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Identification of Bacterial Agents and Resistance Profile of Coagulase-Negative *Staphylococci* Isolated from Heifers Submitted or not to Precalving Treatment

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Abstract

Identify the causative agents of mastitis present in the mammary glands of primiparous heifers submitted or not to precalving treatment and to determine the *in vitro* antimicrobial susceptibility profile of the CoNS isolates. Eighty-seven animals were studied: Farm (1), 40 heifers, commercial farm, all treated precalving; Farm (2), 18 heifers; Farm (3), 29 animals. On Farms 2 and 3, the animals were divided into precalving treated and untreated groups. The treatments consisting of intramammary antibiotic infusion were administered 60 days before calving to all mammary quarters of each heifer after local antisepsis. Samples were collected during the precalving and calving period, 10 days after calving, and monthly. The results showed a predominance of CoNS during the precalving (28.75%) and calving (1.25%) period on Farm 1. On Farm 2, CoNS predominated during the precalving (88.89%) and calving (60%) period in untreated heifers; in the treated group, the precalving frequency of CoNS was 100%. On Farm 3, coagulase-positive *Staphylococci* (CoPS) predominated during the precalving and calving period in the untreated group (69.24% and 39.28%, respectively). The antibiogram revealed the following antibiotic resistance profiles: Farm 1 16.98% ampicillin, and 2.83% oxacillin; Farm 2 31.03% penicillin and 17.24% oxacillin, and Farm 3 52.22% penicillin and 13.33% oxacillin. The presence of CoNS in the mammary gland of antibiotic-resistant heifers suggests these animals to be a source of infection in the herd.

Keywords: Mastitis; Heifers; Antibiotic resistance; Coagulase-negative *Staphylococci*

Introduction

Mastitis is defined as inflammation of the mammary gland, most often due to infection, and is considered to be the main cause of economic losses for dairy farmers and the dairy industry. Dairy farmers adopt preventive measures to control the disease in adult lactating or dry cows, while young animals are considered to be free of infection [1].

Studies have demonstrated the occurrence of intramammary infection in heifers during pregnancy, calving and or early lactation, and coagulase negative *Staphylococci* is the most prevalent. Second Vliegher et al. [2] to review studies the prevalence of IMI ranges between 29 and 75% of quarters before parturition, whereas the immediate prevalence postpartum ranges from 12 to over 57% of quarters infected, though in all studies a major proportion of infection was caused by coagulase negative *Staphylococci.*

Coagulases negative *Staphylococci* are gram-positive cocci are found in the skin of animals and man. Several studies have associated coagulase negative *Staphylococci* from human nosocomial infections and bovine mastitis [3]. Zhou et al. [4] found in milk samples from mammary quarters of lactating cows with mastitis strains resistant to penicillin (18/18, 100%), lincomycin (18/18, 100%), amoxicillin (12/18, 66.7 %) and methicillin (1/18, 5.6%).

The objectives of the present study were to identify the causative agents of mastitis present in the mammary glands of primiparous heifers treated or not during the precalving and calving period and during lactation, to evaluate the efficiency of precalving treatment of primiparous heifer in reducing coagulase-negative *Staphylococci* (CoNS), and to determine the *in vitro* antimicrobial susceptibility profile of the CoNS isolates against the antibiotics most commonly used in veterinary medicine.

Materials and Methods

Characteristics of the herd and animal management

Eighty-seven dairy heifers were evaluated during the precalving and calving period and during lactation; 40 heifers belonged to a farm located in Descalvado, SP (Farm 1), 18 heifers were from an experimental farm in the Vale do Paraíba industrial region located in Pindamonhangaba, SP (Farm 2), and 29 animals belonged to the experimental farm of Instituto de Zootecnia, Nova Odessa, SP (Farm 3). On Farm 1, pre- and post-milking teat dipping in 10% chlorine solution is performed for teat disinfection. The other farms only perform post-milking teat dip in 5% glycerinated iodine.

Treatments

The treatments consisting of intramammary antibiotic infusion were administered 60 days before calving to all mammary quarters of each heifer after local antisepsis. The following treatment regimen was used on each farm:

Farm 1: Since this is a commercial farm, we chose not to modify the management adopted on the farm. The heifers received intramammary

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infusions of 0.25 g anhydrous Cefalonium, a semisynthetic beta-lactam antibiotic with bactericidal activity recommended for the treatment of dry cows.

Farm 2: Ten untreated animals (control group) and eight treated animals (treated group). The animals received intramammary infusions of an antibiotic with bactericidal activity of slow elimination and absorption, recommended for the treatment of dry cows (chemical composition: 677 mg gentamicin sulfate).

Farm 3: Fourteen untreated animals (control group) and 15 treated animals (treated group). The animals received intramammary infusions of an antibiotic with bactericidal activity of slow elimination and absorption, recommended for the treatment of dry cows (chemical composition: 500.000 IU penicillin G potassium, 1,000,000 IU penicillin G procaine, 0.732 g neomycin.

Collection of biological material from heifers

Samples were collected during the precalving and calving period, 10 days after calving, and monthly. Mammary secretions were collected from heifers 60 days before the estimated calving date following strict antisepsis routines. After collection, animals of the treated groups received the antibiotic. All samples (secretion, colostrum and milk) were collected into sterile tubes according to the recommendations of the National Mastitis Council (NMC) [5]. The tubes containing the samples were stored in isotherm boxes with ice cubes and sent to the laboratory for bacterial isolation and identification.

Isolation and identification of microorganisms

Milk aliquots (10 µL) were incubated on plates containing 5% defibrinated sheep blood agar in a bacteriological oven at 37°C under aerobic conditions and were analyzed after 24 and 48 h.

After incubation, the growth characteristics of the colonies on blood agar and the production of catalase were recorded. Next, colony morphology and Gram staining were observed. Colonies identified as catalase positive and Gram-positive cocci were submitted to slide coagulase tests using rabbit plasma [6]. Catalase-positive colonies and Gram-positive rods were classified as *Corynebacterium* spp. [7].

Antimicrobial susceptibility testing

The CoNS species identified were submitted to antimicrobial susceptibility testing according to the standards and recommendations of the National Committee for Clinical Laboratory Standards [8]. Disks impregnated with the following antibiotics were used: ceftiofur (30 µg), neomycin (10 µg), cephalexin (30 µg), gentamicin (10 µg), penicillin (10 g), florfenicol (30 µg), oxacillin (1 g), ampicillin (10 µg), and cefaclor (30 g).

Statistical analysis

To compare the differences between the prevalence of pathogens in treated and untreated groups, we used the Z test for two proportions at 95% confidence. The null hypothesis of the Z test considers equality between the proportions and the alternative hypothesis considers that the proportions differ from each other. For two-sided Z test for two proportions we have: H0: p1-p2=0; H1: p1-p2 ≠ 0. Where, p1 (group of untreated heifers) and p2 (group of heifers) are the proportion of positive samples of milk for the occurrence of pathogens in the population p1 and p2, respectively, which represent the primiparous and p 0 cows indicates that the difference between the two ratios is equal to zero. Software Minitab v. 13 was used for statistical analysis.

The odds ratio (OR), which is an association of intensity measurement was used to calculate how many times the prevalence of the pathogen in the treated group is lower than the prevalence of pathogens in the untreated group [9]. The odds ratio is calculated as the ratio (a / b) / (c / d) or (d) / (b c) in the appendix (a) is presented in a didactic way. Calculate an estimate of the 95% confidence interval associated with the odds ratio. It conducted the statistical analysis using the Chi-square test to assess whether there was a significant difference in the 95% confidence level, when compared to the untreated heifers groups and treated during the antepartum. The test was performed in Minitab Software v.

Results

Isolation profile of CoNS

CoNS were the agents most frequently isolated throughout lactation on Farm 1 (Table 1). On Farm 2, CoNS predominated up to post-calving day 15 in the group of untreated animals, while in treated

Collection	PATÓGENOS						
	CoNS	CoPS	*Strep.* spp	*Cory. spp*	Coccus spp spp -	*S. aureus*	*Bacillus spp G-*
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Precalving	46 (28.75)	11 (6.88)	0	0	3 (1.88)	1 (0.63)	3 (1.88)
Calving	2 (1.25)	0	0	0	0	0	0
3ª	3 (1.88)	0	1 (0.63)	0	0	1 (0.63)	0
4ª	3 (1.88)	0	1 (0.63)	1 (0.63)	0	0	0
5ª	8 (5.0)	0	0	0	0	0	0
6ª	8 (5.0)	0	1 (0.63)	1 (0.63)	0	0	0
7ª	7 (4.38)	0	1 (0.63)	1 (0.63)	0	0	0
8ª	6 (3.75)	0	0	0	1 (0.63)	0	0
9ª	5 (3.13)	0	0	0	0	0	0
10ª	4 (2.5)	0	3 (1.88)	2 (1.25)	1 (0.63)	2 (1.25)	0
11ª	4 (2.5)	0	0	0	0	0	0
12ª	2 (1.25)	0	0	0	0	0	0
13ª	0	0	0	0	0	0	0

N: absolute frequency, %: relative frequency, CoPS: coagulase-positive *Staphylococci*; CoNS: coagulase-negative *Staphylococci*; *; S. aureus*: *Staphylococcus aureus*; *Strep.* spp: *Streptococcus* spp.; *Cory.* spp.: *Corynebacterium* spp.

Table 1: Absolute and relative frequency of microorganisms isolated from mammary secretions, colostrum and milk of heifers submitted to precalving treatment on Farm 1.

animals the highest frequency of isolation of CoNS was observed during the precalving period. In untreated animals of Farm 3, the frequency of isolation of CoNS was higher at the end of lactation, while these pathogens predominated in treated animals until 200 days of lactation (Table 2).

Microbiological profile of the farms

Among the 160 mammary secretion samples collected from heifers during the precalving period on Farm 1, a higher frequency of isolation of CoNS (28.75%). During lactation, the highest frequency of isolation of these pathogens occurred in the 5th and 6th sampling (Table 1). On Farm 2, the highest frequency of isolation of CoNS in the untreated and treated groups occurred during the precalving period (88.89 and 100%, respectively), while during calving the frequency of these pathogens was 60.0 and 33.33% in the untreated and treated groups, respectively. During lactation, the highest frequency of isolation of CoNS in the untreated and treated groups was observed in the 7th and 3rd sampling (62.5 and 40%, respectively).

On Farm 3, in the group of untreated animals, the frequency of isolation of CoPS was 69.24 and 39.28% during the precalving and calving period, respectively, followed by CoNS (15.38% and 28.58%). In the treated group, CoPS were isolated from 100% of the samples during the precalving period. During the calving period, the frequency was 39.28% for CoPS and 25.0% for CoNS. During lactation, the pathogens showing the highest frequency of isolation in the untreated group were CoNS (57.14%) in the 8th sampling, followed by CoPS (42.8%) in the 3rd sampling. In the group of treated animals, the highest frequency of isolation of CoNS was found in the 9th sampling (80%), followed by CoPS in the 11th sampling (33.3%) (Table 2).

A significant difference between treated and untreated heifers (5% level of significance) was observed for *Staphylococcus aureus* and *Corynebacterium* spp. on Farm 2 and for CoPS and *Staphylococcus aureus* on Farm 3 (Table 3). On Farm 2, the prevalence odds ratio of *Staphylococcus aureus* in untreated and treated heifers was 0.46 and was significant at the 5% level. Thus, the probability of occurrence of *Staphylococcus aureus* in the untreated group was 46% compared to the treated group. On Farm 3, the prevalence odds ratio of CoPS was 2.29 times higher in untreated heifers compared to the treated group. The prevalence odds ratio of *Staphylococcus aureus* was 2.45 and was significant at the 5% level. Thus, the prevalence of the pathogen was 2.45 times higher in the untreated group compared to the treated group (Table 4).

Antimicrobial resistance profile of coagulase-negative *Staphylococci*

The resistance rates of the CoNS strains isolated on Farm 1 were 16.98% for ampicillin, 13.21% for penicillin, and 3.77% for gentamicin. Important resistance against oxacillin (2.83%) was also observed. High resistance against penicillin (31.03%) was found on Farm 2, while 17.24% of the strains were resistant to oxacillin and, consequently, to all beta-lactam antibiotics. High resistance to penicillin (52.22%) was observed on Farm 3 and 13.33% of the strains were resistant to oxacillin (Table 5).

Discussion

Coagulase-negative *Staphylococci* were the most frequent agents isolated from mammary secretions, colostrum and milk samples of heifers on Farm 1. The frequencies observed here were lower than those reported by Parker et al. [10] who evaluated 255 heifers during the precalving period and found a prevalence of CoNS of 10.4% in the samples. These authors also observed a reduction in the isolation of CoNS (4.5% during calving) in animals receiving a teat sealant precalving. This rate is higher than that found on Farm 1 (1.25%), probably because of the time of precalving infusion of the teat sealant which was 60 days on Farm 1. A predominance of CoNS (1.88%) was observed on Farm 1 at approximately 15 days of lactation, while Parker et al. [10] analyzing milk samples of animals collected 14 days after calving, found 6.9% of CoNS in mammary quarters with mastitis. The post-calving result observed in the present study is lower than the rates reported by these authors. This difference may be related to the active ingredient and mode of action of the drugs as well as differences in the samples analyzed, since Parker et al. [11] only studied mammary quarters with mastitis.

The frequency of CoNS isolated on Farm 2 at approximately one of

Farm 2															
Untreated mammary	Precalving	Calving	3ª	4ª	5ª	6ª	7ª	8ª	9ª	10ª	11ª	12ª	13ª	14ª	15ª
CoPS	11.11	40.00	28.57	57.14	50.00	0.00	37.50	0.00	50.00	0.00	0.00	0.00	0.00	0.00	0.00
CoNS	88.89	60.00	42.86	28.57	25.00	0.00	62.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S. aureus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	50.00	42.86	10.00	33.33	30.00	25.00	0.00
Treated mammary quarters															
CoPS	0.00	50.00	20.00	57.14	50.00	0.00	0.00	0.00	0.00	0.00	10.00	0.00	0.00	7.14	0.00
CoNS	100.00	33.33	40.00	14.29	0.00	0.00	0.00	0.00	25.00	0.00	10.00	0.00	0.00	0.00	0.00
S. aureus	0.00	33.33	40.00	14.29	0.00	0.00	0.00	0.00	25.00	0.00	10.00	0.00	0.00	0.00	0.00
Farm 3															
Untreated mammary quarters	Precalving	Calving	3ª	4ª	5ª	6ª	7ª	8ª	9ª	10ª	11ª	12ª	13ª	14ª	15ª
CoPS	69.24	39.28	42.85	36.36	40.00	23.52	40.00	28.58	0.00	22.22	0.00	28.57	20.00	26.67	0.00
CoNS	15.38	28.58	35.71	18.19	30.00	41.18	30.00	57.14	40.00	44.44	60.00	0.00	0.00	0.00	0.00
S. aureus	15.38	10.71	7.14	0.00	0.00	5.90	0.00	0.00	0.00	11.12	10.00	35.71	20.00	6.66	0.00
Treated mammary quarters															
CoPS	100.00	39.28	15.50	0.00	9.09	16.66	0.00	0.00	0.00	0.00	33.33	0.00	0.00	10.00	25.00
CoNS	0.00	25.00	30.75	25.00	27.27	50.00	50.00	71.44	80.00	42.85	0.00	60.00	50.00	10.00	0.00
S. aureus	0.00	14.29	7.60	8.34	9.10	0.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

CoPS: coagulase-positive *Staphylococci*; CoNS: coagulase-negative *Staphylococci*; *S. Staphylococcus aureus*.

Table 2: Relative frequency (%) of *Staphylococcus* spp. isolated from milk samples collected from heifers during the precalving and calving period and during lactation on Farms 2 and 3.

Isolates	Heifers	Farm 2			Farm 3		
		Positive (%)	Chi-square (χ^2)	P	Positive (%)	Chi-square (χ^2)	P
Coagulase-positive *Staphylococci*	Untreated	18	0.358 ns	0.549	56	12.174*	0.0001
	Treated	13			26		
Coagulase-negative *Staphylococci*	Untreated	24	1.629 ns	0.202	47	0.274 ns	0.601
	Treated	14			43		
S. *aureus*	Untreated	22	8.512 *	0.004	18	4.767*	0.029
	Treated	43			8		
Corynebacterium spp.	Untreated	34	3.829 *	0.05	----------	----------	-------
	Treated	17			----------		
Streptococcus spp.	Untreated	0	----------	--------	40	0.064 ns	0.8
	Treated	2			43		
Coccus spp -	Untreated	----------	----------	--------	12	3.044 ns	0.081
	Treated	----------			5		
Bacillus spp -	Untreated	2	0.317 ns	0.574	6	1.397 ns	0.23
	Treated	3			11		

*: Significant difference between the treated and untreated groups ($p<0.05$. chi-square test).
ns: not significant
The dashed line indicates values of zero.
P: probability value for the chi-square test.

Table 3: Relative frequency (%) of pathogens isolated from teat samples of treated and untreated heifers throughout the experiment and chi-square value.

Isolates	Farm 2				Farm 3			
	Untreated	Treated	Odds Ratio (OR)	CI 95%	Untreated	Treated	Odds Ratio (OR)	CI 95%
Coagulase-positive SSSSSkS*Staphylococci*	18[a]	13[a]	1.25	0.60-2.58	56[a]	26[b]	2.29	1.42-3.68
Coagulase-negative *Staphylococci*	24[a]	14[a]	1.54	0.79-3.03	47[a]	43[a]	1.12	0.73-1.72
S. aureus	22[a]	43[b]	0.46	0.27-0.78	19[a]	8[b]	2.45	1.07-5.64
Corynebacterium spp	34[a]	17[b]	1.8	0.99-3.27	0	0	-------	----------
Streptococcus spp	0	2	-------	--------	40[a]	43[a]	0.94	0.61-1.47
Coccus spp - .	0	0	-------	--------	12[a]	5[a]	2.47	0.86-7.04
Bacillus spp- .	2[a]	3[a]	0.6	0.10-3.61	6[a]	11[a]	0.55	0.2-1.50
TOTAL	455	410	----------		765	779	----------	

[ab]Frequencies in the same row followed by the same superscript letter do not differ from each other.

Table 4: Absolute frequency, odds ratio and 95% confidence interval of pathogens isolated from treated and untreated heifers (Farms 2 and 3).

week of lactation was 40%. Similar results were obtained by Piepers et al. [11] evaluating 1,354 mammary quarters from all 344 heifers in the first week after calving study were infected 483 quarters (35.7%) shortly after calving. Coagulase negative staphylococci were the most frequently isolated mastitis pathogens (n = 372; 77% of infected quarters) and the most frequent pathogens were CoNS (n= 372, 35.2%). On Farm 2, the variation in pathogens shown in Table 2 is related to the lack of hygiene during milking since no pre-milking teat dipping is performed. The purpose of this procedure is to eliminate pathogens present on the teat skin in order to minimize contamination of the milking equipment, consequently reducing the dissemination of microorganisms between animals.

Coagulase-negative *Staphylococci* are considered to be of low pathogenicity and usually cause subclinical infections. However, intramammary infections in first-lactation animals can affect 10 to 20% of mammary quarters. The heifers can show a high frequency of mastitis caused by CoNS after calving, followed by a rapid decline in cases after the second week of lactation [12]. This fact was not observed in the present study in which CoNS predominated throughout lactation. As can be seen in Table 2, the frequency of CoNS was high during calving (28.58%) and these pathogens continued to be isolated throughout lactation, except for the 12th to 15th sampling, with a peak isolation rate of 80% at 200 days of lactation. The moment of bacteriological colonization and kind of pathogen involved do have an effect on the udder health during first lactation, and the mammary glands of heifer may harbor bacteria precalving, an observation that is supported by the results of the present study [13].

A significant difference (5% level of significance) in the relative frequency of pathogens between untreated and treated animals was observed for *S. aureus* (P=0.004) and *Corynebacterium* spp. (P=0.05)

on Farm 2, and for CPS (P=0.0001) and *S. aureus* (P=0.029) on Farm 3 (Table 3). As can be seen in Table 4, there was a significant difference between the untreated and treated groups in the absolute frequency (with 95% confidence interval) of *S. aureus* (22 and 43) and *Corynebacterium* spp. (34 and 17) on Farm 2, and of CoPS (56 and 26) and *S. aureus* (16 and 8) on Farm 3. The pathogens isolated on Farms 2 and 3 are the causative agents of contagious mastitis, i.e., they are transmitted during milking management. The results suggest that the farms should improve their good milking practices since, according to Zafalon et al. [14], farms employing good milking practices reduce transmission of the infectious agents responsible for bovine mastitis. Another approach of dairy farms to reduce the bacterial load in the mammary gland of heifers is to set up a milking line, i.e., to milk primiparous heifers first since this milking management reduces the transmission of mastitis-causing pathogens from cows to heifers.

Coagulase-negative *Staphylococci* are a common member of the skin microbiota and, by living in balance with this ecosystem, have been described as virulent microorganisms. Progress in the identification of genera, species and subspecies of pathogens has been made over the last decade, permitting clinicians to identify the variety of CoNS present in clinical samples and to imply these microorganisms as the etiological agents of a series of infections. In this respect, CoNS have been the main pathogens isolated from the mammary quarters of animals with mastitis [15,16].

Infections caused by CoNS have been increasing, a fact leading researchers to study existing species which are responsible for different diseases in animals. Animals carrying oxacillin-resistant CNS strains do not respond to treatment with beta-lactam antibiotics except new cephalosporin classified as V generation (ceftobiprole) This resistance is associated with transpeptidase PBP2a no methicilin resistent *Staphylococcus aureus* (MRSA) and methicilin resistant coagulase negative Staphylococci (MR-CoNS) [17].

The resistance rate of CoNS strains isolated on Farm 1 was 16.98% for ampicillin, 13.21% for penicillin, and 3.77% for gentamicin. Important resistance to oxacillin (2.83%) was also observed (Table 5). Soares [18] studied lactating cows and found high resistance of CNS strains to different antimicrobials: penicillin (79%), ampicillin (79%), tetracycline (64%) and oxacillin (29%). These rates are much higher than those observed in the present study involving heifers and the microbial load of resistant bacteria may increase over time in these animals.

High resistance to penicillin (31.03%) was observed on Farm 2, while 17.24% of the strains were resistant to oxacillin and consequently to all beta-lactam antibiotics. Karabasanavar and Singh [19] found strains resistant to penicillin (99.9%), amoxicillin (63.7%) and oxytetracycline (63.7%), and 100% of the strains revealed resistance to erythromycin, amikacin and nitrofurantoin. The high resistance to antimicrobials is associated, according to the authors, the indiscriminate use of antibiotics in India.

On Farm 3, high resistance to penicillin was observed (52.22%) and 13.33% of the strains were resistant to oxacillin. Frey et al. [20] founded 417 coagulase-negative staphylococci in milk samples from cows with clinical and subclinical mastitis (370) 47% de CoNS oxacillin resistance,

Active principle	Profile					
	Resistance		Intermediateo		Susceptibility	
	N	%	N	%	N	%
FARM 1						
Florfenicol 30 µg	4	3.77	2	2.12	100	94.34
Ampicillin 10 µg	18	16.98	-	-----	88	83.02
Cephalexin 30 µg	4	3.77	-	-----	102	96.23
Ceftiofur 30 µg	3	2.83	-	-----	103	97.17
Penicillin 10 µg	14	13.21	-	-----	92	86.79
Neomycin 10 µg	-	-----	-	-----	106	100.00
Oxacillin 1 µg	3	2.83	-	-----	103	97.17
Gentamicin 10 µg	4	4.24	-	-----	102	96.23
FARM 2						
Florfenicol 30 µg	5	17.24	-	-----	24	82.76
Ampicillin 10 µg	5	17.24	-	-----	24	82.76
Cephalexin 30 µg	5	17.24	-	-----	24	82.76
Ceftiofur30 µg	5	17.24	2	6.90	22	75.86
Penicillin 10 µg	9	31.03	-	-----	20	68.97
Neomycin 10 µg	0	0.00	1	3.45	28	96.55
Oxacillin 1 µg	5	17.24	-	-----	24	82.76
Gentamicin 10 µg	5	17.24	-	-----	24	82.76
FARM 3						
Florfenicol 30 µg	12	13.33	-	-----	78	86.67
Ampicillin 10 µg	12	13.33	-	-----	78	86.67
Cephalexin 30 µg	12	13.33	-	-----	78	86.67
Ceftiofur30 µg	12	13.33	6	6.67	72	80.00
Penicillin 10 µg	47	52.22	-	-----	43	47.78
Neomycin 10 µg	1	1.11	2	2.2	87	96.67
Oxacillin 1 µg	12	13.33	-	-----	78	86.67
Gentamicin 10 µg	12	13.33	-	-----	78	86.67

Table 5: Antimicrobial resistance profile of coagulase-negative *Staphylococci* isolated from mammary quarter milk samples of heifers during the precalving and post-calving period and during lactation on Farms.

which is the indicator of MEC gene-mediated methicillin resistance. As a consequence, these animals did not respond to treatment with beta-lactam antibiotics as reported by Mlynarczyk et al. [17]. The drug used for the precalving treatment of heifers was penicillin, an antibiotic to which CoNS were highly resistant (52.22%). This fact may explain the low treatment response of heifers in the treated group compared to untreated animals during the precalving and calving period. Precalving intramammary infusion of antibiotics should not be performed indiscriminately as a routine procedure without knowledge of the microbiological and antibiotic susceptibility profile.

Tarazi et al. [21] found 23% of strains resistant to penicillin, lincomycin 25%, gentamycin 20%, cephalexin 13%, ciprofloxacin 10%. Strains resistant to neomycin (10%) and ampicillin (5%) were also isolated from secretions of mammary quarters of heifers (n=56) in the pre calving, colostrum and regular lactation. Similar results were obtained in the present study on Farms 1, 2 and 3, which demonstrate that heifers need to be given more importance since, as the future of the herd, they should not carry a high frequency of resistant strains at the beginning of lactation.

The high level of resistance among pathogens on the three farms studied should serve as a warning to professionals when prescribing antimicrobial drugs to animals with mastitis [22]. Precalving treatment of heifers may select antibiotic-resistant CNS strains, causing harm to animals and resulting in losses for producers, and could become a public health problem.

Conclusions

The isolation of mastitis-causing infectious agents from mammary secretions of heifers during the precalving and calving period indicates that these animals may become a source of infection in the herd. The presence of oxacillin-resistant CoNS in heifers may affect mastitis treatment in subsequent lactations. Precalving treatment with intramammary antibiotics should be done with caution and should be preceded by microbiological tests and antibiograms to permit the correct use of antimicrobial agents and to obtain the best response to treatment. Antibiotics should not be used indiscriminately as routine treatment on dairy farms.

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