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Isolation of *Streptococci* from Milk Samples of Normal, Acute and Subclinical Mastitis Cows and Determination of Their Antibiotic Susceptibility Patterns

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Abstract: *Streptococci* are frequently isolated from bovine mastitis in dairy cows with only limited information available on the antimicrobial susceptibility of these organisms. A total of 42 *Streptococci* isolated from 148 milk samples of normal, sub acute and acute bovine mastitis cases. Overall, 35% of the strains tested were *Streptococcus dysgalactiae*, *Streptococcus agalactiae* 26%, *Streptococcus uberis* 18 and 4% were *Enterococcus* sp. Differences between the number of isolations in acute and sub acute groups were statistically significant, ($p<0.5$). The antimicrobial susceptibility for these organisms was determined for the following antimicrobial agents: cephalexine, penicillin, clindamycin, cloxaciline, gentamicin, streptomycin, amoxicillin, tetracycline, kanamycin, oxytetracycline, ampicillin, chloramphenicol and erythromycin. *S. agalactiae*, *S. dysgalactiae*, *S. uberis* and *Enterococci* demonstrated high level of resistance against streptomycin, penicillin and cloxaciline. Low level of sensitivity to other tested antimicrobials was demonstrated.

Key words: Dairy cow, milk sample, *Streptococci*, antibiogram, Iran

INTRODUCTION

Mastitis continues to be the most frequent and expensive disease of milking cows. About 150 species of microorganisms were found as the etiological agents of mastitis. Bacteria involved in bovine mastitis are broadly classified as either contagious or environmental pathogens based on their epidemiological association with the disease (Rossitto *et al*., 2002). The main pathogens of environmental group are the gram-negative enteric bacilli (*Escherichia coli*, *Klebsiella* sp., etc.), *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Enterococcus* sp. Rajala-Schultz *et al*. (2004). Exposure to mastitis pathogens can originate from several sources, including the environment of the cow and teat skin flora. Makovec and Ruegg (2003). Environmental mastitis pathogens are now emerging as the most frequent cause of mastitis in many herds, particularly well-managed herds, Giovannini *et al*. (2000). As a matter of practical management on many dairies, antimicrobial therapy of mastitis is initiated prior to microbiological culturing (O,Brien, 2002).

The treatment strategies for *Streptococci* are often chosen with the assumption that the antimicrobial susceptibility of this group is homogenous. Thus, information on the antimicrobial susceptibility of these organisms would guide veterinarians and dairy farmers in the most prudent selection of an antibiotic for therapy of mastitis. The purpose of this study was to compare isolated *Streptococci* from normal, subclinical and clinical mastitis cow milk samples and determination of their antimicrobial susceptibility patterns in the areas of west centre of Iran.

MATERIALS AND METHODS

The study conducted in cow dairies of Shahrekord area in west central Iran and covered 15 months from March 2006. In the present study 50, 50 and 48 milk samples were obtained from normal, sub acute and acute mastitis cows, respectively. The samples were randomly collected from cows reared under conventional and confined housing. Mastitis was diagnosed on the basis of California Mastitis Test (CMT), clinical examination of the udder and macroscopic evaluation of secretion. Definition of normal, sub acute and acute mastitis cows were based on Esron *et al*. (2005).

Milk samples were aseptically collected from each quarter, primary isolates were from milk samples that were streaked onto 5% sheep blood agar plates and incubated

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at 37°C for 18 to 24 h. Microscopic and macroscopic morphology, biochemical and CAMP test were examinations that used for identification of *Streptococci*, Quinn *et al*. (2002).

The percentage of isolations in normal, acute and sub acute groups were compared using Chi-squared test. Isolates were then cultured on 5% sheep blood agar to establish a pure culture and then frozen in Triptose Soy Broth (TSB) containing 15% glycerol at -70°C for future experiments.

Susceptibility to antimicrobial agents was determined for isolated bacteria by the disk diffusion method on Mueller-Hinton agar containing 5% sheep blood following the National Committee for Clinical Laboratory Standards guidelines (NCCLS, 2002). The selected antibiotics for antibiogram were cephalexine, penicillin, clindamycin, cloxaciline, gentamicin, streptomycin, amoxicillin, tetracycline, kanamycin, oxytetracycline, ampicillin, chloramphenicol and erythromycin that were more common in treatment of regional bovine mastitis cases.

RESULTS AND DISCUSSION

A total of 42 *Streptococci* isolates were identified from 148 milk samples cultured (Table 1).

The isolated Streptococci from sub acute, acute and normal groups formed 52.0, 31.25 and 2.0% of all isolations, respectively. Differences between three groups are statistically significant, ($p<0.05$). In sub acute group *S. agalactiae*, *S. uberis* and *S. dysgalactiae* isolates representing 24.0, 18 and 6%, respectively while in acute group the numbers were 2, 0.0 and 14%. In milk samples of normal group only from one sample we have positive culture of *Streptococci*.

All isolates were tested for bacterial resistance. *S. dysgalactiae* strains were resistant to Clindamycin (58.52%), Penicillin, Cloxaciline and Streptomycin (52.94%). *S. agalactiae* showed high resistance rates to Cloxaciline (92.30%), Amoxicillin (76.92%), penicillin and erythromycin (69.23%), clindamycin, streptomycin and ampicillin (61.53%). A high resistance rate was observed among *S. uberis* isolates, in particular, against penicillin (100%), streptomycin and ampicillin (88.88%) and gentamicin (77.77%). Resistance rates for *Enterococcus* sp. to different antimicrobials varied between 33.33 and 66.66% (Table 2).

The isolated Streptococci from sub acute, acute and normal groups formed 52.0, 31.25 and 2.0% of all isolations, respectively. Differences between three groups are statistically significant, ($p<0.05$). We may conclude that as a genus *Streptococci* are more important in bovine subclinical mastitis than acute cases. In clinical mastitis cases the isolated species was principally *S. dysgalactiae* (29.16%). As it was expected, this species is one of the main causal agents of bovine clinical mastitis Quinn *et al.* (2002). The percentage of other *Streptococcus* isolated was: *S. agalactiae* (2.1%), with 67.4% of cultures being negative for *Streptococci*

Table 1: *Streptococcus* sp. isolated from normal, sub acute and acute mastitis cow milk samples

No. and percentage of positives Isolated streptococci	Normal (%)	Sub acute (%)	Acute (%)
S. agalaciae	0 (0.0)	12 (24.0)	1 (2.10)
S. dysgalactiae	0 (0.0)	3 (6.0)	14 (29.16)
S. uberis	0 (0.0)	9 (18.0)	0 (0.00)
Enterococcus sp.	1 (2.0)	2 (4.0)	0 (0.00)
Total	1 (2.0)	26 (52.0)	15 (31.25)

Table 2: Antibiotic susceptibility patterns of *Streptococcus* sp. isolated from normal and mastitis cow milk samples

	S. agalactiae		*S. dysgalactiae*		*S. uberis*		*Enterococcus* sp.	
Streptococci antibiotics	S (%)	NS (%)	S (%)	NS (%)	S (%)	NS (%)	S (%)	NS (%)
Cephalexine	9 (69.23)	4 (30.76)	10 (58.82)	7 (41.17)	6 (66.66)	3 (33.33)	2 (66.66)	1 (33.33)
Penicillin	4 (30.76)	9 (69.23)	8 (47.05)	9 (52.94)	0 (0.00)	9 (100.00)	1 (33.33)	2 (66.66)
Clindamycin	5 (38.46)	8 (61.53)	7 (41.17)	10 (58.82)	4 (44.44)	5 (55.55)	2 (66.66)	1 (33.33)
Cloxaciline	1 (7.69)	12 (92.30)	8 (47.05)	9 (52.94)	2 (22.22)	7 (77.77)	0 (0.00)	3 (100.00)
Gentamicin	10 (76.92)	3 (23.07)	10 (58.82)	7 (41.17)	4 (44.44)	5 (55.55)	1 (33.33)	2 (66.66)
Streptomycin	5 (38.46)	8 (61.53)	8 (47.05)	9 (52.94)	1 (11.11)	8 (88.88)	0 (0.00)	3 (100.00)
Amoxicillin	3 (23.07)	10(76.92)	11 (64.70)	6 (35.29)	3 (33.33)	6 (66.66)	2 (66.66)	1 (33.33)
Tetracycline	8 (61.53)	5 (38.46)	11 (64.70)	6 (35.29)	8 (88.88)	1 (11.11)	1 (33.33)	2 (66.66)
Kanamycin	9 (69.23)	4 (30.76)	11 (64.70)	6 (35.29)	6 (66.66)	3 (33.33)	0 (0.00)	3 (100.00)
Oxytetracycline	10 (76.92)	3 (23.07)	12 (70.58)	5 (29.41)	7 (77.77)	2 (22.22)	1 (33.33)	2 (66.66)
Ampicillin	5 (38.46)	8 (61.53)	12 (70.58)	5 (29.41)	1 (11.11)	8 (88.88)	2 (66.66)	1 (33.33)
Chloramphenicol	10 (76.92)	3 (23.07)	12 (70.58)	5 (29.41)	8 (88.88)	1 (11.11)	2 (66.66)	1 (33.33)
Erythromycin	4 (30.76)	9 (69.23)	8 (47.05)	9 (52.29)	3 (33.33)	6 (66.66)	1 (33.33)	2 (66.66)

S = Sensitive, NS = Non Sensitive

(Table 1). These results were substantially different with respect to bacteriological findings of milk samples of clinical mastitis in Sweden, Hallén-Sandgren (2000) where *S. dysgalactiae* (16%) was the most prevalent isolated *Streptococci*, *S. uberis* (18%) and slightly less than 1% of *S. agalactiae*. Considering *S. agalactiae*, *S. uberis* and *Enterococcus* sp. isolation from clinical mastitis samples, present results are in agree with the results of bacteriological finding in Uruguay, Gianneechini *et al.* (2002). Moreover in subclinical cases of our study also the isolated *Streptococci* are in agree with the last results. It is known that control measures for mastitis such as teat dipping and dry cow therapy are adequate to control contagious pathogens (*S. agalactiae*) but are not effective against environmental pathogens. However, dry cow therapy may be of some value in controlling environmental *Streptococci*. This should serve as a reason for explaining the difference of prevalence among contagious and environmental udder pathogens in clinical cases. In Iran (and likely in Uruguay), these measures have been discontinued, while in Sweden and other Nordic countries they are included in control programmes.

Animal management systems may be another reason, Goldberg *et al.* (1992) reported a lower incidence of environmental pathogens on teat ends in pastured cattle than in confined cattle. In Iran particularly in our area the cows are on confined condition during all year.

The antimicrobial susceptibility data for strains of *S. agalactiae*, *S. dysgalactiae*, *S. uberis* and *Enterococci*, are shown in Table 2. Results of this study demonstrate dramatic insensitivity patterns for the various tested *Streptococci*. For example, the Streptomycin, Penicillin and Cloxaciline are agents that are used to treat mastitis cases and other diseases in cattle None of these three agents is considered a primary agent for streptococcal disease due to their limited activity against these organisms. This is demonstrated by the high percentages of non sensitive isolates exhibited by these agents against the strains of *S. dysgalactiae*, *S. uberis* and *Enterococci* tested in the present study. Interestingly for these three antimicrobials the same is correct for *S. agalactiae*, an important non environmental streptococcal causes of bovine subclinical mastitis.

Antimicrobials that are tested in this study are used in human medicine also, collectively they demonstrated a low sensitivity and relatively high resistance for tested *Streptococci* that is extremely worrisome. More restrictive policies on the use of antibiotics in animals may result in an improvement of the current situation.

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