Prevalence and antimicrobial susceptibilities of coagulasenegative Staphylococci isolated from mammary secretions in pregnant Holstein heifers

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Summary

Mammary secretions of 913 quarters from 229 pregnant Holstein heifers were obtained aseptically to determine the prevalence of coagulase-negative Staphylococci isolated during periparturient period. Quarter samples were collected 14 to 10 days before expected parturition. Analysis of culture data indicated that 78.5% (717/913) of samples were negative, 20.5% (187/913) contained coagulase-negative Staphylococci, 0.33% coagulase-positive Staphylococci, 0.33% E. coli, 0.22% Arcanobacterium pyogenes and 0.11% environmental Streptococci spp. A total of 187 isolates of coagulase-negative Staphylococci were tested in vitro for susceptibility to 8 antimicrobial agents. Susceptibility was detected in 180 (96.3%), 187 (100%), 163 (87.2%), 168 (89.8%), 183 (97.9%), 187 (100%), 174 (93%) and 164 (87.7%) isolates for oxytetracycline, gentamicin, streptomycin, tylosin, cloxacillin, enrofluxacin, trimethoprim+ sulphamethoxazole and penicillin G, respectively. More than 93.8% of the isolates were susceptible to all antimicrobial agents tested. In conclusion, results of this study suggest that coagulase-negative Staphylococci spp. is the most prevalent microorganism isolated from intramammary secretions in pregnant heifers which is susceptible to common antibiotics in vitro.

Key words: Heifer mastitis, Coagulase-negative Staphylococci, Antibiotic susceptibility

Introduction

Mammary glands of pregnant heifers were once thought to be uninfected. However, Oliver and Mitchell (1983) showed that a high percentage of pregnant heifer mammary glands were infected during late gestation, at calving and during early lactation. During the last decade, several studies on the prevalence of mastitis in heifers have been done (Oliver, 1987; Oliver, 1988; Jonsson et al., 1991; Pankey et al., 1991; Matthews et al., 1992; Smith et al., 1994; Fox et al., 1995; Myllys, 1995; Nickerson et al., 1995; Aaerstrup and Jensen, 1997; Malinowski et al., 2003; Oliver et al., 2005). These studies suggest that intramammary infection (IMI) can occur frequently in heifers during the prepartum period. All of these data suggest also that coagulase-negative *Staphylococcus* spp. cause the majority of IMI in unbred and pregnant heifers. However, variation in the prevalence of mastitis caused by other pathogens should be expected among herds (Oliver et al., 2003). Recent studies have indicated that IMI is common in heifers (Oliver et al., 2005); prevalence of IMI ranged from 35 to 97% of heifers (74.6% of quarters) in some dairy herds (Trinidad et al., 1990c). As a result, IMI in dairy heifers may damage the developing secretory tissues and reduce the milk production potential of the animals (Trinidad et al., 1990b).

Traditional programs for the control of bovine IMI or bovine mastitis have focused on reducing the incidence of new IMI and duration of existing IMI in mature dairy cows. Little attention has been made to the IMI status of the mammary glands of prepartum heifers, which were generally regarded as uninfected (Oliver and Sordillo, 1988; Malinowski *et al.*, 2003). As a result, increased concern is being focused on developing programs to control IMI in dairy heifers.

Antimicrobial therapy remains a primary tool for controlling IMI in lactating and dry cows (Oliver et al., 2003). Antimicrobial susceptibility tests provide important information to the veterinarians to select the most effective antimicrobial agent for treating IMI. Several studies have examined the antimicrobial susceptibility of pathogens isolated from IMI of mature dairy cows (Owens and Watts, 1988; Gentilini et al., 2002), but only limited information is the susceptibility available on of microorganisms isolated in heifers (Trinidad et al., 1990d).

The objective of this study was to determine the prevalence and antimicrobial susceptibilities of coagulase-negative Staphylococci isolated from mammary secretions in pregnant Holstein heifers.

Materials and Methods

This study was conducted on 229 pregnant Holstein heifers, belonging to a commercial dairy farm in Tehran province from April to November 2005. Duplicate samples of mammary secretions were collected aseptically 14 to 10 days prior to the expected calving date. All obtained samples were collected using standard procedures described by the National Mastitis Council (NMC, 1999). In summary, before sample collection, teats were dipped in a premilking teat disinfectant, cleaned thoroughly, and dried with individual disposable paper towels and were sanitized with swabs containing 70% ethyl alcohol. Samples were collected in sterile test tubes and were kept on ice at 4°C and then were sent to the laboratory within a maximum of one hr. All samples were frozen and then examined after 24 hrs following procedures as recommended by the National Mastitis Council (NMC, 1999). Mammary secretion

from each mammary gland was cultured onto one quadrant of a trypticase soy agar plate supplemented with 5% defibrinated sheep blood. Plates were incubated at 37°C, and bacterial growth was examined and recorded at 24-hr intervals for two days. Bacteria on primary culture medium were identified tentatively according to colony morphologic features. haemolvtic characteristics, Gram stain reaction, and **Isolates** identified catalase test. presumptively as Staphylococci were tested for coagulase production by the tube coagulase method. Finally, several routine biochemical examinations were conducted to detect and differentiate Staphylococci spp (Quinn et al., 1994). A quarter was considered infected during the prepartum period if the same pathogen was isolated from duplicate samples obtained 14 to 10 days before the expected calving date.

After bacterial isolation, all isolates of coagulase-negative Staphylococci were tested *in vitro* by Kirby-Bauer method for susceptibility to eight antimicrobial agents (Quinn *et al.*, 1994). In this study, oxytetracycline, gentamicin, streptomycin, tylosin, cloxacillin, enrofluxacin, trimethoprim+sulphamethoxazole and penicillin G antibiotic disks were used.

Results

Three quarters were non-functional and excluded from this study. Bacteria were found in 196 (21.5%) of 913 quarters and 105 heifers (45.9%). Pure culture of bacteria was obtained from all quarters; mixed infection (two species of bacteria) was not found in any samples. The most frequently encountered isolates were coagulasenegative Staphylococci (20.5%) from which *S. chromogenes* (16.3%) was commonly isolated (Table 1). In the present study, a mean of 0.83 quarter per each heifer was infected.

A total of 187 isolates of coagulasenegative Staphylococci were tested *in vitro* for susceptibility to eight antimicrobial agents. Susceptibility was detected in 180 (96.3%), 187 (100%), 163 (87.2%), 168 (89.8%), 183 (97.9%), 187 (100%), 174 (93%) and 164 (87.7%) isolates for oxytetracycline, gentamicin, streptomycin, tylosin, cloxacillin, enrofluxacin, trimethoprim+sulphamethoxazole and penicillin G, respectively. More than 93.8% of the isolates were susceptible to all antimicrobial agents tested (Table 2).

Table 1: The frequency of microorganisms isolated from quarter secretion samples in pregnant heifers

Species	No.	%
S. chromogenes	149	76.03
S. hyicus	12	6.12
S. kloosii	6	3.06
S. hominis	5	2.55
S. muscae	4	2.04
S. vitulus	4	2.04
S. aureus	3	1.53
S. caseolyticus	3	1.53
S. capitis	1	0.51
S. pasteuri	1	0.51
S. sacharolyticus	1	0.51
S. schleifer [*]	1	0.51
E. coli	1	0.51
Arcanobacterium pyogenes	3	1.53
Streptococcus spp.	2	1.02
Total	196	100

*Subspecies coagulens

Discussion

Heifers constitute the replacement stock for older cows and are generally expected to have good udder health. In recent years, however, inspection of the significance of IMI in heifers has increased and evidences suggested that IMIs in breeding age and pregnant heifers occur at a much higher rate than that previously thought. Many of these infections can persist for long periods of time, may be associated with elevated SCC, and may impair mammary development during gestation and affect milk production after calving (Oliver et al., 2005).

The results of microbial prevalence in heifer mammary gland secretions agree with those of the previous reports (Trinidad et al., 1990a; Matthews et al., 1992). In spite of a wide variation in the prevalence of contaminated heifers and quarters among various studies, a common denominator is the high proportion of IMI caused by coagulase-negative Staphylococci. The fact that S. chromogenes was the most bacterial species isolated before parturition (16.3%) also confirmed to the previous reports (Trinidad et al., 1990b; Pankey et al., 1991; Matthews et al., 1992; Aaerstrup and Jensen, 1997). The high prevalence of IMI caused by S. chromogenes might be due to its presence on teat skin of heifers (Aaerstrup and Jensen, 1997).

The susceptibility pattern of the isolates against various antimicrobial agents agreed with the findings by Trinidad et al. (1990d) 98.1% who reported that of 146 Staphylococcal isolates (primarily coagulase-negative species) from heifer mammary secretions were susceptible to several antibiotics (Trinidad et al., 1990d). Penicillin is among the most commonly used antibiotics for treatment of mastitis in lactating and dry cows. Overall. susceptibility of isolates to penicillin G in this study was 94.5% which is in agreement with Trinidad et al. (1990d) who reported that 92.3% of all isolates were susceptible to penicillin. Previous researches which were performed on mature cows, have reported 44.2 to 65% susceptibility of isolates to penicillin G. It must be noted that these conducted with isolates studies was

Table 2: Antibiotic susceptibilities of coagulase-negative Staphylococci isolated from mammary secretion samples (n = 187) of heifers to eight antimicrobial agents

Antibiotic	Coagulase-negative Staphylococci				
	S. chromogenes $(n = 149)$	<i>S. hyicus</i> (n = 12)	<i>S. kloosii</i> (n = 6)	S. hominis $(n = 5)$	Other Staphylococci $(n = 15)$
Cloxacillin	100	100	100	100	73.3
Enrofluxacin	100	100	100	100	100
Gentamicin	100	100	100	100	100
Oxytetracycline	100	100	50	100	73.3
Penicillin G	85.9	100	100	100	86.7
Streptomycin	85.9	100	100	100	80
Trimethoprim+Sulphamethoxazole	91.3	100	100	100	100
Tylosin	92.6	100	66.7	60	73.3

collected from herds with mastitis problems (Trinidad et al., 1990d; Owens and Watts, 1988), whereas results reported herein were based on isolates from primigravid heifers with no history of intramammary treatments. Thus, exposure to antimicrobial drugs may account for differences in susceptibilities between heifers and cows. In our study 100% Staphylococci isolated were susceptible to gentamicin and enrofluxacin. Trinidad et al. (1990d) reported 100% susceptibility to cephalothin, gentamicin, oxacillin, sulphamethoxazole+trimethoprim, and vancomycin for staphylococci isolated from heifer mammary secretion samples (Trinidad et al., 1990d). Owens and Watts (1988) reported similar susceptibility pattern of isolates from lactating cows to the same antibiotics with the exception of oxacillin (60.3%). Data of the present study were shown that S. kloosii was not completely susceptible to oxytetracycline (50%) and tylosin (66.7%). S. hominis was not also entirely susceptible to tylosin (60%). S. hyicus were 100% susceptible to all antibiotics and S. chromogenes and other Staphylococci were 100% susceptible to four and three antibiotics, respectively. Trinidad et al. (1990d) reported that S. aureus was 100% susceptible to all except two of the antibiotics tested-novobiocin (96.3%) and streptomycin (81.5%). S. chromogenes and S. hyicus were also found to be 100% susceptible to nine antibiotics, other Staphylococci were and 100% susceptible to eight antibiotics (Trinidad et al., 1990d). Owens and Watts (1988) reported 100% susceptibilities for S. aureus to the same antibiotics with the exceptions ampicillin, erythromycin, oxacillin, of penicillin and tetracycline.

In conclusion, results of this study suggest that coagulase-negative Staphylococci spp is the most prevalent microorganism isolated from intramammary secretions in pregnant heifers. It is susceptible to common antibiotics *in vitro*.

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