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MASTITIS PATHOGENS AND THEIR RESISTANCE AGAINST ANTIMICROBIAL AGENTS IN DAIRY COWS IN NITRA, SLOVAKIA

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ABSTRACT

The objective of this study was to evaluate the effectiveness of different antibiotics against mastitis causing microorganisms in lactating dairy cows in and around Nitra region, Slovakia. Milk samples from quarters were cultured and bacteriologically evaluated. All the bacteria isolated through microbiological procedures were subjected to antimicrobial susceptibility test by disc diffusion method to a large number of antibiotics. The results revealed higher sensitivity against tetradelta (100 % of *Streptococcus agalactiae* and *uberis*, *Escherichia coli* (*E. coli*), Coagulase Negative Staphylococci (CNS)), (97.37 % of *Staphylococcus aureus*) with highest number of bacterial isolates, followed by enrofloxacin (100 % of *Strep. agalactiae and uberis*), (97.37 % *Staph. aureus*), (97.14 % of (CNS), cefalexin + kanamycin (100 % of *Strep. agalactiae* and *uberis*), (97.14 % of CNS), (96.0 % of *E. coli*) and amoxicillin + clavulanat (100 % of *Strep. agalactiae* and *uberis*), (98.57 % of CNS), (94.74 % of *Staph. aureus*), (94.0 % of *E. coli*). Maximum resistance was observed against penicillin (96.0 % of *E. coli*) and streptomycin (66.67 % of *Strep. uberis*). In conclusion, *in vitro* antibiogram studies of bacterial isolates revealed higher sensitivity for tetradelta, enrofloxacin, a combination of cefalexin plus kanamycin and amoxicillin plus clavulanat acid.

Key words: dairy cows; mastitis; antimicrobial agents; bacterial strains; disc diffusion

INTRODUCTION

Mastitis is an inflammation of the mammary gland characterized by physical, chemical, bacteriological and cytological changes in milk. Pathological changes in glandular tissues of the udder and effects on the quality and quantity of milk have been observed (Amir, 2013). This disease is mainly caused by microorganisms usually bacteria, including gram-negative and gram-positive bacteria, mycoplasmas, yeasts and algae (Zadoks *et al.*, 2011).

The majority of mastitis incidences are caused by only a few common bacterial pathogens involved: *Staph.* spp. (*Staph. aureus* & *Staph. epidermidis*), *Strep.* spp. (*Strep. agalactiae, Strep. dysgalactiae, Strep. uberis* & *Strep. bovis*), coliforms (mainly *E. coli* & *Klebsiella pneumoniae*) and *Actinomyces pyogenes* (Sharma, 2010). Coagulase Negative Staphylococci (CNS) and *Corynebacterium bovis*, two other highly prevalent pathogens, are historically considered to be of limited importance and are therefore often described as minor pathogens. The impact of CNS is increasing (Pyörälä and Taponen, 2009), probably because prevalence of major pathogens are decreasing (Sampimon *et al*., 2009).

The most effective procedures to control contagious mastitis pathogens can be obtained by using dry cow therapy, post milking teat disinfectants and effective pre-milking hygiene (Fox and Gay, 1993). The incidence of streptococcal mastitis has been greatly reduced by using antibiotics and improving herd hygiene, but the incidence of staphylococcal mastitis has increased greatly. Treatment of all quarters with antibiotics during drying off is very important (Sharif *et al*., 2009). The majority of antibiotics used are broad-

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spectrum antibiotics acting against Gram-positive and Gram-negative bacteria (NCCLS, 2002). Control of environmental mastitis can be achieved by reducing the number of bacteria to which teat is exposed, increasing immune resistance of the cow, pre milking teat dipping with a germicidal. Animal environment should be as clean and dry as possible.

Antimicrobials are routinely used for treatment of dairy cattle affected with clinical and subclinical infections (Aarestrup, 2005). The use of antimicrobials have, over time, increased the number of antimicrobial-resistant microbes globally, and any use of these agents will to some extent benefit the development of resistant strains and also inappropriate usage of antimicrobials such as wrong dose, drug or duration may contribute the most to the increase in antimicrobial resistance without improving the outcome of treatment (Williams, 2000).

In recent years, antimicrobial susceptibility testing has become under scrutiny because of concerns about antimicrobial resistance, changes in methodology and the relationship between *in vitro* results and on-farm clinical outcomes. Susceptibility tests of milk samples submitted to state diagnostic laboratories that use the disk-diffusion method have demonstrated remarkable agreement but vary from results of a small survey processed using broth dilution (Constable and Morin, 2003).

Our recent study also dealt with the frequency of distribution of pathogens in positive milk samples (Idriss *et al.*, 2013). The present work aimed to study the effectiveness of different antibiotics against isolated microorganisms.

MATERIAL AND METHODS

The study was conducted during the period from 2010-2012 in and surroundings of Nitra region in Slovakia. A total of 390 milk samples were collected from udder quarters of dairy cows at some different small holder dairy farms, and pathogenic bacteria were examined and sensitivity of microorganisms against antibiotics had been tested.

Milk sample collection and laboratory analysis

After a quarter had been cleaned up by removing any possible dirt and washed with tap water, the teat end was dried and swabbed with cotton soaked in 70 % ethylalcohol. Approximately 100 ml of milk was collected aseptically into sterile bottles, after discarding the first 3 milking streams. Milk samples from each quarter were transported to the Animal Production Research Center Laboratory in an ice cooled box at 4ºC and analysed immediately (max. 4 h after collection) either for identification of the clinical mastitis

Table 1: Results of microbiological culture of milk samples collected from mastitis cows in Nitra region

Isolated microorganisms	Total. No.	%
Staphylococcus aureus	38	9.74
Streptococcus agalactiae	6	1.54
Streptococcus uberis	16	4.10
E. coli	50	12.82
Enterococcus spp.	12	3.08
Bacillus spp.	25	6.41
Corynebacterium pyogenes	5	1.28
CNS	70	17.95
Pseudomonas aeruginosa	13	3.33
Staphylococcus epidermidis	14	3.59
Staphylococcus chromogenes	4	1.03
Yeasts	22	5.64
Others (bacteria and mould)	13	3.33
infected quarters	288	73.85
non-infected quarters	102	26.15
Total dairy cows in herd	390	100

T. no- Total number of isolate, %- percentage of bacteria, T.no. , CNS- Coagulase Negative Staphylococci.

pathogen or to determine the reason for an increased somatic cell count (SCC). The milk samples were investigated for pathogenic mastitis in accordance with a standard procedure (IDF, 1981).

Antimicrobial susceptibility test

All the bacteria isolated through microbiological procedures were subjected to antimicrobial susceptibility test by disc diffusion method to identify the most effective drugs for mastitis treatment in the study area (Hameed, 2008). The sensitivity against amoxicillin, amoxicillin + clavulanat acid, cefalexin + kanamycin, ceftiofur, cloxcillin, enrofloxacin, lincomycin, nafpenzal, neomycin, penicillin, rifaximin, streptomycin and tetradelta were determined on Mueller Hinton agar as described by National Committee for Clinical Laboratory Standards (NCCLS, 2002). The results were obtained by measuring the diameter of the growth inhibition zone around the antibiotic disc for each isolated bacterial strain and recorded as sensitive, intermediate and resistant.

Statistics: Statistical evaluation of data was done by Excel program.

RESULTS AND DISCUSSION

From our previous study a total of 390 milk samples were investigated, 288 (73.8 5 %) samples were positive. No pathogens were isolated from 102 (26.15 %) milk samples as given in Table 1 (Idriss *et al*., 2013).

The study of the frequency of susceptibility of *Staph. aureus* (n = 38) to antibiotics has revealed a higher sensitivity to the enrofloxacin, tetradelta (97.37 % to each), combinations of amoxicillin plus clavant acid and cefallexin plus kanamycin (94.74 % to each) and rifaximin (94.74 %). A certain resistance has been noted to amoxicillin and streptomycin (18.42 % to each), lincomycin (13.16 %) and penicillin (10.53 %). More number of isolates showed moderate sensitivity or resistance to streptomycin (10.53 %), amoxicillin and penicillin (2.63 % to each) (Table 2).

Staphylococci were mostly susceptible to antimicrobials tested but, Muhamed *et al*. (2012) found that *Staph. aureus* was resistant to penicillin and streptomycin (41.44 % and 25.65 % respectively). Similar results were obtained by Sumathi *et al*. (2008) where *Staphylococcus* and *Streptococcus* spp. were resistant to streptomycin and penicillin. Those results are in accordance with our findings.

In contrast, CNS (n = 70) have been found to show a complete sensitivity to the rifaximin and tetradelta (100 % to each), and higher sensitivity to amoxicillin combination plus clavulanat acid (98.57 %), cefalexin plus kanamycin, ceftiofur, cloxcillin, enrofloxacin, lincomycin, nafpenzal (97.14 % to each). Apart from these unexpected results of CNS strain sensitivity for all antibiotic except to streptomycin (14.29 %), penicillin and amoxicillin (5.71 % to each), some strains showed intermediate sensitivity or resistance to amoxicillin and penicillin (7.14 % to each). Whereas the antibiogram

Table 2: Frequency of susceptibility of Staphylococcus aureus (n = 38) and Coagulase negative staphylococci (CNS) (n = 70) to antibiotics

Bacterial strains	*Staphylococcus aureus* (n = 38)			CNS (n = 70)		
Antibiotic agent	S %	IM %	R %	S %	IM %	R %
Amoxicillin	78.95	2.63	18.42	87.14	7.14	5.71
Amoxicillin + clavulanat	94.74	0.00	5.26	98.57	0.00	1.43
Cephalexin + kanamycin	94.74	0.00	5.26	97.14	1.43	1.43
Ceftiofur	94.74	0.00	5.26	97.14	0.00	2.86
Cloxcillin	92.11	0.00	7.89	97.14	0.00	2.86
Enrofloxacin	97.37	0.00	2.63	97.14	0.00	2.86
Lincomycin	86.84	0.00	13.16	97.14	0.00	2.86
Nafpenzal	94.74	0.00	5.26	97.14	0.00	2.86
Penicillin	86.84	2.63	10.53	87.14	7.14	5.71
Rifaximin	94.74	0.00	5.26	100.00	0.00	0.00
Streptomycin	71.05	10.53	18.42	85.71	0.00	14.29
Tetradelta	97.37	0.00	2.63	100.00	0.00	0.00

CNS- Coagulase negative staphylococci, n- number of bacteria strains, S- Sensitivity, IM- Intermediate, R- Resistant.

test to various antibiotics revealed that the isolates of CNS was resistant to streptomycin (14.29 %), followed by amoxicillin and penicillin were (5.71 % to each) (Table 2).

In the present study *Staph. aureus* was resistant to amoxicillin, streptomycin, lincomycin and penicillin and CNS was resistant to streptomycin, penicillin and amoxicillin, which is consistent with previous findings (Bengtsson *et al.*, 2009).

It is interesting to note that the present study has revealed a complete susceptibility (100 %) of *Strep. agalactiae* and *Strep. uberis* to all anibiotics, except *Strep. agalactiae* was resistant to lincomycin (16.67 %) and streptomycin (33.33 %), and *Strep. uberis* to cloxcillin (20 %) and streptomycin (66.67 %) (Table 3).

In our study we have found that all *Strep. agalactiae* and *Strep. uberis* were susceptible to a lot of antibiotics. In contrast, Erskine *et al.* (2002) and Makovec and Ruegg (2003) have found congruent results that *Staph.* other than *Staph. aureus* were sensitive to penicillin, ceftiofur and cephalothin and *Staph. aureus* was sensitive to ceftiofur and cephalothin and resistant to penicillin.

Vasiľ (2009) tested 14, 52 and 30 strains of *Strep. agalactaie*, *Strep. uberis* and CNS and has found that *Strep. agalactaie* strains were sensitive to all antibiotics except to neomycin, streptomycin, while *Strep. uberis* was a complete sensitive to a combination of amoxicillin + clavulanat and ampicillin, followed by cefalotin, lincomycin, whilst it is resistant to streptomycin, novobiocin and neomycin and CNS was sensitive to a combination of amoxicillin + clavulanat and resistant to streptomycin and penicillin. These results are in accordance with our findings that CNS, *Strep. agalactiae*, *Strep. uberis* and *E. coli* were completely sensitive (100 %) to tetradelta, while *Staph. aureus* showed sensitivity of 97.37 %. *Strep. agalactiae*, *Strep. uberis* and *E. coli* were complete sensitive (100 %) to enrofloxacin, followed by *Staph. aureus* and CNS (97.37 %) and (97.14 %), respectively. *Strep. agalactiae* was (100 %) sensitive to cefalexin + kanamycin, followed by CNS, *E. coli* and *Staph. aureus* (97.14 %), (96.0 %) and (94.74 %), respectively. *Strep. agalactiae* was (100 %) sensitive to amoxicillin + clavulanat, followed by CNS, *Staph. aureus* and *E. coli* (98.57 %), (94.74 %) and (94.0 %), respectively.

The percentage of susceptibility of *E. coli* (n = 50) isolates, revealed complete sensitivity to ceftiofur, enrofloxacin and tetradelta (100 %) isolates, followed by a combination of amoxicillin plus clavant acid and neomycin (96 % to each). A highly resistance has been noted to cloxcillin (98 %), lincomycin and penicillin with (96 % to each) and amoxicillin (82 %). Among the *E. coli* isolates, intermediate susceptibility was observed with streptomycin (6 %) and combinations of amoxicillin plus clavant acid (4 %) (Table 4).

Results of the current study demonstrated that *E. coli* was resistant to amoxicillin and penicillin. Similar result was obtained by Onerba (2006) who reported that *E. coli* was resistant to amoxicillin (85 %).

Table 3: Frequency of susceptibility of *Streptococcus agalactiae* (n = 6) and *Streptococcus uberis* (n = 15) to antibiotics

Bacterial strains	*Streptococcus agalactiae* (n = 6)			*Streptococcus uberis* (n = 15)		
Antibiotic agent	S %	IM %	R %	S %	IM %	R %
Amoxicillin	100	0	0	100	0	0
Amoxicillin + clavulanat	100	0	0	100	0	0
Cephalexin + kanamycin	100	0	0	100	0	0
Ceftiofur	100	0	0	100	0	0
Cloxcillin	100	0	0	80	0	20.00
Enrofloxacin	100	0	0	100	0	0
Lincomycin	83.33	0	16.67	100	0	0
Nafpenzal	100	0	0	100	0	0
Penicillin	100	0	0	100	0	0
Rifaximin	50	0	50	100	0	0
Streptomycin	66.67	0	33.33	33.33	0	66.67
Tetradelta	100	0.0	0	100	0	0

S- Sensitivity, IM- Intermediate, R- Resistant, n- number of bacteria strains

Table 4: Frequency of susceptibility of *Escherichia coli* (n = 50) to antibiotics

Bacterial strains	*Escherichia coli* (n = 50)		
Name of antibiotic	S %	IM %	R %
Amoxicillin	18.00	0.00	82.00
Amoxicillin + clavulanat	94.00	4.00	2.00
Cephalexin + kanamycin	96.00	2.00	2.00
Ceftiofur	100.00	0.00	0.00
Cloxcillin	2.00	0.00	98.00
Enrofloxacin	100.00	0.00	0.00
Lincomycin	4.00	0.00	96.00
Nafpenzal	90.00	0.00	10.00
Neomycin	96.00	0.00	4.00
Penicillin	4.00	0.00	96.00
Rifaximin	62.00	0.00	38.00
Streptomycin	84.00	6.00	10.00
Tetradelta	100.00	0.00	0.00

S- Sensitivity, IM- Intermediate, R- Resistant, n- number of bacteria strains

Foltys and Kirchnerová (2005) tested 60, 62 and 77 strains of *Staph. aureus*, *Strep. agalactiae* and *E. coli*, respectively to various antibiotics and they reported that *Staph. aureus* was sensitive to all antibiotics except lincomycin and streptomycin, whilst *Strep. agalactiae* was 100 % sensitive to amoxicillin and ampicillin and resistant to streptomycin, neomycin and tetracycillin and *E. coli* was resistant to all antibiotics. These findings are in complete accordance with the results of the present study except *E. coli* which was sensitive to ceftiofur and enrofloxacin (100 % to each of them) and to neomycin (96.0 %).

CONCLUSION

Antibiotic susceptibility tests should be done to determine the effectiveness of drug that can be used for successful treatment of diseases. Proper isolation and identification of the causative organism play significant role in prevention and control of the diseases. In our study a combinations of amoxicillin plus clavulanat acid, cefalexin plus kanamycin, enrofloxacin and tetradelta were the most effective antibiotics for control of bovine mastitis in Nitra area. Thus, there is a need to routinely investigate and record the epidemiology of bovine mastitis and antibiogram sensitivity of bacterial isolates in various parts of Slovakia.

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