

STUDY ON BOVINE SUB-CLINICAL MASTITIS ON FARM CONDITION WITH SPECIAL EMPHASIS ON ANTIBIOGRAM OF THE CAUSATIVE BACTERIA

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ABSTRACT

The study was conducted to find out the prevalence of sub-clinical mastitis (SCM) and antibiogram of the causative bacteria in dairy cows at the Sylhet govt. Dairy Farm (SGDF) and Local Farms of Sylhet (LFS) during the period of July 2014 to June 2015. These farms were selected to assess the predominant types of bacteria involved in causing sub-clinical mastitis and to know the in vitro antibiotic sensitivity spectrum of these bacterial isolates against the commonly used antibiotics and to study the economic effects due to SCM. Use of California Mastitis Test (CMT) for the detection of sub-clinical mastitis showed 42 out of 100 samples were test positive, among which +(Trace) 22.0%, ++(Distinct) 12.0%, +++(Strong) 3.0%, ±(Doubtful) 5.0%. Bacteriological examination of milk samples of 100 milch cows (400 quarters) revealed that 42 cows (42.0%) had suffering from SCM with different bacterial infection with 95% confidence limit was 32.1574-51.8426. Among 42 positive samples 31 (31.0%) cows had mono-bacterial infection and 11 (11.0%) cows had mixed bacterial infections. Statistical analysis of the result of single and mixed bacterial infections in the milk of apparently healthy milch cows revealed that the single infection was significantly ($P < 0.05$) higher than mixed bacterial infection. Of the 31 mono-bacterial isolates, of which 23 (23.0%) isolates were Staphylococci, 3 (3.0%) isolates were *Escherichia coli*, 5 (5.0%) isolated *Streptococcus* spp. The 11 cows had mixed infection, of which 5 had *Staphylococcus* spp. + *Streptococcus* spp., 3 had *E. coli* + *Staphylococcus* spp., and 3 had *Streptococcus* spp. + *E. coli*. Of 42 positive cases of SCM *Staphylococcus* spp. isolated from 23 samples. Among these 23 positive samples 13 obtained from SGDF (37.14%) and 10 obtained from LFS (15.38%). *Escherichia coli* isolated from 3 samples. Among these 3 positive samples 3 obtained from LFS (4.62%). *Streptococcus* spp. isolated from 5 samples. Among these 5 positive samples 1 obtained from SGDF (2.86%) and 4 obtained from LFS (6.15%). Ceftriaxone, ciprofloxacin and gentamicin were the best drug for treating sub-clinical mastitis.

Key words: Bovine sub-clinical mastitis, prevalence, antibiogram.

INTRODUCTION

Mastitis is an inflammatory condition of the mammary gland, characterized by changes in the physical characteristics of the udder or milk (Nazifi *et al.*, 2011). Mastitis can be classified into three major types: clinical mastitis (CM), sub-clinical mastitis (SCM) and chronic mastitis (ChM) (Anonymous, 2003). In CM, there is swelling, heat, pain, and indurations observed in the mammary gland and also changes in milk color, clots are present in the milk and there are large numbers of leukocytes in the milk (Sharma and Maiti, 2010). In SCM, there are no gross inflammatory changes in the udder tissue and no changes physical appearance on milk. Only increased somatic cell count (SCC) in the milk, the presence of pathogenic organisms in the milk, and an inflammatory response that can only be detected by screening tests such as California Mastitis Test (CMT), White Side Test (WST), Surf Field Mastitis Test (SFMT) (Sachin and Suresh, 2006; Madut *et al.*, 2009). In ChM, udder becomes hard due to fibrosis, the quarters may become thickened, firm, nodular and atrophic. The milk may appear as yellowish fluid or white with clots and flakes. Sometimes it may look as green or yellow-green and foul smelling (Chakrabarti, 2007). The sub-clinical form of mastitis in dairy cows is important because this form is (a) 15 to 40 times more prevalent than the clinical form, (b) it usually precedes the clinical form, (c) it is of long duration, (d) it is difficult to detect, (e) It reduces milk production, and (f) it adversely affects milk quality (Seegers *et al.*, 2003). It is now a well-known fact that the SCM is more serious and is responsible for much greater loss to the dairy industry in Bangladesh (Kader *et al.*, 2003). There are also economic losses in dairy farms due to (a) reduction in milk production, (b) discarding abnormal milk and milk withheld from cows treated

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with antibiotics, (c) degradation of milk quality and price due to high bacterial presence or SCC, (d) costs of drugs, (e) veterinary services and increased labor costs, (f) increased risk of subsequent mastitis, (g) herd replacement, and (h) problems related to antibiotics residues in milk and its products (Bramely, 1996). The prevalence of sub-clinical mastitis in milch cow has been reported to be 15.8% with California Mastitis Test (CMT) from Baghabarighat, Sirajgonj district by Prodhan *et al.* (1996). Rahman *et al.* (2009) reported that the prevalence of bovine SCM as 19.9% and 44.8% in dry & wet seasons, respectively at Sirajgonj districts. There also found the prevalence of SCM was 43.3% in Sylhet Govt. dairy farm and 53.1% in local dairy farms, respectively in Sylhet district (Rahman *et al.*, 2010). The CM can easily be detected by inspection of udder or systemic signs of inflammation, whereas, diagnosis of SCM is more problematic since the milk appears normal but usually has an elevated somatic cell count (Islam *et al.*, 2010). Various methods, based on physical and chemical changes of milk and cultural isolation of organisms, are used for diagnosis of SCM. Therefore, indirect tests such as CMT, WST, SFMT etc. can be used which are simple and do not require any complex laboratory equipment. The CMT and SCC are arguably the only reliable cow side screening tests for SCM that can easily be applied. The CMT is a simple, inexpensive, rapid screening test for SCM, based upon the amount of cellular nuclear protein present in the milk sample. CMT showed better performance in detecting SCM (37.58%) among three indirect tests used WST (36.67%) and SFMT (35.15%) (Islam *et al.*, 2010). Various indirect tests have been described (Schalm *et al.*, 1971) to detect the SCM, but each test has got some limitations as it fails to detect SCM at all stages. The somatic cell count and bacterial load count (BLC) are two methods accepted reliably for detecting the early infection (Schalm *et al.*, 1971). The efficacy of antibiotic therapy for intramammary infections (IMIs) early in lactation is scarce and few, with the ones carried out reporting mixed results. Rosenberg *et al.*, (2002) evaluated the response to therapy with intramammary (IMM) Cephapirin Sodium on CMT positive quarters in lactating cows on cure rates and SCC. It was determined that by the 4-week post-calving evaluation, quarters treated with Cephapirin Sodium had significantly increased cure rates, and SCC were significantly reduced. Wallace *et al.* (2004) also randomly assigning cows with CMT-positive quarters to receive either IMM Cephapirin Sodium or no treatment, found that there was no difference in cure rates for IMM antibiotic-treated quarters for major pathogens compared to the untreated cows. However, there was an advantage for cure rates using antibiotics against environmental streptococcal infections. Quarters with streptococci infections were 3.5 times more likely to cure if treated with Cephapirin Sodium. A field studies have found that IMM antibiotic therapy was beneficial for Gram-positive organisms and coagulase-negative staphylococci, but ineffective for Gram-negative organism (Roberson *et al.*, 2004), because Gram-negative bacteria tend to have a more complex layering in their cell wall structure (Beveridge, 1999).

Additionally, the advent of postmilking teat antiseptics has been important in contributing to decreasing contagious IMI. Teats were dipped after milking and cows were treated with penicillin-dihydrostreptomycin at dry-off, IMI caused by major mastitis pathogens decreased by 75% and 45%, respectively. Post-dipping alone has been estimated to decrease the rate of new IMI by 50% (Nickerson and Boddie, 1997). Antimicrobials are the most important tool in SCM control programs in cows. But there are not properly known by dairy farmers to infuse of intramammary antibiotics in SCM affected lactating cows. However, the reports on the efficacy of intramammary infusion in lactating cows are very limited and the information on the prevalence of SCM in the area is lacking or very less. The main objectives of the research are to isolate and identify the causative bacteria involved in sub-clinical mastitis and in-vitro antibiotic sensitivity test of the bacteria and to know the economic effect of sub clinical mastitis in Dairy cows.

MATERIALS AND METHODS

The research work was conducted in the Department of Medicine and the laboratory of Microbiology and Immunology, Sylhet Agricultural University, Sylhet, during the period of July2014 to June2015. Study was conducted on Sylhet govt. Dairy Farm (SGDF) and Local Farms of Sylhet (LFS). A total number of thirty (35) apparently healthy milch cows of the SGDF and Sixty five (65) milch cows of LFS were randomly selected for this study. Before taking the milk sample, a teatful of milk was milked into a tube and later discarded. This was to flush out any bacteria that might be present in the teat canal. The teat and teat tips were disinfected with a swab soaked in 70% alcohol. Milk samples were collected aseptically from the udder at the time of morning milking. All the milk collected vials were labeled with the identification number of cow (and others symbol). Immediately after collection the samples were subjected to CMT.

Each milk sample was stained with Gram's Method and was examined under microscope as described by Samad (1996). The findings were recorded and then the samples which were found positive for bacteria used for the isolation and identification of bacteria. Each of the collected milk samples was kept in the incubator at 37°C for 24 hours and then stained with Gram's Method as described by Samad (1996). The samples which were found positive for bacteria were selected for the isolation of the organisms. Cultural media were used for the isolation and identification of the bacteria were, Nutrient Broth (Hi media, India), Blood agar media (Hi Media Lab. Pvt. Ltd., Bombay), EMB agar (EMB, Oxiod, UK), Mannitol-salt agar (Hi media, India), Muller Hinton Agar (MHA).

Statistical analysis

Data was analyzed with SAS version 9.4. A95% Confidence Limits for prevalence percent was estimated.

RESULTS AND DISCUSSION

Milk samples from 35 milch cows of SGDF (140 quarters), Sylhet and from 65 milch cows of different LFS (260 quarters), were randomly collected aseptically and examined bacteriologically, of which SGDF showed 15 (42.86%) with 95% Confidence Limits 25.6095-60.1048, Home Fresh Dairy Farm 14 (70%) limit 47.9957-92.0043, Baby Dairy Farm 4 (20%) limit 0.7931-39.2069 , Bulbul Dairy Farm 5 (33.33%) limit 6.3116-60.3551, Rahman Dairy Farm 4 (40%) positive SCM limit 3.0591-76.9409. Highest prevalence of SCM was recorded in milch cows of Home Fresh Dairy Farm, which is 70%. Overall prevalence of sub-clinical mastitis was found 42% with 95% confidence limits 32.1574-51.8426(Table 1).

Use of California Mastitis Test (CMT) for the detection of sub clinical mastitis showed 42 samples were test positive out of 100, among which +(Trace) 22.0%, ++(Distinct) 12.0%, +++(Strong) 3.0%, ±(Doubtful) 5.0%. 48 samples were negative showed in (Table 2).

Table 1. Distribution of sub-clinical mastitis in Sylhet govt. dairy farm and local farms of Sylhet

Sources	Total no. of cows tested	Test positive	Test negative sample	Prevalence of SCM (%)	95% Confidence limits
SGDF(Sylhet Govt. Dairy Farm)	35	15	20	42.86	25.60-60.10
Home Fresh Dairy Farm	20	14	06	70	47.99-92.00
Baby Dairy Farm	20	4	16	20	0.79-39.20
LFS Bulbul Dairy Farm	15	5	10	33.33	6.31-60.35
Rahman Dairy Farm	10	4	06	40	3.06-76.94
Total	100	42	48	42	32.16-51.84

Table 2. Use of California Mastitis Test (CMT) for the detection of sub-clinical mastitis in dairy cows

Test	Total sample	Nature of reaction	No. of sample reacted	Percentage (%)
California Mastitis Test (CMT)	100	+(Trace)	22	22.0
		++(Distinct)	12	12.0
		+++ (Strong)	3	3.0
		±(Doubtful)	5	5.0
		-(Negative)	48	48.0

Bacteriological examination of milk samples of 100 milch cows (400 quarters) revealed that 42 cows (42.0%) had suffering from SCM with different bacterial infection, of which 31 (31.0%) cows had mono-bacterial infection and 11 (11.0%) cows had mixed bacterial infections (Table4). Statistical analysis of the result of single and mixed bacterial infection in the milk of apparently healthy milch cows revealed that the single infection was significantly (P < 0.05) higher than mixed bacterial infection (Table 3). Of the 31 mono-bacterial isolates, of which 23 (23.0%) isolates were Staphylococci, 3 (3.0%) isolates were *Escherichia coli*, 5 (5.0%) isolated *Streptococcus* spp. (Table 3). The 11 (11.0%) cows had mixed infection, of which 5 (5.0%) had *Staphylococcus* spp. + *Streptococcus* spp., 3 (3.0%) had *E. coli* + *Staphylococcus* spp., and 3 (3.0%) had *Streptococcus* spp. + *E. coli*. Among 42 positive cases of SCM *Staphylococcus* spp. isolated from 23 samples. Among these 23 positive

samples 13 obtained from SGDF (37.14%) and 10 obtained from LFS (15.38%). *Escherichia coli* isolated from 3 samples. Among these 3 positive samples 3 obtained from LFS (4.62%). *Streptococcus* spp. isolated from 5 samples. Among these 5 positive samples 1 obtained from SGDF (2.86%) and 4 obtained from LFS (6.15%). Of the 11 mixed infections, 5 were *Staph. + Strep.* Among these 5 positive samples 1 obtained from SGDF (2.86%) and 4 obtained from LFS (6.15%). *Staph. + E. coli* obtained from LFS that is 3 (4.62%). *Strep. + E. coli* combination obtained from 3 samples also from LFS. The overall mean milk yield of dairy cows who had bacterial infection (SCM) reduce from 7.7 litre to 6.2 litre i.e. - 1.50 litre/cow/day (Table 3).

Table 3. Etiology and effects of sub-clinical mastitis on milk production in dairy cows

Sl No.	Organisms isolated	Farms	No. of cows tested	Positive		Mean milk yield (L/cow/day)		
				No.	%	Pre-testing records	Records at testing time	Difference
01	<i>Staphylococcus</i> spp.	SGDF	35	13	37.14	5.5	4.7	-0.8
		LFS	65	10	15.38	10.5	7.8	-2.70
		Single	100	23	23	8	6.25	-1.75
02	<i>Escherichia coli</i>	SGDF	35	0.00	0.00	0.00	0.00	
		LFS	65	03	4.62	11.2	8.5	-2.70
03	<i>Streptococcus</i> spp.	SGDF	35	01	2.86	4.5	4.1	-0.40
		LFS	65	04	6.15	9.6	7.1	-2.50
04	<i>Staph. + Strep.</i>	Single	100	05	05	7.05	5.6	-1.45
		SGDF	35	01	2.86	4.5	4.2	-0.30
		LFS	65	04	6.15	10.3	7.5	-2.80
05	<i>Staph. + E. coli</i>	Mixed	100	05	05	7.4	5.85	-1.55
		SGDF	35	0.00	0.00	0.00	0.00	
		LFS	65	03	4.62	10.5	9.5	-1.00
06	<i>Strep. + E. coli</i>	SGDF	35	0.00	0.00	0.00	0.00	
		LFS	65	03	4.62	11.2	8.7	-2.50
07	Total	SGDF	35	15	42.86	4.8	4.3	-0.50
		LFS	65	27	41.54	10.6	8.1	-2.50
	Overall		100	42	42	7.7	6.2	-1.50

Among all the bacterial isolates, each of ten (10) isolates of *Staphylococcus* spp. *Streptococcus* spp. *E. coli* were selected randomly for the antibiotic sensitivity and resistance pattern against commonly used antibiotic. The result of sensitivity against antibiotics discs (zone of inhibition) were categorized as resistance (-), less sensitive (+), moderately sensitive (++) , highly sensitive (+++). The results of antibiotics sensitivity are given in the (Table4). From the antibiotic sensitivity test of sub-clinical mastitis, it was found that 30% of the isolated *Staphylococcus* spp. was moderately sensitive and 70% were highly sensitive to gentamicin. 30% of the isolates were moderately sensitive and 70% were highly sensitive to ciprofloxacin. The organisms showed that 30% were less sensitive and 70% were moderately sensitive to erythromycin. Among the isolates, 70% were resistance and 30% were less sensitive to ampicillin. The organisms showed that 70% were resistance and 30% were less sensitive to amoxicillin. Among the isolates, 30% were moderately sensitive and 70% were highly sensitive to ceftriaxone (Table 4). The different isolates of *Staphylococcus* spp., *Streptococcus* spp., and *E. coli* showed identical results in different biochemical tests including sugar fermentation, catalase, methyl red, voges-proskauer (VP) and indole test. Variability on the frequency distributions of different species of bacterial isolates in different milk samples was found. Results of the present study indicate that all the three different types of bacteria were not present in the same milk sample collected from the mastitic cows. *Staphylococcus* spp. has been isolated as the main pathogens of mastitic cows. This finding is in agreement with the earlier reports made by Gupta *et al.* (1992), Jha *et al.* (1994), Boscos *et al.* (1996), Tuteja *et al.* (1993), Misra *et al.* (1993), Shike *et al.* (1998), Goswami *et al.* (2002), Seyoum *et al.* (2003), Saxena *et al.* (1993) with slight variation. In the antibiotic sensitivity test showed that 30% of the isolated *Streptococcus* spp. was moderately sensitive and 70% were highly sensitive to gentamicin. 30% of the isolates were moderately sensitive and 70% were highly sensitive to ciprofloxacin. The organisms showed that 30% were less sensitive and 70% were moderately sensitive to

erythromycin. Among the isolates, 70% were resistance and 30% were less sensitive to ampicillin. The organisms showed that 100% were resistance to amoxicillin. Among the isolates 30% were moderately sensitive and 70% were highly sensitive to ceftriaxone.

It was found that 30% of the isolated *Escherichia coli* were moderately sensitive and 70% were highly sensitive to gentamicin. Among the isolates, 30% were moderately sensitive and 70% were highly sensitive to ciprofloxacin. The organisms showed 70% were moderate sensitive and 30% were highly sensitive to erythromycin. The isolates showed that 30% were moderately sensitive and 70% were less sensitive to ampicillin. The isolates showed that 70% were less sensitive and 30% were resistant to amoxicillin. The organisms showed that 30% were moderate sensitive and 70% were highly sensitive to ceftriaxone. From the study we observed ceftriaxone, ciprofloxacin and gentamicin are the best drug for treating sub-clinical mastitis, though these antibiotics showed maximum sensitivity to the organism responsible to cause sub-clinical mastitis. The results of isolation, identification, biochemical test, frequency distribution and antibiotic sensitivity of the bacteria isolated from milk of sub-clinical mastitic cows in the present study, indicated that the microbial factors play an important role for the development of mastitis. Detailed further epidemiological study requires about the extrinsic and intrinsic factors, which might have direct or indirect influence on the development of mastitis in association with microbes, are required.

Table 4. Results of antibiotic sensitivity tests of the isolated bacteria obtained from milk samples

Name of bacteria (No of isolates)	Sensitivity	% of isolated strains sensitive to various antibiotics					
		GN	CIP	AMP	AMX	CTR	ERY
<i>Staphylococcus</i> spp. (n=10)	Resistance	00.00	00.00	70.00	70.00	00.00	00.00
	Less sensitive	00.00	00.00	30.00	30.00	00.00	30.00
	Moderate sensitive	30.00	30.00	00.00	00.00	30.00	70.00
	Highly sensitive	70.00	70.00	00.00	00.00	70.00	00.00
<i>Streptococcus</i> spp. (n=10)	Resistance	00.00	00.00	70.00	100.00	00.00	00.00
	Less sensitive	00.00	00.00	30.00	00.00	00.00	30.00
	Moderate sensitive	30.00	30.00	00.00	00.00	30.00	70.00
	Highly sensitive	70.00	70.00	00.00	00.00	70.00	00.00
<i>E. coli</i> (n=10)	Resistance	00.00	00.00	00.00	30.00	00.00	00.00
	Less sensitive	00.00	00.00	70.00	70.00	00.00	00.00
	Moderate sensitive	30.00	30.00	30.00	00.00	30.00	70.00
	Highly sensitive	70.00	70.00	00.00	00.00	70.00	30.00

GN = Gentamicin; AMP = Ampicillin; CIP = Ciprofloxacin; AMX = Amoxicillin; CTR = Ceftriaxone; ERY = Erythromycin

From the results of the present study, it may be concluded that single infection is higher than mixed infection; the mixed bacterial infections are the major causes of sub-clinical mastitis in cows. *Staphylococcus* spp. *Streptococcus* spp. and *E. coli*, are established as the major etiological agents of sub-clinical mastitis in cows from the present study. Antibiogram results indicated that gentamicin, ciprofloxacin, and ceftriaxone in optimum doses would be the drug of choice to resolve the most cases of sub-clinical mastitis. Antibiotic sensitivity results suggested not to recommend amoxicillin, ampicillin for therapeutic uses against mastitis since isolated pathogens were resistant to these drugs.

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