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Prevalence of bacterial agents isolated from clinical cases of bovine mastitis in the dry period and the determination of their antibiotic sensitivity in Tabriz, Iran

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ABSTRACT

Objective: To determine the prevalence of mastitis-causing bacteria in the dry period and its antibiotic sensitivity.**Methods:** In this study, 852 dry cows were examined. A total of 30 cows with clinical mastitis symptoms were detected and their milk samples were collected. In order to purify the bacteria, brain heart infusion and blood agar media were applied and single colonies were used for Gram staining, oxidase and catalase testing, cultivating in O-F medium to determine the genus and species of bacteria. Then, antimicrobial susceptibility was tested by the agar disk diffusion method.**Results:** The prevalence of isolated bacteria was 2.46%, in which coagulase positive *Staphylococcus*, coagulase negative *Staphylococcus*, *Streptococcus dysgalactiae*, *Streptococcus faecalis*, *Escherichia coli*, *Pseudomonas*, *Bacillus* and yeast were (9/99)%, (6/66)%, (13/32)%, (3/33)%, (6/66)%, (13/32)%, (9/99)% and (6/66)%, respectively. After tests of antibiotic susceptibility, the most and the least sensitivity were reported to enrofloxacin and ampicillin respectively.**Conclusions:** This study indicated that *Streptococcus dysgalactiae* is the most commonly isolated bacteria with the greatest sensitivity to enrofloxacin and tetracycline which can be used to treat mastitis in the dry period in Tabriz.

1. Introduction

Mastitis, as an inflammatory condition of the breast glands, is the most important disease of cattle in response to wounding, physiological and metabolic changes, trauma, environmental and known contagious pathogenic microorganisms. It causes neutralization of infectious agents, leading to recovery and return to normal function[1,2]. More than 100 organisms from a wide

range of the phylogenetic spectrum can cause bovine mastitis[2]. Many different environmental and contagious bacteria cause mastitis. The infectious pathogenic bacteria live and multiply in infected mammary glands and could be passed from one cattle to another through Cartier mammary glands. These bacteria include *Staphylococcus aureus* (*S. aureus*), *Streptococcus agalactiae* (*S. agalactiae*) and several species of *Mycobacterium* and *Arcanobacterium*. Pathogenic bacteria are present in the animal environment and their presence in the nipple can cause infection in the mammary gland. This group includes *Escherichia coli* (*E. coli*), *Streptococcus dysgalactiae* (*S. dysgalactiae*), *Streptococcus uberis*, *Klebsiella* and *Bacillus* species[1]. This disease causes considerable changes in the breast and milk of cows, and large financial losses in the dairy industry have ensued. In such a way that in Hokkaido, one of the main areas of milk production in Japan, \$100 million is lost annually[3,4]. Dry period is important

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for the health of the breast glands[5]. This period is a crucial time in the lactation cycle, the appropriate time for the treatment of existing intramammary infections and also a period with the greatest susceptibility to new infections[6,7]. Studies regarding to the significance of infections during the dry period have demonstrated that such infections will affect the next lactation[7]. It is supposed that the subclinical mastitis-causing bacteria have entered the dry period during the delivery[8]. Injection of antibiotics is a known method for improving the health of mammary gland in order to prevent the new infections during the dry period and reduce the amount of common intermammary infections[9]. Use of broad-spectrum antibiotics to prevent new intramammary infections is important, highlighting the pivotal role of this infection in the incidence and causes of clinical mastitis during the next lactation in the dry period[6]. Thus, the aim of this study was to determine the prevalence of bovine mastitis-causing bacteria in the dry period and their antimicrobial susceptibility which was done by using bacterial cultures and antibiogram method.

2. Materials and methods

This study was done on 14 industrial dairy farms in the vicinity of Tabriz, including 2090 milch Holstein-Friesian breed of dairy cattle with 852 circulating dry cows during the weekly visits. Sampling was conducted under sterile conditions and samples were transported to the Laboratory of Microbiology, Faculty of Veterinary Medicine, Islamic University of Tabriz. In order to purify the bacteria, blood agar and brain heart infusion culture media were used. After bacterial growth in brain heart infusion medium (after purification), single colonies were used for Gram staining, oxidase and catalase tests, cultivating in O-F medium, and antibiogram test was used for differential diagnosis of the genus and species of the bacteria. It should also be noted that MacConkey medium was used for the study of Gram-negative bacteria. In order to determine the bacterial sensitivity towards antibiotics, modified Kirby-Bauer method in Muller Hinton agar medium was applied with enrofloxacin, trimethoprim, sulphamethoxazole, lincomycin, tetracyclin, ampicillin, amoxicillin, penicillin, and ceftriaxone discs according to the National Committee for Clinical Laboratory guidelines. Pure cultures of bacteria were used to prepare microbial suspension. The bacterial samples were taken from the top of the 3 to 4 colonies by using sterile swab. The swab was then inserted into a tube containing physiological serum to reach the same turbidity as standard tube (0.5 McFarland standard). A sterile swab was dipped into the bacterial suspension, and it was then rubbed on the surface of the Muller Hinton agar medium in three directions. Consequently, the disks were placed on the surface of the medium by using forceps, and were incubated at 35 °C for 16 to 18 h. A ruler was used to measure the diameter of inhibition zone (mm), and the sensitivity of bacteria toward antibiotics were reported as sensitive (S), intermediate sensitive (I) and resistant (R) according to the reference[10].

3. Results

3.1. Culture results

After checking 852 circulating dry cows during 6 months, 30 cows (3.52%) showed signs of clinical mastitis in the dry period. Results of the prevalence of different disease-causing agents in understudied cattle after process of recognition and identification of bacteria were shown in Table 1.

The prevalence of bacteria isolated from bacterial cultures was 2.46% in which *S. dysgalactiae* and *Streptococcus faecalis* were as the most common (13.32%) and the least common (3.33%) isolated bacteria, respectively. It should be noted that in the bacterial culture medium, 4 samples (13.32%) were contaminated and in 5 samples (16.66%) no bacterial growth was detected, hence they were excluded from our study.

Table 1

Bacteria isolated from clinical cases of bovine mastitis during the dry period.

Bacterial agents	%	Number
No growth	16/66	5
<i>S. dysgalactiae</i>	13/32	4
<i>Streptococcus faecalis</i>	3/33	1
Coagulase positive staphylococci	9/99	3
Coagulase negative staphylococci	6/66	2
<i>Pseudomonas</i> spp	13/32	4
<i>Bacillus</i> spp	9/99	3
Yeast	6/66	2
<i>E. coli</i>	6/66	2
Contaminated	13/32	4
Total	100	30

3.2. Results of antibiogram test

The isolated bacteria showed the most sensitivity to enrofloxacin, tetracycline, ceftriaxone, lincomycin, cotrimoxazole and amoxicillin, respectively. In other hand, the least sensitivity was shown to ampicillin and penicillin.

4. Discussion

After testing 30 milk samples transported to the laboratory, the prevalence of isolated bacteria was 2.46%, in which *S. dysgalactiae* and *Streptococcus faecalis* were reported as the most and as the least isolated bacteria with the rate of 13.32% and 3.33%, respectively. In the antibiotic susceptibility testing of isolated bacteria, the most sensitivity was shown to enrofloxacin, tetracycline, ceftriaxone, lincomycin, cotrimoxazole, and amoxicillin, respectively and the least sensitivity was shown to ampicillin and penicillin. Some studies in other countries have demonstrated different results. For example, in a research performed on 5 cow herds during the dry period in Canada and America by Dingwell *et al.*[5], the following results were obtained: coagulase-positive staphylococci (*S. aureus*) (11%), coagulase-negative staphylococci (15%), *E. coli* (14%) and *Pseudomonas* (5%) in which the most common isolated bacteria were related to *Streptococcus* spp. In another research conducted by Pantoja *et al.*[11] in America, during the dry period, the most isolated

bacteria were related to coagulase-negative staphylococci. In an investigation conducted on 480 dairy cows in America by Green et al.[12], entitled “the effect of dry period intramammary bacteria on clinical mastitis in dairy cows”, *E. coli*, as Gram-negative bacteria, was the most common bacteria isolated from samples. The most common bacterial species isolated from mastitis in all three conducted studies differ from those we isolated. In a research conducted on 120 milk samples using PCR method by Moatamedi et al.[13], *S. agalactiae*, *S. dysgalactiae*, and *Streptococcus uberis* were 20%, 12.5% and 0.83%, respectively. In another research conducted in Tehran by Atyabi et al. during 4 years[14], to determine the incidence of mastitis-causing bacteria, the frequency of coagulase-negative staphylococcus, *S. agalactiae*, *S. dysgalactiae*, *E. coli*, and *S. aureus* was reported as 30.27%, 22.11%, 11.43%, 10.16% and 2.89%, respectively. The most common isolated bacteria in our study differ from those of other studies in Iran. Results of antibiotic susceptibility testing with disk diffusion toward samples isolated from mastitis in the dry period revealed that isolated bacteria showed the most sensitivity to enrofloxacin and tetracycline, respectively and the least sensitivity to ampicillin and penicillin, respectively. Whereas in a study done in Romania by Brinda[15], to investigate the antibiotic susceptibility of mastitis-causing bacteria, the most sensitivity was reported to ampicillin and the most resistance was reported to tetracycline. In another study conducted in Turkey by Kenar[16], it was shown that the most sensitive and resistant antibiotic isolated from isolates of coagulase-negative staphylococci were cephalothin and trimethoprim, sulphamethoxazole, respectively. These results are not consistent with our antibiotic susceptibility results. The cause of differences among the results of the conducted studies might be due to the climatic and hygiene conditions of the areas where these studies were done, so it requires further investigations on this field. The results of this study show that the best antibiotics for treatment of mastitis in the dry period in this area are enrofloxacin and tetracycline.

Regarding the results of studies done worldwide and various mastitis-causing bacteria and their antibiotic sensitivity, it is proposed that the type of antibiotic should be chosen in accordance with the type of bacteria which cause mastitis in the special region in order to prevent antimicrobial resistance.

Conflict of interest statement

We declare that we have no conflict of interest.

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