.05
n=12	<i>Streptococcus equinus</i>	91.6	83.3	87.5	5.87	4.15
n=128	<i>Streptococcus agalactiae</i>	92.9	78.9	85.9	9.89	7.00
n=176	<i>Staphylococcus epidermidis</i>	94.8	91.5	93.2	2.34	1.65
n=166	<i>Staphylococcus aureus</i>	97.5	93.9	95.7	2.55	1.80
n=28	<i>Staphylococcus intermedius</i>	85.7	89.3	87.5	2.55	1.80
n=136	<i>Staphylococcus hyicus</i>	86.8	84.6	85.7	1.56	1.10
Mean		90.5	84.9			
Std.dev		4.83	6.11			

While comparing effectiveness of different molecules of penicillins against tested species of bacteria it could be outlined that almost in all cases the most frequent effectiveness demonstrated combination of amoxicillin and clavulanic acid, with an exception against enterococci. This genus was more frequently susceptible to penicillins without beta-lactamase inhibitor.

Table 3 illustrates the first-generation cephalosporin class susceptibility for all bacterial species except *Enterococcus spp.* in this study. From this table there can be seen that cephalothin and cephalexin would be predicted to be effective in the treatment of mastitis caused by streptococci and staphylococci. Overall, *Staphylococcus spp.* was the most susceptible to cephalothin and cephalexin. *S. dysgalactiae* and *S. agalactiae* and particularly *S. uberis* were more frequently resistant to cephalosporins.

Discussion. Gram positive bacteria were the most predominated in our tested material. This confirms data obtained by other authors. Current date indicate that *S. aureus*, coagulase negative staphylococci, *S. agalactiae* and environmental streptococci are predominant aetiological agents in both subclinical and clinical forms

of mastitis (Olde Riekerink et al., 2006; Ferguson et al., 2007). There is indicated that environmental mastitis pathogens are now emerging as the most frequent cause of mastitis in many herds, particularly well-managed herds (Ebrahimi et al., 2008). Our study indicates that more species of pathogens are involved in mastitis aetiology however, the predominated genus was *Staphylococcus* (58.1 %) with statistically reliability ($p < 0.05$). The prevalence of *S. epidermidis* and *S. aureus* was higher, respectively 20.2 % and 19.05 %. The majority of the intramammary infection (IMI) (78.07 %) were caused by non-*aureus* staphylococci. The distribution of pathogens isolated from mastitis samples differ considerably among countries and even among studies within a country. *Staphylococcus spp.* also had the highest prevalence in the reports from the United States and Norway (Makovec and Ruegg, 2003; Reksen et al., 2006). Although the control of *S. aureus* mastitis is often deemed to be difficult, many herds have successfully achieved this through implementation of the standard mastitis prevention program. As a result the prevalence of *S. aureus* IMI has decreased in several European countries (Pitkälä et al., 2004; Barkema et al., 2006; Bradley et al.,

2007). However, *S. aureus* was one of the most frequent pathogens in our study. Besides under laboratory diagnostic data in Lithuania Klimienė et al. (2005) confirm that in 57.6 % cases of mastitis *S. aureus* was detected.

According to Ebrahimi et al. (2008), Olde Riekerink et al. (2008) not only *S. agalactiae* but *S. dysgalactiae* (16-42.4%), *S. uberis* (18-39.9%), *S. bovis* and *Enterococci* spp. as well were discovered from mastitis milk in the USA, Norway, Uruguay, Sweden and New Zealand. These are well known as important pathogens, spread in the environment and often causing cow mastitis and increasing general milk containers impurity with bacteria. Authors indicate that *S. agalactiae* was found in 1-13.1% of the positive samples in Sweden (Ebrahimi et al., 2008) and in 7.7% of the positive samples in USA (Makovec and Ruegg, 2003). Though Makovec and Ruegg (2003), Andersen et al. (2003), Olde Riekerink et al. (2006), Piepers et al. (2007), Sampimon et al. (2009) described a constant decline of the prevalence of *S. agalactiae* in their diagnostic material. The reports also suggest the re-emergence of *S. agalactiae* in some European countries e.g., Finland (Pyörälä, 2008). Several other mastitis pathogens are thought to have the potential to spread in a contagious manner. This is particularly true for *S. dysgalactiae* and to a lesser extent for *S. canis*, and *S. uberis* (Hassan et al., 2005; Tikofsky and Zadoks, 2005). In our study we usually found *S. agalactiae* (14.7 % of all pathogens). While *S. dysgalactiae* (3.6 %), *S. uberis* (2.7 %) and *S. equinus* (1.3 percent) contained non-significant part among other pathogens of mastitis. Klimienė et al. (2009) indicates that *S. agalactiae* was the causative agent of mastitis, detected in 18.04 % cases.

Little information is available on enterococcal pathogens isolated from milk samples, and if any, most studies focus on the two major species – *E. faecalis* and *E. faecium*. While data are sparse, the incidence of enterococci as aetiological agents of bovine mastitis varies from 0 to 21.2% (Pitkälä et al., 2004; Bradley et al., 2007). The enterococci are generally only a minor component of the environmental streptococcal/enterococcal mastitis complex and a major herd problem caused by the enterococci is relatively rare. In our study *E. durans/hirae* dominated among isolated enterococci. The quantity of this agent was identical to isolated quantities of *S. hyicus* and *S. agalactiae*.

The sensitivity of the identified bacteria to *beta*-lactam depends on the species of bacteria and particular antibiotic. Staphylococci demonstrated low sensitivity to aminopenicillin (meanly 47.05 %), though – only 28.2% of *S. aureus* in our study. That coincides to research executed by Pavilonytė et al. (2008) in Lithuania who identified that the highest resistance of *S. aureus*, is to aminopenicillins (33.9–58 %) in medicine clinics. Besides, *S. aureus* strain which was isolated from the public and clinic visitors reached 80.5–83.1 % of resistance to penicillin. The data of other authors are controversial and depends on the region where studies were performed. E.g., Bennedsgaard et al. (2006) determined rare *S. aureus* distribution which was isolated

from cow mastitis and its resistance to penicillin was 4-12 % in the countries of Scandinavia. Whereas the resistance of *S. aureus* was frequent (51-64 %) in Belgium and Germany (Martel and Coudert, 1993; Trolldenier, 1999). Significant resistance of *S. aureus* to penicillin and ampicillin was reported in Argentina, North of Palestine, and Finland (Erskine et al., 2002; Pitkälä, 2004; Ghaleb, 2006). According Ružauskas et al. (2009) ampicillin, while being the antibiotic of wide spectrum of activity, however naturally is not so suggestible from bacteria. Other species of staphylococci were less frequently resistant than *S. aureus*, though they often (approximately half of all strains) were resistant to penicillin but were susceptible to combination of amoxicillin and clavulanic acid. The rare resistance of staphylococci to amoxicillin and clavulanic acid combination was identified by other authors as well (De Oliveira et al., 2000). The combination of amoxicillin and clavulanic acid was statistically reliably effective and even 58.4 % of *S. aureus* strains were susceptible to the combination of these agents in our study.

The data for streptococci resistance to antimicrobial drugs are not as extensive as for *S. aureus*. Variability in drug resistance is more announced among individual streptococcal species than among studies of staphylococci. Though, some researches prove that *S. agalactiae* may demonstrate medium resistance to penicillin. Erskine et al. (2002) reported virtually no resistance among strains of *S. agalactiae* for beta-lactam antibiotics like penicillin and ampicillin. A small proportion of resistant strains were reported for these molecules 26 years ago. However, little evidence has been reported to suggest this increase is significant. Item Ebrahimi et al. (2008) identified that *S. agalactiae* showed high resistance rates to amoxicillin (76.92%), penicillin (69.23%), and ampicillin (61.53%). Authors reported that MIC levels for *S. agalactiae* isolates were higher for herds that reported dry cow treating all cows, as opposed to herds that did not dry cow treat or only selectively treated cows. This study reiterated that some strains of *S. agalactiae* are resistant to beta-lactam antibacterial drugs. *S. agalactiae* and *S. dysgalactiae* demonstrated resistance to beta-lactams, though they were more frequently susceptible to amoxicillin and clavulanic acid combination. Still it is known that mastitis, evoked by *S. dysgalactiae* and *S. agalactiae*, is much more easily cured than this which is caused by *S. aureus* (Rositto et al., 2002). Therefore the results performed should be judged integrally, taking in to account not only sensitivity of the agent *in vitro* and his elements produced for the resistance for antibiotics but other biological features and particular pharmacokinetic and pharmacodynamic data as well.

According to Ebrahimi et al. (2008) a high resistance rate was observed among *S. uberis* isolates, in particular, against penicillin (100 %), ampicillin (88.88 %). According to Piepers et al., (2007) *S. uberis* was more frequently resistant to the penicillin within the class of penicillins. According to our data 11.5 % of *S. uberis* were resistant to penicillin.

S. equinus is rare isolated species and there is not much information regarding the diseases caused by it. There is some information about human endocarditis caused by this bacteria (Klein et al., 1980). Nevertheless, there is scarce data regarding the susceptibility of this agent to antibiotics. While estimating the susceptibility of *S. equinus* to penicillin we determined that susceptibility of *S. equinus* is not in extreme contrast to other streptococci. Four strains were found to be susceptible to amoxicillin (out of 12) in our study. One strain was resistant to combination of amoxicillin and clavulanic acid.

Enterococcus spp. are used as indicator bacteria for the development of antimicrobial resistance (DANMAP, 2008) and provide accurate information on previous antibiotic treatment of the animals. As bovine mastitis is the reason of the most common usage of antibiotics in dairy farms knowledge of resistance profiles of enterococci isolates from milk samples would be useful for assessing the scale of the resistance problem and monitoring changes in resistance rates of dairy enterococci isolates. The analysis of the literature shows that *E. faecalis* as well as *E. faecium* may produce beta-lactamases. Piepers et al., (2007) reported, that the *Enterococcus* spp. were even more resistant than streptococci to beta-lactams. This is in coincidence to data obtained by us. *E. faecium* is also often resistant to penicillins in our study (35.8-57.2 %, depending on antibiotic). Previous study in Lithuania demonstrates that different species of enterococci should be isolated from the same specimen as different species within the same genus carrying different resistance patterns. Thus, during this study different types of colonies of enterococci were selected for further testing and different species and resistance patterns from the same milk specimens were found as well.

According Makovec et Ruegg (2003), Erskine et al. (2002) the resistance to beta-lactams of the gram-positive mastitis pathogens did not increase, over the course of the study. However (Ebrahimi, 2008; Ghaleb, 2006) indicated that the resistance to beta-lactams is increasing and it was indicated in the most reports. Our results indicate a frequent resistance of staphylococci (53.9 %), streptococci (22.7 %) and enterococci (29.7 %) to beta-lactams. This could be potentially hazardous to humans and animals, e.g., in the case of milk consumption after inappropriate milk processing and the breached withdrawal period. It is necessary to monitor mastitis pathogens to assess any changes in their antibiotic resistance patterns.

Conclusions

1. *S. epidermidis*, *S. aureus*, *S. hyicus*, *E. durans/hirae* and *S. agalactiae* were the most frequent mastitis pathogens in cows in Lithuania.

2. Fifty nine percent of all tested agents were susceptible to amoxicillin, 57.9 % – to ampicillin, 61.4 % – to penicillin. Amoxicillin and clavulanic acid complex was effective in 79.3 % of tested agents, cephalexin – in 84.9%, while cephalotin – in 90.5 %.

3. The most frequent resistance to ampicillin,

amoxicillin and penicillin was demonstrated by *S. aureus*, other staphylococci, *S. agalactiae* and *E. faecium*. The data of our study demonstrated that the resistance of staphylococci to aminopenicillins remain high.

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Received 13 January 2011

Accepted 15 November 2011