Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

Effect of Infusions of Non-Antibiotic Antibacterials Alone and in Combination with Cephradine on Milk Yield of Buffaloes Affected with Clinical Mastitis

M. Yousaf*, G. Muhammad[1], M. Z. Khan[2] and S. U. Rahman[3]

Department of Livestock & Dairy Development Bunir, NWFP; Department of Clinical Medicine & Surgery; Department of Veterinary Pathology; Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan
*Corresponding author: dr.myousaf@yahoo.com

ARTICLE HISTORY

Received: June 03, 2009
Revised: June 06, 2009
Accepted: July 06, 2009

Key words:
Non-antibiotic antibacterials
Milk yield
Mastitis
Dairy buffalo

ABSTRACT

The objective of the present study was to evaluate the effect of four non-antibiotic antibacterials alone or in combination with cephradine in buffaloes on milk yield of mastitis affected quarters. For this purpose, 270 clinically mastitic quarters were grouped in randomized pattern. Non-antibiotic antibacterials viz., 2.5% chlorpromazine (2 ml), 4% lidocaine (10 ml), 10% povidone-iodine (10 ml) and 99.5% dimethylsulphoxide (20 ml) alone and in combination with first generation cephalosporin (cephradine 500 mg) were instilled into clinically mastitic quarters daily for five days. The group administered cephradine alone served as control. Mean milk yield (L/quarter per day) was recorded before administration of treatment and over a period of 4 weeks post initiation of treatment. Among the 4 non-antibiotic antibacterials tested alone, chlorpromazine (CPZ) showed significantly higher ($P<0.05$) recuperative effect on the milk yield of clinically mastitic quarters of dairy buffaloes. However, dimethylsulphoxide (DMSO) when infused alone, further aggravated ($P<0.05$) the milk yield loss, indicating negative effect on milk yield improvement. Adjuncting cephradine with each of the non-antibiotic antibacterials, the lidocaine-cephradine group showed the highest effect ($p<0.05$) on net recovery of milk yield on day 28 post initiation of treatment. It was concluded that that CPZ can be used in clinical mastitis in buffaloes as a low cost alternative to expensive branded antibiotics. Further, the use of lidocaine with cepheradnie was superior to all other combination regimens in milk yield recovery.

To cite this article: Yousaf M, G Muhammad, MZ Khan and SU Rahman, 2010. Effect of infusions of non-antibiotic antibacterials alone and in combination with Cephradine on milk yield of buffaloes affected with clinical mastitis. Pakistan Vet J, 30(1): 39-43.

INTRODUCTION

Mastitis is the most common and economically the most important disease of the dairy industry throughout the world (Sharif and Muhammad 2009). Bubaline mastitis is the disease of milk producing organ of dairy buffalo (*Bubalus bubalis*) which is also called the ‘black gold’ of South Asia, where 95% of the buffalo milk is produced (Javaid *et al*., 2009). Buffalo is also recognized as the world second most important milk producing species (McDowell *et al.*, 1995; Bhatti *et al*., 2009). Mastitis is the single most common reason for antibiotics use in lactating dairy animals (Erskine *et al*., 2004). However, the poor treatment response of mastitis to antibiotics therapy is a major area of concern for dairy farmers, veterinarians and mastitis researchers. The use of antibiotics for mastitis treatment is attendant with the following important problems: i) Response to antibiotic therapy, in particular in quarters infected with *Staphylococcus aureus*, is generally very poor, and bacteriological cure rates in clinical *S. aureus* mastitis vary from 9.7 to 52% (Sole *et al.*, 1994; Sole *et al.*, 2000), ii) Antibacterial therapy of mastitis has been incriminated as a catalyst for developing resistance in pathogenic bacteria both in treated and healthy individuals within a herd (Berghash *et al.*, 1983; Griggs *et al.*, 1994; Teuber, 2001) and iii) The use of antibiotics for mastitis treatment is one of the most important causes of violative antibiotic residues in milk and meat of treated animals (Erskine, 1996).

Thus, there is a pressing need to try some alternative compounds endowed with antibacterial properties to overcome these problems. Recently, a variety of compounds commonly employed in the treatment of pathological conditions of non-infectious etiology have been shown to modify cell permeability and to exhibit broad-spectrum antimicrobial activity *in vitro* against bacteria and other micro-organisms (Cederiund and Mardh, 1993; Martin *et al.*, 2008). Such compounds have been given the name 'non-antibiotics' (Kristiansen and Amaral, 1997). In addition, these compounds have been found to enhance the *in vitro* activity of certain antibiotics against specific bacteria (Kristiansen, 1990), to render *in vitro* antibiotic-resistant bacteria susceptible to previously ineffective drugs (Kristiansen *et al.*, 2007; Martins *et al.*, 2008) and to exhibit strong *in vitro* antimycobacterial activity against clinical strains resistant to one or more conventional antibiotics (Kristiansen, 1990; Williams, 1995; Rodgrigues *et al.*, 2008). These compounds, primarily phenothiazines, thioxanthenes and other agents with affinities for cellular transport systems, are characterized by their effects on the plasma membrane of eukaryotic cells (Martin *et al.* 2008) and have been termed membrane stabilizers (Kristiansen, 1990). Martin *et al.* (2008) have reviewed the potential role of non-antibiotics (helper compounds) in the treatment of multidrug-resistant gram-negative infections.

However, these compounds have not been evaluated in the treatment of mastitis. The objective of the present preliminary study was to determine the effect of infusions of 4 non-antibiotic antibacterials (chlorpromazine, lidocaine, povidone-iodine and dimethylsulphoxide) with and without cephradine (a first generation cephalosporin) on milk yield improvement in mastitic quarters in dairy buffaloes after treatment with these compounds.

MATERIALS AND METHODS

A total of 270 clinically mastitic quarters of 249 Nili-Ravi lactating dairy buffaloes (*Bubalus bubalis*) were selected. Foremilk samples from the affected quarters were collected for bacteriological examination before initiation of treatment and then on day 28 post initiation of treatment (National Mastitis Council, Inc. 1990). Principles of the design of clinical trials with special reference to mastitis therapy, as described by International Dairy Federation (Thorburn, 1990), were followed for selecting trial quarters and their allocation to different treatments and control groups by randomization. Animals previously treated for mastitis during the current lactation were not included in the panel of experimental subjects. Similarly, only those quarters were selected which had contralateral normal quarters.

Animals selected were from buffaloes managed at the Livestock Experimental Station, University of Agriculture, Faisalabad and six private buffalo dairy farms. All experimental buffaloes were managed in tie-stall and loose housing system during the experimental period. These buffaloes received a diet of concentrate mixture and green fodder. In a cut-and-carry feeding system, chopped green fodder plus chaffed wheat straw were fed. Standard mastitis control practices (e.g. post milking antiseptic teat dipping, dry period antibiotic therapy, segregation or culling of mastitic animals) were not in practice at any of the farms.

The milk yield of the quarters affected with mastitis was subtracted from the milk yield of contralateral normal quarters before infusion of non-antibiotic antibacterials into mastitic quarters. Similarly, the milk yield of mastitis affected quarters treated with non-antibiotics was subtracted from the milk yield of opposite normal quarter. Usually, treated quarters do not regain their milk production in a week or two. So, a period of 28 days after the commencement of treatment was adopted for milk yield record to compare the mean yield of different groups.

The present study involved two experiments. The experiment 1 aimed at evaluation of non-antibiotic antibacterials alone in the treatment of bubaline clinical mastitis. For this purpose, 30 mastitic quarters were treated by intramammary route with each of the 4 non-antibiotic antibacterials (Table 1), using human intravenous catheter no. 22 (Vasocan Braunule™) attached to 50 ml plastic syringe. Only 2-3 mm anterior tip of the catheter was introduced into the teat (partial insertion) for infusion.

Immediately before treatment, mean milk yields of the mastitis quarters as well as opposite normal quarters were recorded. Similarly, at day 28 post initiation of treatment, mean milk yield of treated quarters and contralateral normal quarters were recorded.

Table 1: Evaluation of non-antibiotic antibacterials alone in the treatment of bubaline clinical mastitis

Non-antibiotic antibacterials	Product and manufacturer	Volume and amount infused into each quarter per day	No. of quarters infused	Duration of treatment (days)
Chlropromazine HCl (CPZ)	Inj. Largactil™, Aventis Pharma, Pakistan	2 ml (50mg) + 38 ml normal saline	30	5
Lidocaine HCl (Lid)	Xylocaine™ (4%) Barrett Hodgson, Pakistan	10 ml + 30 ml normal saline	30	5
Povidone-iodine (PI)	Pyodine™ solution (10%), Brookes Pharmaceutical Lab., Pakistan	10 ml + 30 ml normal saline	30	5
Dimethylsulphoxide (DMSO)	Dimethylsulphoxide (99.5%) Sigma-Aldrich, GhbH, Germany	20 ml + 20 ml normal saline	30	5
Cephradine (control) (Ceph)	Inj. Velosef, Bristol-Mayer[s] Squibb, Pakistan	500 mg + 40 ml normal saline	30	5

Experiment II involved the evaluation of non-antibiotic antibacterials in combination with antibiotic (cephradine) in the treatment of bubaline clinical mastitis. All 4 non-antibiotic antibacterials (chlorpromazine, lidocaine, povidone-iodine and dimethylsulphoxide) were evaluated in regimens similar to those in experiment I, except that 500 mg cephradine (Inj. Velosef™, Bristol-Myers Squibb, Pakistan) was added to daily infusions of each non-antibiotics antibacterial in 30 mastitis quarter in each group. Mean milk yield loss of the affected quarters was analyzed by analysis of variance to compare means before and after treatment (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

The overall effects of infusions of non-antibiotic antibacterials with and without cephradine on milk yield of the buffalo quarters affected with mastitis are shown in Table 2. Statistical prioritization of mean milk yield loss before and at day 28 post-initiation of treatment is given in Table 3.

The pre-treatment milk yield losses in quarters treated with CPZ, Lid, PI and DMSO alone were 31.69, 30.06, 33.08 and 24.80%, respectively. The corresponding values on day 28 post-initiation of treatment were 16.31, 22.53, 27.4 and 34.55% (Table 2).

Thus, the highest percent net recovery of milk yield was observed in CPZ group (15.38), followed by Lid (7.53) and PI (5.68) group. Contrarily, in DMSO treated group, the milk yield loss increased further instead of decline. In other words, a significant ($P<0.05$) reduction (Table 3) in losses of milk yield was observed in CPZ group as compared to other treatment groups. This may be due to strong antibacterial effect of CPZ (Williams, 1995; Amaral *et al*., 1996; Martins *et al*., 2008). On the other hand, the quarters treated with DMSO registered a further significant ($P<0.05$) increase in loss of milk yield. This may be attributed to inability of dimethylsulphoxide to control bacterial infections, as this chemical possesses only a weak antibacterial activity (Plumb, 1999). The net recovery of milk yield in quarters treated with povidone-iodine was very poor (5.68%), which might have been due to its irritability on udder tissue. Udder irritation produced by antimastitic preparations is one of the main criteria in the evaluation experiments (Muhammad *et al.*, 1990). More irritant the drug, the lesser it is desirable for intramammary infusion (Uvrov, 1971).

In the present study, although all four non-antibiotic antibacterials with and without cephradine were administered by intramammary infusion, no attempt was made to study their comparative irritability in terms of increases in milk somatic cell counts. This is one of the shortcomings of the present study which should be addressed in any similar future investigation on the use of non-antibiotic antibacterials in mastitis treatment. Milk somatic cell count has been shown to increase due to mastitis (Khan and Khan, 2006; Sharif *et al*., 2007).

Considering combination regimens (Table 2), the pre-treatment milk yield losses in quarters treated with CPZ, Lid, PI and DMSO in combination with cephradine were 29.10, 34.75, 31.42 and 33.10%, respectively. The corresponding values on day 28 post-initiation of treatment were 11.85, 13.47, 19.01 and 15.38%, with a resultant net recovery of milk yields of 17.25, 21.28, 12.41 and 17.72%.

Table 2: Effect of four non-antibiotic antibacterials infusions alone and in combination with cephradine on milk yield in buffaloes over a period of 4 weeks post initiation of treatment

Non-antibiotic antibacterials alone and in combination with cephradine		Milk yield of mastitic quarters before and after treatment	Milk yield of contralateral normal quarters	Difference of mean	Reduction in milk yield loss of affected quarters (%)	Net recovery of milk yield of treated quarters on day 28 (%)
CPZ	A	0.97 ± 0.10	1.42 ± 0.11	0.45 ± 0.05	31.69	15.38
	B	1.18 ± 0.13	1.41 ± 0.11	0.23 ± 0.07	16.31	
Lid	A	1.0 ± 0.09	1.43 ± 0.11	0.43 ± 0.12	30.06	7.53
	B	1.10 ± 0.07	1.42 ± 0.09	0.32 ± 0.02	22.53	
PI	A	0.89 ± 0.11	1.33 ± 0.12	0.44 ± 0.08	33.08	5.68
	B	0.98 ± 0.17	1.35 ± 0.11	0.37 ± 0.11	27.4	
DMSO	A	1.03 ± 0.07	1.37 ± 0.07	0.34 ± 0.09	24.8	-9.75
	B	0.89 ± 0.08	1.36 ± 0.07	0.47 ± 0.08	34.55	
Ceph (control)	A	0.95 ± 0.09	1.45 ± 0.09	0.50 ± 0.08	34.48	13.94
	B	1.16 ± 0.13	1.46 ± 0.08	0.30± 0.09	20.54	
CPZ + Ceph	A	0.95 ± 0.12	1.34 ± 0.10	0.39 ± 0.06	29.10	17.25
	B	1.19 ± 0.11	1.35 ± 0.10	0.16 ± 0.09	11.85	
Lid+ Ceph	A	0.92 ± 0.11	1.41 ± 0.10	0.49 ± 0.10	34.75	21.28
	B	1.22 ± 0.14	1.41 ± 0.10	0.19 ± 0.08	13.47	
Pi + Ceph	A	0.96 ± 0.26	1.40 ± 0.11	0.44 ± 0.07	31.42	12.41
	B	1.15 ± 0.11	1.42 ± 0.11	0.27 ± 0.07	19.01	
DMSO + Ceph	A	0.97 ± 0.19	1.45 ± 0.10	0.48 ± 0.10	33.10	17.72
	B	1.21 ± 0.14	1.43 ± 0.09	0.22 ± 0.12	15.38	

CPZ = Chlorpromazine, Lid = Lidocaine, DMSO = Dimethylsulphoxide PI = Povidone – iodine, Ceph = Cephradine
A = Pre-treatment; B = Post-treatment

Table 3: Comparison of different treatments on the basis of mean milk yield loss (mean ± SE) of buffalo mastitic quarters

Non-antibiotic antibacterials alone/ and in combination with cephradine	Pre-treatment milk yield loss (L/quarter/day)	Post-treatment milk yield loss (L/quarter/day)
CPZ	0.45 ± 0.05 abc	0.23 ± 0.07 hi
Lid	0.43 ± 0.12 c	0.32 ± 0.02 ef
PI	0.44 ± 0.08 bc	0.37 ± 0.11 de
DMSO	0.34 ± 0.09 def	0.47 ± 0.08 abc
Cephradine (control)	0.50 ± 0.08 a	0.30 ± 0.09 fb
CPZ + cephradine	0.39 ± 0.06 d	0.16 ± 0.09 j
Lid+ cephradine	0.49 ± 0.10 ab	0.19 ± 0.08 ij
PI + cephradine	0.44 ± 0.07 bc	0.27 ± 0.07 gh
DMSO + cephradine	0.48 ± 0.10 ab	0.22 ± 0.12 hi

Means sharing different letters in a column or row are significantly different (P<0.05).
CPZ = Chlorpromazine HCl; Lid = Lidocaine HCl; PI = Povidone - Iodine; DMSO = Dimethylsulphoxide

Thus, the highest net recovery of milk yield on day 28 post-initiation of treatment was 21.28% for Lid + Ceph group, followed DMSO + Ceph, CPZ + Ceph and PI + Ceph groups. Among the quarters receiving combination treatments, higher reduction (P<0.05) in the loss of milk yield was observed in Lid + Ceph, group (Table 3) compared to other treatment groups. This may be due to strong antibacterial effect of lidocaine against Gram negative bacteria and that of cephradine against Gram positive bacteria (Schmidt and Rosenkranz, 1970).

In summary, among the 4 non-antibiotic anti-bacterials tested alone, chlopromazine showed a relatively more promising recuperative effect on the milk yield of clinically mastitic quarters of dairy buffaloes. Dimethylsulphoxide, when infused alone, aggravated the milk loss of clinically mastitic quarters. Adjuncting cephradine with each of the 4 non-antibiotic antibacterials showed that the lidocaine-cephradine combination had the highest effect (P<0.05) on the net recovery of milk yield loss at day 28 post initiation of treatment.

The present study was the first one on the use of chlorpromazine and lidocaine in the treatment of mastitis. In view of a small number of quarters (n=30) on which each of the 4 non-antibiotic antibacterials were tested, a larger field trials involving a larger number of mastitis affected animals and quarters is clearly warranted.

REFERENCES

Amaral L, JE Kristiansen, LS Abebe and W Millett, 1996. Inhibition of the respiration of multi-drug resistant clinical isolates of *Mycobacterium tuberculosis* by thioridazine: potential use for initial therapy of freshly diagnosed tuberculosis. J Antimicrob Chemother, 38: 1049-1053.

Berghash SR, JN Davidson, JC Armstrong and GM Dunny, 1983. Effects of antibiotic treatment of nonlactating dairy cows on antibiotic resistance patterns of bovine mastitis pathogens. Antimicrob Agents Chemother, 32: 355-365.

Bhatti JA, M Younas, M Abdullah, ME Babar and H Nawaz, 2009. Feed intake, weight gain and haematology in Nili-Ravi buffalo heifers fed on mott grass and Berseem fodder substituted with saltbush (*Atriplex amnicola*). Pakistan Vet J, 29(3): 133-137.

Cederiund H and PA Mardh, 1993. Antibacterial activity of non-antibiotic drugs. J Antimicrob Chemother, 32: 355-365.

Erskine RJ, 1996. Why do antibiotic residues in milk happen? Michigan Dairy Review, 1: 16-17.

Erskine R, J Cullor, M Schaellibaum, B Yancey and A Zecconi, 2004. Bovine mastitis pathogens and trends in resistance to antibacterial drugs. Proc 43rd Ann Meeting National Mastitis Council, Inc, Feb 1-4, Charlotte North Carolina, USA, pp: 400-407.

Griggs DJ, MC Hall, YF Jin and LJV Piddock, 1994. Quinolone resistance in veterinary isolates of *Salmonella*. J Antimicrob Chemother, 33: 1173-1189.

Javaid SB, JA Gadahi, M Khaskeli, MB Bhutto, S Kumbher and AH Panhwar, 2009. Physical and chemical quality of market milk sold at Tandojam, Pakistan. Pakistan Vet J, 29(1): 27-31.

Khan MZ and A Khan, 2006. Basic facts of mastitis in dairy animals: A review. Pakistan Vet J, 26(4): 204-208.

Kristiansen JE, 1990. The antibacterial activity of psychotherapeutic drugs and stereo-isomeric analogues (Thesis MSc). Danish Medical Bulletin, 37: 165-182.

Kristiansen JE and L Amaral, 1997. The potential management of resistant infections with non-antibiotics. J Antimicrob Chemother, 40: 319-327.

Kristiansen JE, L Amaral and VF Thomsen, 1993. The reversal of antibiotic resistance by phenothiazines. 18th Intl. Congress Chemother. Stockholm, Sweden, Abst 510: 65-66.

Kristiansen JE, U Hendricks, T Delvin, TS Butterworth, L Aagaard, JB Kristiansen, VC Flores and H Keyzer. 2007. Reversal of resistance in microorganisms by help of non-antibiotics. J Antimicrob Chemother, 59(6): 1271-1279.

Martins M, SG Dastidar, S Fannings, JE Kristiansen, J Molnar, JM Pages, Z Schelz, G Spengler, M Viveiros and L Amaral, 2008. Potential role of non-antibiotics (helper compounds) in the treatment of multidrug-resistant Gram-negative infections: mechanisms for their direct and indirect activities. Intl J Antimicrob Agents, 31: 198-208.

McDowell RE, JC Wilk, SK Shah, DS Balain and GH Metyry, 1995. Potential for Commercial Dairying with Buffalo. North Carolina State University, USA.

Muhammad G, T Aziz, NI Chaudhry and M Nawaz, 1990. Comparative efficacy of four intramammary antibiotics for the treatment of subacute mastitis in buffaloes. Pakistan Vet J, 10(1): 5-10.

National Mastitis Council Inc, 1990. Microbiological Procedures for the Diagnosis of Bovine Udder Infection. Natl Mastitis Council Inc, Arlington, Virginia, USA.

Plumb DC, 1999. Veterinary Drug Handbook. 3rd Ed, The Iowa State University Press, Ames, Iowa, USA, pp: 208-210.

Rodrigues LDW, M Viveiros, D Sampaio, I Couto, M Vavra, WN Kern and L Amaral, 2008. Thioridazine and chlorpromazine inhibition of ethedium bromide efflux in Mycobacterium. J Antimicrob Chemother, 61(5): 1076-1082.

Schmidt RM and HS Rosenkranz, 1970. Antimicrobial activity of local anaesthetics-Lidocaine and procaine. J Infect Dis, 121(6): 597-607.

Sharif A and G Muhammad, 2009. Mastitis control in dairy animals. Pakistan Vet J, 29(3): 145-148.

Sharif A, T Ahmad, MQ Bilal, A Yousaf and G Muhammad, 2007. Effect of severity of sub-clinical mastitis on somatic cell count and lactose contents of buffalo milk. Pakistan Vet J, 27(3): 142-144.

Sole J, OC Sampimon, JJ Snoep and YH Schukken, 1994. Factors associated with bacteriological cure after dry cow treatment of subclinical Staphylococcal mastitis. J Dairy Sci, 77: 75-79.

Sole J, OC Sampimon, JJ Snoep and YH Schukken, 2000. Factors associated with bacteriological cure during lactation after therapy for subclinical mastitis caused by *Staphylococcus aureus.* J Dairy Sci, 83: 278-284.

Steel, RGD, JH Torrie and DA Dickey, 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd Ed, McGraw Hill Book Co Inc, New York, USA.

Teuber M, 2001. Veterinary use and antibiotic resistance. Current Opinion in Microbiology, 4: 493-499.

Thorburn MA, 1990 General principles for the design of clinical trials with special reference to mastitis therapy. Bulletin of the International Dairy Federation (No. 247) Brussels, Belgium, pp: 39-51.

Uvrov O, 1971. Drugs against mastitis. Vet Rec, 88: 674-675.

Williams JD, 1995. Selective toxicity and concordant pharmacodynamics of antibiotics and other drugs. J Antimicrob Chemother, 35: 721-737.