

Prevalence and antimicrobial susceptibilities of coagulase-negative Staphylococci isolated from mammary secretions in pregnant Holstein heifers

Ataee, O.^{1*}; Hovareshti, P.²; Bolourchi, M.²;
Niasari-Naslaji, A.² and Barin, A.²

¹Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University of Karadj, Karadj, Iran; ²Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

*Correspondence: O. Ataee, Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University of Karadj, Karadj, Iran. E-mail: ataee@kiauu.ac.ir

(Received 7 Mar 2006; revised version 28 Jun 2006; accepted 9 Aug 2006)

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, enrofloxacin, 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 available on the susceptibility 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, haemolytic characteristics, Gram stain reaction, and catalase test. Isolates identified 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, enrofloxacin, 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 coagulase-negative 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 coagulase-negative 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, enrofloxacin, 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.	%
<i>S. chromogenes</i>	149	76.03
<i>S. hyicus</i>	12	6.12
<i>S. kloosii</i>	6	3.06
<i>S. hominis</i>	5	2.55
<i>S. muscae</i>	4	2.04
<i>S. vitulus</i>	4	2.04
<i>S. aureus</i>	3	1.53
<i>S. caseolyticus</i>	3	1.53
<i>S. capitis</i>	1	0.51
<i>S. pasteurii</i>	1	0.51
<i>S. sacharolyticus</i>	1	0.51
<i>S. schleifer</i> *	1	0.51
<i>E. coli</i>	1	0.51
<i>Arcanobacterium pyogenes</i>	3	1.53
<i>Streptococcus</i> 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) who reported that 98.1% 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 studies was conducted with isolates

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				
	<i>S. chromogenes</i> (n = 149)	<i>S. hyicus</i> (n = 12)	<i>S. kloosii</i> (n = 6)	<i>S. hominis</i> (n = 5)	Other Staphylococci (n = 15)
Cloxacillin	100	100	100	100	73.3
Enrofloxacin	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 Staphylococci isolated were 100% susceptible to gentamicin and enrofloxacin. 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, and other Staphylococci were 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 of ampicillin, erythromycin, oxacillin, 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*.

References

- Aaerstrup, FM and Jensen, NE (1997). Prevalence and duration of intramammary infection in Danish heifers during the peripartum period. *J. Dairy Sci.*, 80: 307-312.
- Fox, LK; Chester, ST; Hallberg, JW; Nickerson, SC; Pankey, JW and Weaver, LD (1995). Survey of intramammary infections in dairy heifers at breeding age and first parturition. *J. Dairy Sci.*, 78: 1619-1628.
- Gentilini, E; Denamiel, G; Betancor, A; Rebuelto, M; Rodriguez Fermepin, M and De Torres, RA (2002). Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine mastitis in Argentina. *J. Dairy Sci.*, 85: 1913-1917.
- Jonsson, P; Olsson, SO; Olofson, AS; Faith, C; Holmeberg, O and Funke, H (1991). Bacteriological investigations of clinical mastitis in heifers in Sweden. *J. Dairy Res.*, 58: 179-185.
- Malinowski, E; Klossowska, A; Kaczmarowski, M and Kuzma, K (2003). Prevalence of intramammary infections in pregnant heifers. *Bull. Vet. Inst. Pulawy.* 47: 165-170.
- Matthews, KR; Harmon, RJ and Langlois, BE (1992). Prevalence of *Staphylococcus* species during the periparturient period in primiparous and multiparous cows. *J. Dairy Sci.*, 75: 1835-1839.
- Myllys, V (1995). Staphylococci in heifer mastitis before and after parturition. *J. Dairy Res.*, 62: 51-60.
- Nickerson, SC; Owen, WE and Boddie, RL (1995). Mastitis in dairy heifers: initial studies on prevalence and control. *J. Dairy Sci.*, 78: 1607-1618.
- NMC, (1999). *Laboratory handbook on bovine mastitis*. Rev. Ed. Natl. Mastitis Council, Madison, WI, USA. PP: 71-82.
- Oliver, SP (1987). Intramammary infections in heifers at parturition and during early lactation in a herd with a high prevalence of environmental mastitis. *Tenn. Farm Home Sci.*, 143: 18-22.
- Oliver, SP (1988). Frequency of isolation of environmental mastitis causing pathogens and incidence of new intramammary infection during the nonlactating period. *Am. J. Vet. Res.*, 48: 1789-1793.
- Oliver, SP; Gillespie, BE; Headrick, SJ; Lewis, MJ and Dowlen, HH (2005). Prevalence, risk factors, and strategies for controlling mastitis in heifers during the periparturient period. *Intern. J. Appl. Res. Vet. Med.*, 3: 150-161.
- Oliver, SP; Lewis, MJ; Gillespie, BE; Dowlen, HH; Jaenicke, EC and Roberts, RK (2003). Milk production, milk quality and economic benefit associated with prepartum antibiotic treatment of heifers. *J. Dairy Sci.*, 86: 1187-1193.
- Oliver, SP and Mitchell, BA (1983). Intramammary infections in primigravid heifers near parturition. *J. Dairy Sci.*, 66:

- 1180-1183.
- Oliver, SP and Sordillo, LM (1988). Udder health in periparturient period. *J. Dairy Sci.*, 71: 2584-2606.
- Owens, WE and Watts, JL (1988). Antimicrobial susceptibility and β -lactamase testing of staphylococci isolated from dairy herds. *J. Dairy Sci.*, 71: 1934-1941.
- Pankey, JW; Dreschsler, PA and Wildman, EE (1991). Mastitis prevalence in primigravid heifers at parturition. *J. Dairy Sci.*, 74: 1550-1552.
- Quinn, PJ; Carter, ME; Mrkey, B and Carter, GR (1994). *Clinical veterinary microbiology*. 1st. Edn., London, Wolf Publishing. PP: 95-102.
- Smith, KL; Hogan, JS; Todhunter, DA; Weiss, WP and Schoenberger, PS (1994). Intramammary infection and clinical mastitis in heifers at calving and dynamics over a 14 year period in a dairy herd. *J. Dairy Sci.*, (Suppl. 1), 77: 197-215.
- Trinidad, P; Nickerson, SC and Alley, TK (1990a). Prevalence of intramammary infection and teat canal colonization in unbred and primigravid dairy heifers. *J. Dairy Sci.*, 73: 107-114.
- Trinidad, P; Nickerson, SC and Adkinson, RW (1990b). Histopathology of staphylococcal mastitis in unbred heifers. *J. Dairy Sci.*, 73: 639-647.
- Trinidad, P; Nickerson, SC; Alley, TK and Adkinson, RW (1990c). Efficacy of intramammary treatment in unbred and primigravid dairy heifers. *J. Am. Vet. Med. Assoc.*, 197: 465-470.
- Trinidad, P; Nickerson, SC and Luther, DG (1990d). Antimicrobial susceptibilities of staphylococcal species isolated from mammary glands of unbred and primigravid dairy heifers. *J. Dairy Sci.*, 73: 357-362.