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Research Article

EFFECT OF EXTENDED INTRAMAMMARY ANTIBIOTIC THERAPY ON BACTERIOLOGICAL CURE IN BOVINE CLINICAL MASTITIS

NEELAM*, V.K. JAIN[1], MAHAVIR SINGH[2], RICKY JHAMBH, V.G. JOSHI[3], Y.S. RANA and PARVEEN GOEL
Department of Veterinary Medicine, [1]Department of Veterinary Clinical Complex,
[2]College Central Laboratory, [3]Department of Animal Biotechnology.
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar -125004, India

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ABSTRACT

Intramammary administration of drugs for treatment of mastitis leads to direct delivery and high concentration of drugs at the site of infection. The present study was conducted to evaluate effect of extended intramammary antibiotic therapy on bacteriological cure in bovine clinical mastitis. A total of 144 infected quarters from 80 clinical cases of bovine mastitis were divided and treated with recommended (group A and group B) and extended therapeutic regimens (group C and group D) through intramammary administration of cefoperazone and cefuroxime, respectively. Bacteriological cure was assessed after five days of completion of therapy. Extended therapy using cefuroxime and cefoperazone for treatment of bovine clinical mastitis showed higher bacteriological cure as compared to recommended therapeutic regimen by approximately 10 and 17 per cent, respectively. The findings of the present study will be helpful for veterinary practitioners for selection of treatment regimens as an effort to improve the bacteriological cure in clinical cases of bovine mastitis.

Keywords: Bovine, Clinical mastitis, Intramammary, Cefoperazone, Cefuroxime, Extended therapy

Mastitis is a complex and most frequently observed disease in bovines characterized by inflammation of mammary glands mainly due to bacterial infections (Constable *et al.*, 2017). It continues to be a great challenge in dairy industry because it is difficult to manage and has negative economic impact on dairy industry worldwide (Down *et al.*, 2013). A total of approximately 200 different microbial pathogens have been found to be associated with bovine mammary gland infection(Sharma *et al.*, 2012) and among these staphylococci, streptococci and *E. coli* are most prevalent pathogens (Sharma *et al.*, 2018).

Intramammary administration of antibiotics permits direct delivery of drug to the mammary gland (Sudhan and Sharma, 2010) and high concentration of antibiotic is achieved at the site of the infection (Gehring and Smith, 2006). Cefuroxime and cefoperazone (cephalosporins) are commonly used for treatment of both Gram-positive and Gram-negative bacteria as they are relatively resistant to β-lactamase bacteria (Aiello and Moses, 2016). Some authors reported better therapeutic efficacy of extended duration of antimicrobial therapy over standard treatment protocols (Swinkels *et al.*, 2014; Rainard *et al.*, 2018). However, scanty literature is available on efficacy of extended duration of intramammary treatment with cefuroxime and cefoperazone on bacteriological cure in mastitis. Therefore, the present study was planned and conducted to evaluate effect of extended intramammary antibiotic therapy on bacteriological cure in bovine clinical mastitis.

MATERIALS AND METHODS

Bacteriological examination of milk samples: A total 278 quarters' milk samples, which were collected aseptically and received at College Central Laboratory, LUVAS from 80 bovines (29 cattle and 51 buffaloes) with clinical mastitis viz. signs of change in udder secretions such as flakes, clots, abnormal colour, change in consistency, abnormal taste, swelling and oedema in mammary glands, were included in the study. The cases showing febrile response viz. anorexia, depression and fever were excluded from the study. Milk samples were subjected to bacterial isolation by inoculating 10 µl milk sample on 5% defibrinated sheep blood agar and MacConkey Lactose agar plates (Carter *et al.*, 1995). Growth of micro-organisms was observed after incubation at 37 °C for 16-18 hrs and bacteria were identified on the basis of colony morphology, Gram's staining and catalase test.

Antimicrobial susceptibility testing and therapeutic regimen: Antimicrobial susceptibility testing was determined through disc-diffusion method on Mueller-Hinton agar by using commercially available antibiotic discs, as per method of Markey *et al.* (2011). Based on antimicrobial sensitivity testing, the two antimicrobials, cefoperazone and/or cefuroxime were selected for treatment in four groups, each consisting of 20 animals (total 80 animals with 144 infected quarters) as per the following therapeutic regimen.

Group A (29 quarters)**:** Cefoperazone @ 250 mg per affected quarter intramammary single shot

Group B (26 quarters)**:** Cefuroxime @ 250 mg per affected quarter intramammary; three shots administered at an interval of 12 hours

Group C (20 quarters)**:** Cefoperazone @ 250 mg per

*Corresponding author: neelamaps2011@gmail.com

affected quarter intramammary; two shots administered at an interval of 36 hours

Group D (25 quarters)**:** Cefuroxime @ 250 mg per affected quarter intramammary; six shots administered at an interval of 12 hours

Along with the intramammary antibiotics, supportive therapy consisting of anti-inflammatory (Inj. Ketoprofen @ 3 mg/kg b.wt. IM), antioxidants (ascorbic acid @ 15 mg/kg b.wt. IM), Galactogogues (orally) and mineral mixture (orally) etc. were administered as per the need of case in all groups.

Assessment of bacteriological cure: To determine the bacteriological cure of treatment, milk samples were again subjected to bacteriological examination after five days of completion of therapy in order to determine the efficacy of treatment. Bacteriological cure was defined as non-existence of the initial pathogen in milk sample after therapy.

RESULTS AND DISCUSSION

A total of 154 quarters out of 278 quarters were found to be infected with various bacterial pathogens. Among these, staphylococci were highest in occurrence, followed by streptococci and *E. coli*. These findings are in agreement with Bhat *et al.* (2017) and Sharma *et al.* (2018). Contrary to this, Tomazi *et al.* (2018) reported *E. coli* as most frequently isolated pathogen, followed by *Streptococcus uberis* and *Streptococcus agalactiae*. The distribution of pathogens varies with adoption of different managemental and hygienic practices for rearing of animals (Kumar *et al.*, 2010 and Constable *et al.*, 2017).

Antimicrobial sensitivity of clinical isolates is depicted in Table 1. The sensitivity pattern of antimicrobials against staphylococci observed in the present study is in line with Chandrasekaran *et al.* (2014).Contrary to this, Kumar *et al.* (2011) and Sharma *et al.* (2018) reported high sensitivity towards chloramphenicol, ampicillin and cefoperazone, respectively against staphylococci. Streptococci were found to be highly sensitive to cefoperazone, cefuroxime and chloramphenicol. Contrary to this, Schabauer *et al.* (2018) reported high sensitivity towards penicillin and Sharma *et al.* (2018) reported high sensitivity of streptococci against all the tested antimicrobials than the present study. *E. coli* showed highest sensitivity towards enrofloxacin and levofloxacin and least sensitivity was observed towards antimicrobials of penicillin group and amikacin, neomycin and oxytetracycline. These findings are in close agreement with Chandrasekaran *et al.* (2014). They also reported high sensitivity of *E. coli* towards enrofloxacin (79%), gentamicin (73.1%) and ceftriaxone (69%) while, high resistance towards penicillin (63.5%), amoxicillin (61.5%) and oxytetracycline (49%). Contrary to this, Wu *et al.* (2016) reported high sensitivity towards tetracycline (93.3%), gentamicin (50%) and streptomycin (78.3%). The enormous increase in resistance of pathogens may be due to immense use of antimicrobials for treatment and mainly caused by administration of antimicrobials without exploiting the antimicrobial profile of the pathogen, which leads to emergence of antimicrobial resistance or it may be due to the colonization of resistant strains of pathogen, infecting bovine mammary glands (Kumar *et al.*, 2010).

Therapeutic efficacy was assessed on the basis of bacteriological cure in response to treatment regimen as depicted in Table 2. In group A, 43.18% quarters were bacteriologically cured. However, Wilson *et al.* (1986) reported high bacteriological cure (69%) with administration of same dose of cefoperazone in 434 cases of bovine clinical mastitis. In group B, 19 quarters (54.29%) were found to be bacteriologically cured out of 35 quarters. On contrary, McDougall *et al.* (2007) reported comparatively high bacteriological cure (69.9%) in clinical cases of bovine mastitis through administration of same dose of cefuroxime intramammary. In group C, 23 quarters (60.53%) were found to be bacteriologically cured out of 38 quarters. Bacteriological cure in extended treatment regimen through administration of cefoperazone is approximately 17% higher than that of recommended regimen. In group D animals, which were treated with extended therapeutic regimen of cefuroxime, 24 quarters (64.9%) were found to be bacteriologically cured out of 37 quarters which is approximately 10% higher than that of recommended regimen of the cefuroxime. However, the difference was statistically non-significant.

Several authors reported that strategies with extended antimicrobial therapies for treatment of mastitis results in higher probability of bacteriological cure (Swinkels *et al.*, 2014; Rainard *et al.*, 2018) as compared to short duration treatment of the bovine clinical mastitis cases. Swinkles *et al.* (2014) compared the efficacy of standard versus extended treatment through intramammary administration of a cephalosporin antimicrobial, cefquinome in clinical cases of cows and reported that with extended treatment, overall bacteriological cure was 78 per cent and with standard treatment was 72 per cent. Contrary to this, McDougall *et al.* (2019) reported no significant difference in bacteriological cure in cows through administration of a combination of amoxicillin, clavulanic acid and prednisolone three times at 12 hours interval verses five times at 12 hours interval with bacteriological cure with 73.3±7.8% and 72.0±7.4%, respectively. However, they observed fewer clinical failures through increasing the duration of treatment. High

Table 1

Antimicrobial sensitivity of different bacterial isolates (n=154) from mastitic milk samples

Antimicrobial Group	Antimicrobial	Per cent Sensitivity		
		Staphylococci (n=100)	Streptococci (n=45)	*E. coli* (n=9)
Penicillins	Penicillin	30.00	42.22	33.33
	Amoxicillin	35.00	46.67	33.33
	Ampicillin	35.00	48.89	33.33
	Cloxacillin	41.00	42.22	22.22
Cephalosporins	Ceftriaxone	63.00	64.44	55.56
	Cefoperazone	75.00	93.33	88.89
	Cefuroxime	84.00	93.33	88.89
Amphenicol	Chloramphenicol	74.00	84.44	55.56
Aminoglycosides	Amikacin	46.00	35.56	22.22
	Gentamicin	64.00	57.78	77.78
	Neomycin	37.00	33.33	22.22
	Streptomycin	45.00	48.89	77.78
Fluoroquinolones	Enrofloxacin	61.00	44.44	100.00
	Levofloxacin	53.00	53.33	100.00
	Moxifloxacin	67.00	71.11	55.56
Tetracycline	Oxytetracycline	38.00	46.67	44.44

Table 2

Bacteriological cure of quarters (144) affected with bovine clinical mastitis

Bacteria	Recommended regimen				Extended regimen			
	Group A		Group B		Group C		Group D	
	No. of quarters cured/total number of quarters	%	No. of quarters cured/total number of quarters	%	No. of quarters cured/total number of quarters	%	No. of quarters cured/total number of quarters	%
Staphylococci	11/29	37.93	13/26	50	8/20	40	14/25	56
Streptococci	7/12	58.33	5/7	71.43	13/16	81.25	8/10	80
E. coli	1/3	33.33	1/2	50	2/2	100	2/2	100
Total	19/44	43.18	19/35	54.29	23/38	60.53	24/37	64.9

bacteriological cure with extended therapeutic regimen may be due to longer persistence of the drug in the infected quarter, thus increasing the exposure time of the drug against the micro-organism thus leading to enhanced efficacy of the drug towards microorganism (Cagnardi *et al.*, 2010).

Although extended therapy leads to increase in cost of treatment and may lead to trauma to teat canal with every intramammary infusion; however, both of these intramammary infusions are supplied with short canula to minimise the damage to teat canal. Also, the extension in therapy increases the milk withdrawal period, but it is necessary to increase the success of treatment especially for chronic or recurrent infections (Prescott, 2013) through maintenance of minimum inhibitory concentration of drug against the micro-organism for longer duration (Giguère *et al.*, 2013). Thus, the extension in duration of treatment may be opted for higher bacteriological cure in bovine clinical mastitis. These findings will be helpful for veterinary practitioners for selection of treatment regimens as an effort to improve the outcomes of clinical cases of mastitis.

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