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Antimicrobial Susceptibility of Environmental Bovine Mastitis Pathogens in West Central Iran

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Abstract: The purpose of this study was to describe antimicrobial susceptibility of environmental mastitis pathogens isolated from dairy herds of Chahar Mahal province in west central Iran. Out of the 620 milk samples collected from the four districts, 180 were CMT (California mastitis test) positive that cultured and yield, 7 (3.88%) coagulase negative *Staphylococci* (CNS), 15 (8.33%) *Streptococci* other than agalactiae and 17 (9.44%) *E. coli*. CNS resistance to penicillin was 14.28% but for streptomycin, Oxytetracycline and Colistin, were 28.57%. *Non agalactiae Streptococci* resistance to Oxytetracycline and Kanamycin were 20 and 13.33%, respectively. *E. coli* resistance to penicillin, oxytetracycline, streptomycin, erythromycin and Colistin were 88.23, 82.35, 76.47, 76.47 and 52.94%, respectively.

Key words: Antibiotic, resistance, cow milk, Iran

INTRODUCTION

In recent years, antimicrobial resistance has been a growing concern worldwide. Bovine mastitis is a frequent cause of economic loss in dairy herds and is also the single most common reason for antimicrobial use in them. 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).

Some studies showing an increasing trend over time in resistance among mastitis pathogens (Hirvela-Koski *et al*., 1998). Exposure to mastitis pathogens can originate from several sources, including the environment of the cow and teat skin flora (Makovec and Ruegg, 2003).

Probably the most important change in mastitis epidemiology over the past decade has been the rise in importance of environmental pathogens, primarily coliforms and streptococci other than agalactiae. The objective of this study was to describe susceptibility patterns of environmental mastitis pathogens isolated from dairy herds of Chahar Mahal province in west central Iran.

MATERIALS AND METHODS

Chahar Mahal region is a semi-arid area and receives an average annual Rain fall of 400 mm. The study areas were four districts (namely Shahrekord, Broujen, Lordegan and Farsan). A two-stage random selection procedure was adopted where study herds were randomly selected from a sampling frame comprising all herds in each district in 2004.

On average, herds selected to participate in the study constituted about 10% of all herds in the study area. All lactating animals on the day of visit were listed from which 10-15 cows were randomly selected for examination and sample collection.

During farm visits, a structured questionnaire was used to collect animal-and herd-level information on knowledge on clinical and sub clinical mastitis and any antibiotic treatments related to mastitis control. Unfortunately history of past mastitis's was not available in the herd files.

All cows (155) selected in the four districts were at least one month post calving and clinically examined for evidence of clinical mastitis as manifested by visible changes in milk and in the udder.

The examination was complemented by testing milk from lactating quarters (n = 620) for sub clinical mastitis using California Mastitis Test (CMT). The results were classified as either negative, 1 +, 2 + or 3 + depending on the amount of gel formed (Esron *et al*., 2005).

Positive CMT Milk samples were submitted for bacteriological examination and inoculated onto blood (BA) and MacConkey (MC) agars and incubated at 37°C.

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At 24 h, the plates were examined for bacterial growth. Bacterial isolates were characterized by macro- and micro-morphology, gram staining and biochemical tests using different sugars (Quinn *et al*., 1994).

Other tests including motility, coagulase, catalase and oxidase reactions were carried out to assist identification of different bacterial isolates.

Susceptibility to antimicrobial agents was determined for isolated bacteria by the disk diffusion method on Mueller-Hinton agar following the National Committee for Clinical Laboratory Standards guidelines (NCCLS, 2002). The selected antibiotics for antibiogram were Lincospectine, Penicillin, Colistin, Oxytetracycline, Streptomycin, Enrofloxacin, Chloramphenicole, Gentamicin, Erythromycin, Furazolidone and Kanamycin that were more common in treatment of regional bovine mastitis cases.

RESULTS

The study revealed no significant differences in the average of the herd sizes in the four districts. No clinical mastitis cases were diagnosed on days of farm visits.

Out of the 620 milk samples collected from the four districts (Table 1), 180 were CMT positive that cultured and yield, 7 (3.88%) coagulase negative *Staphylococci* (CNS), 15 (8.33%) *Streptococci* other than agalactiae and 17 (9.44%) *E. coli*. Also in 122 cases contagious pathogens (including coagulase positive *Staphylococcus aureus* and *Streptococcus agalactiae*) were isolated. The proportions of pathogens recovered were comparable in all four districts and also two seasons.

CNS resistance to penicillin was 14.28% while that for Streptomycin, Oxytetracycline and Colistin, were 28.57% (Table 2).

Non agalactiae Streptococci resistance to Oxytetracycline and Kanamycin were 20 and 13.33%, respectively while that for Streptomycin, Erythromycin, Gentamicin and Colistin was 6.66%.

E. coli resistance to Penicillin, Oxytetracycline, Streptomycin and Erythromycin and Colistin were 88.23, 82.35, 76.47 and 52.94%, respectively.

However some of our isolates have not criteria for inhibition zones of susceptible or resistant and were considered as medium.

Table 1: Distribution of herds, samples and CMT positives in four districts of Chahar Mahal province

Districts	No. of visited farms	No. of samples	No. of positives
Shahrekord	6	356	99 (27.8)
Farsan	2	116	35 (30.2)
Broujen	2	112	27 (24.1)
Lordegan	1	36	19 (52.8)
Total	11	620	180 (29.0)

Values in parenthesis shows percentage

Table 2: Antibiotic susceptibilities of 7, 15 and 17 isolates of CNS, *Streptococci* and *E. coli*, respectively

Bacteria / Antibiotics	No. of susceptible: CNS	*Streptococci*	*E. coli*
Lincospectine	1 (14.28)	15 (100)	17 (100)
Penicillin	4 (57.14.3)	4 (26.66)	2 (11.76)
Colistin	4 (57.14)	8 (53.33)	5 (29.14)
Oxytetracycline	4 (57.14)	3 (20)	2 (11.76)
Streptomycin	4 (57.14)	12 (80)	2 (11.76)
Enrofloxacin	7 (100)	15 (100)	17 (100)
Chloramphenicole	7 (100)	14 (93.33)	7 (100)
Gentamicin	6 (85.71)	13 (86.66)	5 (29.41)
Erythromycin	4 (57.14)	9 (60)	2 (11.76)
Furazolidone	4 (57.14)	10 (66.66)	17 (100)
Kanamycin	4 (57.14)	12 (80)	5 (29.41)

Values in parenthesis shows percentage

DISCUSSION

A number of factors have been reported to influence the occurrence of mastitis in dairy cattle. They include managerial factors particularly those related to poor milking hygiene, environmental population of mastitis pathogens, predisposing factors such as teat injuries, sores and incomplete emptying of mammary gland quarters (Jarret *et al*., 1981; Karimuribo *et al*., 2002).

In this study *E. coli* and Coagulase-negative *Staphylococci* were the most frequently isolated bacteria in agreement with other reports (Myllys *et al*., 1998; Watts *et al*., 1995).

Escherichia coli is an environmental pathogen and *E. coli* mastitis is a major disease in cows. Certain strains of *E. coli* are capable of epithelial adherence and invasion leading to chronic IMI (Intramamary infection) and recurrent clinical mastitis (Dopfer *et al*., 1999).

Oxytetracycline, Streptomycin, Erythromycin and Colistin resistance of *E. coli* reported here (82.35, 76.47, 76.47 and 52.94%, respectively) was higher than 11.5% reported by Myllys *et al*. (1998).

The role of CNS as a cause of bovine mastitis is not completely clear. CNS infections are associated with damage to milk secretary tissue of the mammary gland by increased connective tissue stroma (Trinidad *et al*., 1990).

Susceptibility of CNS isolated from bovine IMI to selected antimicrobial agents has been previously reported (Rajala-Schultz *et al*., 2004). Penicillin predicts susceptibility to other β-lactamase-sensitive antimicrobial agents, e.g., ampicillin. CNS β-lactamase producers will be resistant to penicillins and some cephalosporins.

A number of international reports have shown that Streptococci genus is highly sensitive to bta-lactam and some other groups of antibiotics (Myllys *et al*., 1998; Sobiraj *et al*., 1997). This was the case in this study: A high proportion of *Streptococci* species were sensitive to tested antibiotics (Table 2).

Although little information is available about resistance of *Streptococci* to enrofloxacin it is important to note that Myllys *et al.* (1998) found 7% of *Streptococcus* strains isolated from bovine mastitis in Finland were resistant. This result was not remarkably different from our record of 0.0%. Penicillin-dihydrostreptomycin combination are normally used in these herds as dry cow therapy. Unfortunately, we did not have the individual animal treatment records, but in general, the observed resistance patterns agree with the antibiotic usage patterns. The results in the present study clearly show that our country is at risk for the world hazard of bacterial resistance.

REFERENCES

Dopfer, D., H.W. Barkema, T.J.G.M. Lam, Y.H. Schukken and W. Caastra, 1999. Recurrent clinical mastitis caused by *E. coli* in dairy cows. J. Dairy Sci., 82: 80-85.

Esron, D.K., J.K. Lughano, H.M. Robinson, M.K. Angolwisye, C. Sindato and M.K. Dominic, 2005. Studies on mastitis, milk quality and health risks associated with consumption of milk from pastoral herds in Dodoma and Morogoro regions, Tanzania. J. Vet. Sci., 6: 213-221.

Hirvela-Koski, V., T. Honkanen-Buzalski, J. Juntilla, L. Kulkas, O. Myllykangas, M. Niskanen, H. Saloniemi, M. Sandholm and T. Saranppa, 1998. Bovine mastitis in Finland in 1998 and 1995-Changes in prevalence and antimicrobial resistance. Acta Vet. Scand., 39: 119-126.

Jarret, J.A., 1981. Mastitis in dairy cows. Vet Clin North Am. Large Anim. Prac., 3: 447-454.

Karimuribo, E.D., 2002. Epidemiological studies of mastitis in small holder dairy farms in Tanzania. Ph.D Thesis, University of Reading, Reading.

Makovec, J.A. and P.L. Ruegg, 2003. Results of milk samples submitted for microbiological examination in wisconsin from 1994 to 2001. J. Dairy Sci., 86: 3466-3472.

National Committee for Clinical Laboratory Standards (NCCLS), 2002. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved Standard. NCCLS Document M31-A2, Wayne, PA.

Quinn, P.J., M.E. Carter, B.K. Markey and G.R. Carter, 1994. Clinical Vet. Microbiol. Mosby, London, pp: 22-91.

Rajala-Schultz, P.J., K.L. Smith, J.S. Hogan and B.C. Love, 2004. Antimicrobial susceptibility of mastitis pathogens from first lactation and older cows. Vet. Microbiol., 102: 33-42.

Rossitto, P.V., L. Ruiz, Y. Kikuchi, K. Glenn, K. Luiz, J.L. Watts and J.S. Cullor, 2002. Antibiotic susceptibility patterns for environmental streptococci isolated from bovine mastitis in central *California dairies*. J. Dairy Sci., 85: 132-138.

Sobiraj, A., A. Kron, U. Schollmeyer and K. Failing, 1997. Federal investigations on the distribution and *in vitro* resistance of udder pathogenic bacteria in the milk of cows with subclinical mastitis. Tierärztl Prax, 25: 108-115.

Trinidad, P., S.C. Nickerson and R.W. Adkinson, 1990. Histopathology of *Staphylococcal mastitis* in unbred dairy heifers. J. Dairy Sci., 73: 639-647.

Watts, J.L., S.A. Salmon, R.J. Yancey, Jr.S.C. Nickerson, L.J. Weaver, C. Holmberg, J.W. Pankey and L.K. Fox, 1995. Antimicrobial susceptibility of micro-organisms isolated from the mammary glands of dairy heifers. J. Dairy Sci., 78: 1637-1648.