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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(8): 198-203

www.thepharmajournal.com
Received: 29-06-2017
Accepted: 30-07-2017

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Therapeutic management of sub clinical mastitis in cows

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Abstract
The present study ''Therapeutic Management of Sub Clinical Mastitis in Cows'' was undertaken to study the bacteriogram of the milk isolates and therapeutic efficacy of two different antibiotics. A total of 20 cows (33 quarters) were selected for the present study and *In vitro* drug sensitivity test was conducted on sub clinically positive whole milk cultures of which, highest sensitivity was shown to Ceftriaxone (89.83%) followed Amoxyclav (81.36%), Enrofloxacin (77.97%), Gentamicin (71.19%), Amikacin (54.23%), Chloramphenicol (50.84%), Tetracycline (44.06%) and least to Penicillin G (8.47%). For therapeutic trials a total of 20 cows with subclinical mastitis were randomly divided into two groups i.e., group II and group III with 10 cows in each. An apparently healthy control group of 10 cows (which were culturally negative) was also included in the present study. Cows of group II were treated with Ceftriaxone whereas cows of group III were treated with Amoxicillin-Cloxacillin.

Keywords: Sub Clinical Mastitis, Cows, Ceftriaxone, Amoxicillin-Cloxacillin.

Introduction
The term subclinical mastitis refers to a state of mild inflammation of the udder where the symptoms are not manifested but the milk is culturally positive for pathogenic bacteria. Among the animal diseases which affect the profitability of rearing animals, mastitis is considered to be one of the expensive diseases in terms of production losses. Subclinical mastitis affects milk quality and quantity causing great economic loss for producers [30, 8]. The economic consequences of losses due to subclinical form of mastitis were assessed in terms of reduction in milk yield, medicine and veterinary expenses incurred and additional resources used. An annual economic loss of over Rs. 6000 crore due to mastitis has been recorded, of this, Rs. 4300 crore are lost due to sub clinical mastitis [7]. Treatment of mastitis in subclinical stage itself is more important in large scale mastitis control programs to avoid losses which are apt to occur even after treatment of clinical form of mastitis. Antibiotic treatment of mastitis leads to significant increase in milk quantity and quality, lower somatic cell count and is likely associated with reduction in prevalence of clinical mastitis among herds, which is economically beneficial [16]. Intramuscular treatment of subclinical Staphylococcal mastitis in lactating cows with penicillin G and Methicillin recorded efficacy in the elimination of infection [38]. Pefloxacin, Amoxicillin-Cloxacillin combination and neomycin for the treatment of subclinical mastitis in crossbred cows and reported that Pefloxacin was highly effective (95%) in treating infected animals followed by Amoxicillin/Cloxacillin combination [3].
The present study "Therapeutic Management of Sub Clinical Mastitis in Cows" was undertaken to study therapeutic efficacy of two different antibiotics for the treatment of subclinical mastitis in cows.

Materials and Methods
The study was carried out on cases of subclinical mastitis from three different dairy farms and individual holdings during the period from February 2015 to August 2015. A total of 20 cows (33 quarters) were selected for the present study.

Screening for Sub Clinical Mastitis
All the crossbred cows which have calved more than 15 days were taken for the investigation. The milk samples from different quarters were subjected to California mastitis test (CMT), White side test (WST) Surf field mastitis test (SFMT) and Somatic Cell Count (SCC) to diagnose subclinical mastitis. CMT results were graded as per Schalm *et al.* [24] as negative, 1+ (there is precipitate but no gel formation), 2+ (the precipitate thickens and forms gel towards

the centre of the paddle) and 3+ (distinct gel that adheres to the bottom of the paddle). In WST Milk thickened and flakes appeared when the sample was positive for mastitis. Based on the degree of thickening and appearance of flakes, the results were graded as negative, 1+ (background is less opaque, with larger particles of coagulated materials thickly scattered and slight degree of clumping), 2+ (background is more watery with large clumps of coagulated materials. If the stirring is been rapid, fine threads or strings may be present) and 3+ (background is very watery and whey-like, with large masses of coagulated material forming into strings and shreds). In Surf Field Mastitis Test (SFMT) the reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of reaction was obtained within 30 seconds and immediately scored as negative, 1+, 2+ and 3+ basing on the formation of floccules or gel of varying degrees [15]. Total Somatic Cell Count (SCC) was estimated using *Ekomilk scan* somatic cells analyzer supplied by M/s Eon Traders, as per the manufacturer's instructions. The values obtained were expressed as × 10^5 cells/ml of milk. The milk samples containing SCC range of 0-2 lakh cells/ml were considered negative, 2-4 lakh cells/ml as healthy or sub clinically infected and more than 4 lakh cells/ml as positive for clinical or subclinical mastitis.

Based on the above test results the animals were randomly divided into two groups, *viz.*, group II and III with ten cows in each. 10 cows with 18 infected quarters were included in group II. Though all the 18 quarters were treated, only 15 were included in the therapeutic trial to maintain proportionate number of quarters in the groups. In group III, 10 cows with 15 infected quarters were included. The apparently healthy cows were taken as group I (10 cows, 15 quarters). The animal was considered as free from the disease when milk samples showed a negative reaction for CMT, WST, SFMT and SCC along with culturally negative results.

***In vitro* Drug Sensitivity:** The antibiotic sensitivity of the whole milk sample cultures was done *in vitro* by disc diffusion method on brain heart infusion (BHI) agar plates.

The following discs were used for antimicrobial sensitivity testing.

S. No.	Antimicrobial agent	Symbol	Concentration
1.	Amoxyclav	AMC	30 mcg
2.	Amikacin	AK	30 mcg
3.	Ceftriaxone	CTR	30 mcg
4.	Chloramphenicol	C	10 mcg
5.	Enrofloxacin	EN	10 mcg
6.	Gentamicin	GEN	10 mcg
7.	Penicillin – G	P	10 units
8.	Tetracycline	TE	30 mcg

Two antibiotics *viz.*, Ceftriaxone and Amoxicillin-Cloxacillin were chosen for the present therapeutic trial Both the groups were given a common supportive therapy with post milking teat dip Mastidip liquid in 1:1 dilution twice daily immediately after milking.

Results

All the cows were treated for a period of 5 days and monitored daily by CMT, WST, SFMT and SCC and number of days taken for complete recovery. Treated cows showing negative reaction for CMT, WST and SFMT and yielding culturally negative results were considered as completely cured.

In vitro drug sensitivity was tested on whole milk cultures rather than on individual organisms and the results are presented in the table 1, fig. 1. The mean milk pH values in cows with subclinical mastitis under group II (6.917±0.052) and group III (6.94±0.075) before treatment were found significantly (P<0.01) elevated when compared to that of the apparently healthy control group (6.588±0.024). The post treatment milk pH values of group II and group III reduced significantly (P<0.01) to 6.596±0.027 and 6.606±0.023, respectively, compared to the mean pre-treatment milk pH values (table 2, 3). The reduced post treatment milk pH values of group II and III were comparable to apparently healthy cow

Table 1: *In vitro* drug sensitivity of bacteria from whole milk cultures affected with subclinical mastitis in cows (n=59)

S. No	Symbol	Name of the antibiotic sensitivity disc	Concentration (mcg)	Number of milk samples tested	Number of sensitive bacteria				Resistant bacteria	
					More Sensitive	Intermediate sensitive	Total	% Sensitivity	Number	%
1.	CTR	Ceftriaxone	30	59	49	4	53	89.83	6	10.17
2.	AMC	Amoxyclav	30	59	41	7	48	81.36	11	18.64
3.	EN	Enrofloxacin	10	59	40	6	46	77.97	13	22.03
4.	GEN	Gentamicin	10	59	35	7	42	71.19	17	28.81
5.	AK	Amikacin	30	59	24	8	32	54.23	27	45.77
6.	C	Chloramphenicol	10	59	20	10	30	50.84	29	49.16
7.	TE	Tetracycline	30	59	19	7	26	44.06	33	55.94
8.	P	Penicillin- G	10 units	59	5	-	5	8.47	54	91.53

Fig. 1: Antibiogram of whole milk culture

The mean pre-treatment milk SCC of cows under group II and group III were 7.47±0.843 (×10^5 cells/ml) and 8.29±0.911 (×10^5 cells/ml), respectively, which showed highly significant difference (P<0.01) when compared to that of the apparently healthy control group (2.207±0.156×10^5 cells/ml). The post treatment values of SCC of group II and group III reduced significantly (P<0.01) to 2.471±0.18 (×10^5 cells/ml) and 2.436±0.251 (×10^5 cells/ml), respectively, compared to the mean pre-treatment values (table 2, 3).

Table 2: Mean pH and SCC ($\times 10^5$ cells/ml) of group II before and after treatment (n=15)

Parameter	Apparently healthy control (Group I)	Before treatment	After treatment
pH	6.588± 0.024	6.917 ±0.052*	6.596±0.027*
SCC ($\times 10^5$ cells/ml)	2.207± 0.156	7.47 ±0.843*	2.471±0.18*

* Significant T (P< 0.01)

Table 3: Mean pH and SCC ($\times 10^5$ cells/ml) of group III before and after treatment (n=15)

Parameter	Apparently healthy control (Group I)	Before treatment	After treatment
pH	6.588± 0.024	6.94± 0.075*	6.606 ±0.023*
SCC ($\times 10^5$ cells/ml)	2.207± 0.156	8.29 ±0.911*	2.436± 0.251*

* Significant T (P< 0.01)

The efficacy of Ceftriaxone and Amoxicillin-Cloxacillin for the treatment of subclinical mastitis and the observations of pre and post treatment from the affected cows. Out of 15 sub clinically affected quarters (Group II) from 10 cows subjected to Ceftriaxone treatment @ 5mg/kg body weight IM for 5 days, 4 cows with 4 quarters recovered on 2nd day, followed by 3 cows with 6 quarters on 3rd day and 3 cows with 5 quarters on 4thday of therapy giving an overall cure rate of 100% animal wise and quarter wise, respectively (table 4).

Table 4: Therapeutic efficacy of Ceftriaxone in cows of group II

No. of animals (n=10)			No. of quarters (n=15)			No. of days taken for complete recovery
Treated	Cured	% cure	Treated	Cured	% cure	
10	10	100%	15	15	100%	2 - 4

15 quarters of 10 cows affected with subclinical mastitis of group III were treated with Amoxicillin-Cloxacillin @ 10mg/kg body weight IM for 5 days and the therapeutic efficacy was studied. Results showed 60% and 66.67% animal wise and quarter wise cure rate, respectively (table 5). At the end of 3rd day of therapy, 2 cows with 2 quarters recovered, followed by 2 cows with 4 quarters on 4th day and 2 cows with 4 quarters on 5th day of therapy, respectively. However, 4 cows with 5 quarters did not recover at the end of 5th day of therapy, and hence treatment was continued for 2 more days.

Table 5: Therapeutic efficacy of Amoxicillin-Cloxacillin in cows of group III

No. of animals (n-10)			No. of quarters (n=15)			No. of days taken for complete recovery
Treated	**Cured**	**% cure**	**Treated**	**Cured**	**% cure**	
10	6	60%	15	10	66.67%	3 – 5

Both the groups were given a common supportive therapy with post milking teat dip Mastidip liquid in 1:1 dilution twice daily immediately after milking and the combined therapeutic efficacy was studied 15 days post treatment. The results indicate that therapy with Ceftriaxone has a rapid cure rate compared to the therapy with Amoxicillin-Cloxacillin, though both the drugs were effective in treating subclinical mastitis and employing Mastidip post milking reduced the occurrence of new intramammary infections.

The normal milk yield of cows of groups II and III, prior to diagnosis, on the first day of treatment and on the 15th day after therapy were taken for calculating the difference in milk yield and to study the therapeutic efficacy (table 6). The normal daily milk yield of cows of group II had a range from 6.32 lts to 13.61 lts with a mean value of 8.99±0.70 lts, which decreased to 7.76±0.54 lts, on the first day of treatment showing a percentage decrease of 13.68%. The mean milk yield on 15th day after therapy was 8.88±0.26 lts, showing a significant increase (P<0.05) in between pre and post therapy milk yield levels with a percentage increase of 14.43%. It is also evident that the cows of group III had a normal daily milk yield range of 6.67 lts. – 15.11lts, with a mean value of 11.12±0.95 lts, and on the day of treatment it was 9.76±0.48 lts. Table 6 show a percentage decrease in milk yield as 12.23% prior to diagnosis and after diagnosis. The mean value of milk yield was 10.81±0.66 lts, after therapy with Amoxicillin-Cloxacillin which increased significantly (P<0.05) compared to the pre-treatment milk yield values with a percentage increase of 10.76%.

Table 6: Variation in milk yield of affected cows before and after therapy (n=10)

S. No.	Groups of cows	Mean±SE milk yield (15 days prior to diagnosis)	Mean±SE milk yield of affected cows (on the 1st day of treatment)	% decrease in milk yield[a]	Mean±SE milk yield of treated cows (15 days after therapy)	% increase in milk yield *
1.	Group II (n = 10)	8.99± 0.70	7.76 ±0.54	13.68	8.88± 0.26**	14.43
2.	Group III (n = 10)	11.12±0.95	9.76 ±0.48	12.23	10.81 ±0.66**	10.76

** Significant at (P<0.05)

* As compared to 15 days post treatment and 1st day of treatment

[a] As compared to 15 days prior to diagnosis and 1st day of treatment

Discussion

The emergence of drug resistance is due to indiscriminate use of antibiotics, inadequate therapy or certain other epidemiological factors. The susceptibility of the bacterial pathogens to different antimicrobials varies. Due to this fact the rational treatment of the animal with subclinical mastitis based on *in vitro* susceptibility of isolated organisms from individual cases has assumed great importance [23]. It is considered necessary to perform sensitivity of grown microflora from whole milk samples because of the possibility of mixed infections occurring in most of the cases and that the antibiotic has to act against all the infective organisms present in the udder and not on a single organism [28]. In the current study, *in vitro* drug sensitivity was performed on 59 whole milk cultures out of which highest percentage of isolates (53) were sensitive to Ceftriaxone (89.83%), followed by Amoxicillin+Clavulanic acid (81.36%), Enrofloxacin (79.7%), gentamicin (71.19%), Amikacin (54.23%), Chloromphenicol (50.84%), Tetracycline (44.06%) and least number of isolates were sensitive to penicillin G (8.47%). Doriasamy *et al.* [6] reported that Ceftriaxone (92.66%), Enrofloxacin (89.83%) and Ciprofloxacin (82.3%) were most effective, Gentamicin (63.16%) and Chloromphenicol (55.93%) were moderately effective and tetracycline (32.20%) and ampicillin (30.50%) were least effective drugs. Sharma *et al.* [26] showed 100% sensitivity to Ceftriaxone, Cloxacillin and Cefoperazone and 90.9-100% towards enrofloxacin and gentamicin.

Constituent of milk responsible for pH are casein citrate, phosphate and dissolved carbon dioxide and bicarbonates which are balanced with permeability of udder cells to the blood capillaries. In mastitis increased permeability of the gland to blood components viz. bicarbonate ions results higher values of pH in the milk [21]. In the present study, the mean pH of normal healthy group of cows (group I) was 6.588±0.024. The findings of Batavani *et al.* [2] and Hassan [9] showed that the mean milk pH of normal healthy cows was 6.59±0.02 and 6.62±0.02, respectively, which are in agreement with the current findings. In the present study, mean milk pH values in cows with subclinical mastitis under group II and group III were 6.917±0.052 and 6.94±0.075, respectively. These findings are in agreement with Hassan [9] who reported pH of 6.94± 0.06 in the milk from cows with subclinical mastitis. Almost similar results were reported by Anirban and Sandeep [1], [11, 35, 4]. However, higher pH of 7.32±0.06 with SCM was reported by Patil *et al.* [17]. Joshi *et al.* [10] suggested that the increase in chloride concentration might be responsible for increase in pH of milk in subclinical mastitis. Neutrophils, macrophages, lymphocytes, erythrocytes and the small percentage of epithelial cells are found in normal bovine milk and are termed as somatic cells which form second barrier to infection. Somatic cells are indicators of both resistance and susceptibility of cows to mastitis and can be used to monitor the level or occurrence of subclinical mastitis in herds or individual cows [18]. In the current study, the milk samples containing SCC range of 0-2 lakh cells/ml were considered negative, 2-4 lakh cells/ml as healthy or sub clinically infected and more than 4 lakh cells/ml as positive for clinical or subclinical mastitis [22]. In the present investigation, the average number of SCC in normal healthy group of cows (group I) was 2.207±0.156 $\times 10^5$ cells/ml. The total milk somatic cell count from normal uninfected lactating glands of cattle ranged from 1-3 x 10^5 cells/ml in the study conducted by Singh *et al.* [29]. A SCC of 2,00,000 cells per ml of milk in a sample of composite milk is commonly regarded as the threshold for the change of status from non-infectious to infectious level [25]. It can be inferred from the data that SCC ranged from 3.89 to 15 x 10^5 in group II and 3.97 to 18.06 x 10^5 in group III with mean values of 7.47±0.843 ($\times 10^5$ cells/ml) and 8.29±0.911 ($\times 10^5$ cells/ml), respectively. The present findings are in agreement with Maiti *et al.* [13] who reported the mean SCC of 8.75×10^5 cells/ml and 1.13×10^5 cells/ml in milk of SCM affected cows and healthy cows, respectively. Similar results were presented by Matei *et al.* [14] who reported that the SCC of milk from sub clinically infected cows ranged between 5 lakhs/ml to 15 lakhs/ml, with the counts not being more than 2.7 lakhs/ml in healthy animals. SCC is a useful predictor of intramammary infection and, therefore, an important component of milk in assessment of aspects of quality, hygiene, and mastitis control. However, it is evident from the literature that physiological stress, seasonal effects, age, number and stage of lactation, husbandry system and feed practices influence somatic cell variations.

It is evident from the results that the intra muscular treatment of 10 cows having 15 sub clinically infected quarters in group II with Ceftriaxone cured 4 cows (4 quarters), 3 cows (6 quarters) and 3 cows (5 quarters) on 2nd day, 3rd and 4th day of treatment, respectively, showing 100% cure rate both animal wise and quarter wise. These findings are in agreement with those of Kumar *et al* [12] who employed Ceftriaxone for a period of 5 days in the treatment of subclinical mastitis in cows and reported 100% cure rate animal and quarter wise, respectively. Singh [27] successfully treated clinical and subclinical cases of mastitis with Ceftriaxone @ 5-10 mg/kg intramuscularly. Ceftriaxone is a bactericidal drug, belonging to third generation cephalosporin, which act by inhibiting bacterial cell wall synthesis. It had excellent efficacy against *Streptococcus* spp., good against *Staphylococcus* spp. and remarkable activity against Enterobacteriaceae [19]. The therapy with Intamox in 15 sub clinically affected quarters of 10 cows of group III, showed 60% and 66.67% animal and quarter wise cure rate, respectively, over a period of 3-5 days. Almost similar findings were noticed in the clinical trials of Kumar *et al.* [12] who reported 60 per cent animal cure rate and 68.75 per cent quarter cure rate with Amoxicillin - Cloxacillin. Also, the present study findings are in agreement with Tiwari and Sisodia [31] who reported 50 per cent animal cure rate and 61.53 per cent quarter cure rate in a clinical trial. The lower efficacy of Amoxicillin - Cloxacillin noticed in the current study might be due to development of resistance from pathogenic bacteria towards commonly available antibiotics because of their widespread and indiscriminate use. However, Bhalerao *et al.* [3] employed Amoxicillin – Cloxacillin for treating subclinical mastitis in crossbred cows and reported that the drug had 87.5% therapeutic efficacy. Similarly, Tufani *et al.* [32] recorded 75% recovery rate with Amoxicillin-Cloxacillin (10mg/kg, IM, BID) within 4.75 days in the therapeutic management of bovine mastitis.

Both the groups were given a common supportive therapy with Mastidip liquid, a post milking herbal teat dip, employed at a dilution of 1:1 twice daily immediately after milking for a period of 15 days. This is a polyherbal dip comprising of Berberis lyceum, Curcuma longa and Eucalyptus globulus as chief ingredients which are well known to possess antimicrobial, anti-inflammatory and immune modulatory activities. These properties might be responsible for normalizing the SCC and pH of the milk, thus leading to

increase milk yield in subclinical mastitic animals. Ramprabhu *et al*. [20] employed Mastidip for a period of 30 days and concluded that the teat dip was effective in reducing the incidence of subclinical mastitis. Results of Patil *et al.* [17] showed that the herbal teat dip at 1:2 dilution was effective in reducing the incidence as well as occurrence of new intramammary infections. The major economic losses due to subclinical mastitis have been attributed with the loss of milk yield. It is well recognised that the milk production is reduced in cows with subclinical mastitis and the loss may vary from 10 to 25 per cent depending on the severity and the type of organisms associated with SCM [5, 36]. In the present investigation milk yield of all the animals under study was recorded 15 days prior to therapy and 15 days post therapy. It is evident from the current study that the cows under group II and group III showed a decrease of 13.68% and 12.23% on the first day of treatment compared to 15 days prior to therapy. Verma [33] and Zingeser *et al.* [37] noted 6.8 and 6.68% reduction in milk yield in subclinical mastitis, respectively. An increase in milk yield of 14.43% and 10.76% was recorded in group II and group III, 15 days post therapy with Ceftriaxone and Intamox, respectively, compared to the production yield of pre-treatment fortnight. Vijaya [34] employed Enrofloxacin and Ampicillin-Cloxacillin in the therapeutic management of subclinical mastitis and recorded 6.6% and 4.95% increase in milk yield/animal, respectively, during a period of 15 days compared to the milk yield 15 days prior to therapy. The findings of the present study with respect to improvement in milk yield following therapy are in accordance with the above authors.

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