Prevalence and identification of sub-clinical mastitis in the commercial cattle of Rupandehi District, Nepal

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ABSTRACT

Mastitis is a major enemy of dairy farmers causing loss of milk production and degrading quality of milk leading to great economic losses. This study was carried out to assess the sub clinical mastitis, its prevalence, causative organisms and their resistance pattern to major antibiotics used. A cross sectional study was conducted in the commercial cattle farms of Rupandehi district, taking 94 milking animals randomly from the herd along with questionnaire survey with owners. California Mastitis Test (CMT) was performed at the farmer's shed and 10 ml of milk sample from the infected quarter was collected. Milk samples were subjected to culture and causative organisms were identified based on colony characteristics, Gram's staining and biochemical tests. Antibiogram was performed to find out the resistance pattern of microbes. The preventive measures of sub-clinical mastitis (SCM) were found loosely followed in the farms. Out of total 94 animals screened for SCM, 62.77% were found positive in CMT. LF (39.36%) quarter has more prevalence followed by RF (30.85%), RH (29.79%) and LH (21.28%). In total, out of 376 quarters tested for SCM, 30.32% were found positive. Among positive cases, causative organisms were coliform (57.63%), Staphylococcus (11.86%) and Streptococcus (8.47%). In 22% CMT positive samples, organisms did not grow in the culture medium. Gentamicin (68.57%) was found more sensitive antibiotics on antibiogram followed by Amikacin (62.86%), Tetracycline (57.14%) and Ciprofloxacin (51.43%). 23.9 % of the etiologic agents were found multidrug resistant. It is very crucial to follow precautionary actions like milking order, teat dipping, farm biosecurity as well as early detection and treatment with judicious use of antimicrobials to minimize economic losses.

***Keywords**: Cattle, Prevalence, Quarter, Sub-clinical mastitis*

INTRODUCTION

Dairy is an important agricultural sector which contributes about 12% to the agricultural gross domestic product (AGDP) and 62% to the livestock GDP (DLS 2014). Cattle and buffaloes are the main milk producing animals of Nepal which share 24.7% and 32.7% in milk and milk products respectively (Regmi et al., 2017). Rupandehi is one of the districts in the western region where large number (98,384) of cattle are reared for dairy purpose (MOLD, 2017). Total 43,118 Mt. milk is produced in this district, out of which 13,133 Mt. milk is contributed by 12,914 milking cows (SINA, 2017). This shows that Rupandehi district only contributes about 2.3% in the total national milk production where as 2% in the total cow milk production.

Mastitis is a multi-etiological and complex disease characterized by pathological changes in the mammary tissue followed by physical, biochemical and usually bacteriological changes in the milk. The sub-clinical form is 15-40 times more prevalent than the clinical form, and usually precedes the clinical form and is of long duration (Seegers et al., 2003). Detection of sub-clinical mastitis (SCM) is more difficult because cardinal signs are not readily apparent. It is also important to emphasize that the sub-clinically affected animals remain a continuing source of infection for herd mates (Islam et al., 2011).

SCM is financially important as besides causing loss in milk production and its quality, it also increases the chances of clinical mastitis. It can lead to a 10% to 20% decrease in milk production (Iraguha et al, 2015). In addition, it has an undesirable effect on the constituents and nutritional value of the milk, rendering it of low quality and less fit for processing (Iraguha et al., 2015). If the animal can be screened early for SCM and treated, it will be more cost effective than treating for clinical mastitis later. CMT is the most preferable test for SCM screening (Iraguha et al., 2017). Therefore, this study is aimed to find out the prevalence of SCM using CMT, identification of causative organisms and their antibiotic sensitivity pattern in the commercial cattle of Rupandehi district.

MATERIALS AND METHODS

Study Site

This study was conducted in Siddharthanagar municipality of Rupandehi district, which is the pocket area for milk production. The laboratory analysis of the collected milk sample was carried out at the Veterinary Laboratory, Pokhara.

Sampling Technique and Laboratory Examination

94 milking animals were randomly selected, out of which 50 were Jersey cross, 37 Holstein cross, 5 local cross bred and 2 were Hariyana. CMT was performed at the farmer's shed and ten ml of milk sample from each quarter was collected in a sterile bottle from the animal infected sub-clinically. They were numbered and marked as right front (RF), left front (LF), right hind (RH) and left hind (LH). All the samples brought to the laboratory were subjected to cultural examination on Nutrient agar, Mac Conkey agar and EMB media. They were incubated at 37º C for 24 hours. Cultural isolates were identified on the basis of colony characteristics, Gram's staining and different biochemical tests; IMViC, Oxidase, Catalase and Motility (Table-1). Finally, antibiotic sensitivity test (AST) was performed using commercially available Penicillin, Aminoglycosides, Quinolones, Tetracycline and Cephalosporin group of antibiotics.

Table 1. Biochemical test used for identification of cultural isolates

Bacterial isolates	Oxidase	Catalase	IMViC	Motility
E. coli	-	+	+ + - -	+
Klebsiella spp	-	+	- - - -	+
Staphylococcus spp	-		- - - -	–
Streptococcus spp	-	-	- - - -	–

RESULTS AND DISCUSSION

The husbandry practices were almost similar in all farms. Biosecurity measures were poor as well as other preventive measures of sub-clinical mastitis like teat dipping, milking order and calf weaning practice were not properly followed though all the farms had clinically infected mastitis animals (Table 2).

Table 2 Husbandry practices of five commercial cattle farmers

Husbandry practices	**no of farms**	**no of animals**
Biosecurity measures followed	1	51
Stall floor:		
Concrete	*5*	94
Concrete+mattress	*1*	51
Feeding method:		
Stall	*5*	94
Bedding:		
No bedding	*4*	89
straw	*1*	5
Use of disinfectant:		
Occasionally (Lime)	*2*	29
Calf suckling	3	44
History of clinical mastitis	5	94
Milking order	3	34
Teat dipping:		
Regular	*3*	24
Only to infected animals	*2*	56
Never	*1*	14

The prevalence of SCM was found 62.77 % which is lower than the findings of Lamsal (2018) in a study conducted in the commercial cattle of Chitwan district where the prevalence was reported 69%. Out of the total 376 quarters tested, 30.32 % were found to be affected with SCM. The highest incidence was in the LF quarter (37%) followed by RF (29%), RH (28%) and LH (20%) as shown in Table-3, which is similar to the findings of Jha et al. (1993). Khanal & Pandit, (2013) have also found highest incidence (26%) in the left fore quarter which is similar to our result.

Table 3 Screening of milking animals for SCM based on CMT

Screening Result; n (%)		Quarter wise distribution of SCM			
Positive	Negative	Teat Location	Positive	Negative	Total Prevalence n (%)
59 (62.77)	35 (37.23)	RF	29	65	29 (30.85)
		LF	37	57	37 (39.36)
		RH	28	66	28 (29.79)
Total: 94 milking cows		LH	20	74	20 (21.28)

Abbr.: RF, Right Front; LF, Left Front; RH, Right Hind; LH, Left Hind

The main causative organism causing SCM was coliform (57.63%) followed by *Staphylococcus* (11.86%) and *Streptococcus* (8.47%) as shown in Table-4. In the 22% affected milk sample, the organisms could not be grown that may be due to the antibiotics used to treat the decrease in milk production because of suspicion of SCM or it may be due to the organisms other than bacteria (fungus). Thapa & Kaphle, (2002) & Subedi & Dhakal, (2002) had also found the coliform as the main causative organism followed by *Staphylococcus*.

Table 4 Distribution of different types of subclinical mastitis on animal and quarter basis

Culture Report	No of animals (94) n (%)	No of quarters (376) n (%)
Mastitis Negative	35 (37.23)	262 (69.68)
Coliform mastitis	34 (57.63)	114 (30.32)
***Staphylococcus* mastitis**	7 (11.86)	
***Streptococcus* mastitis**	5 (8.47)	
No growth	13 (22)	

Out of the six important antibiotics used namely Amikacin, Ampicillin, Ciprofloxacin, Ceftriaxone, Tetracycline and Gentamicin (Figure 1), antibiogram revealed that majority of the isolates were sensitive to Gentamicin (68.57%) which is in concordance with Thapa & Kaphle, (2002) and Subedi & Dhakal, (2002), followed by Amikacin (62.86%), Tetracyclin (57.14%) and Ciprofloxacin (51.43%). Coliform showed a mean antimicrobial susceptibility of 27.03% to Gentamicin, 21.62% Amikacin, 21.62% Tetracyclin, 16.22% Ciprofloxacin, 9.5% Ceftriaxone and 4.05% Ampicillin (Figure 2). 23.9% isolated organisms were found multidrug resistant (Figure 3).

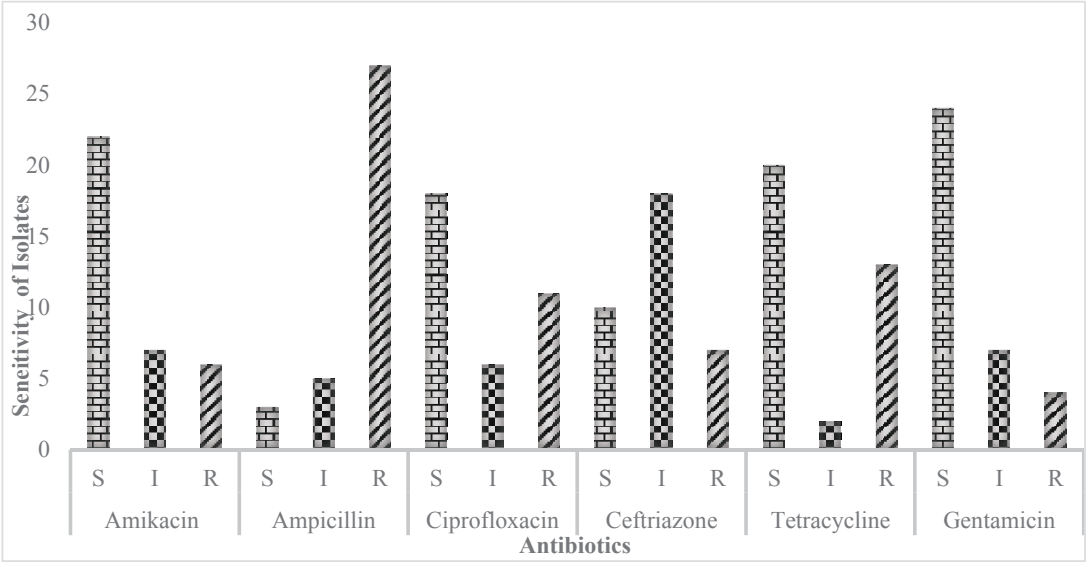

Fig 1 Antibiotic sensitivity Test (AST) of isolates (Abbr.: S, Sensitive; I, Intermediate; R, Resistant)

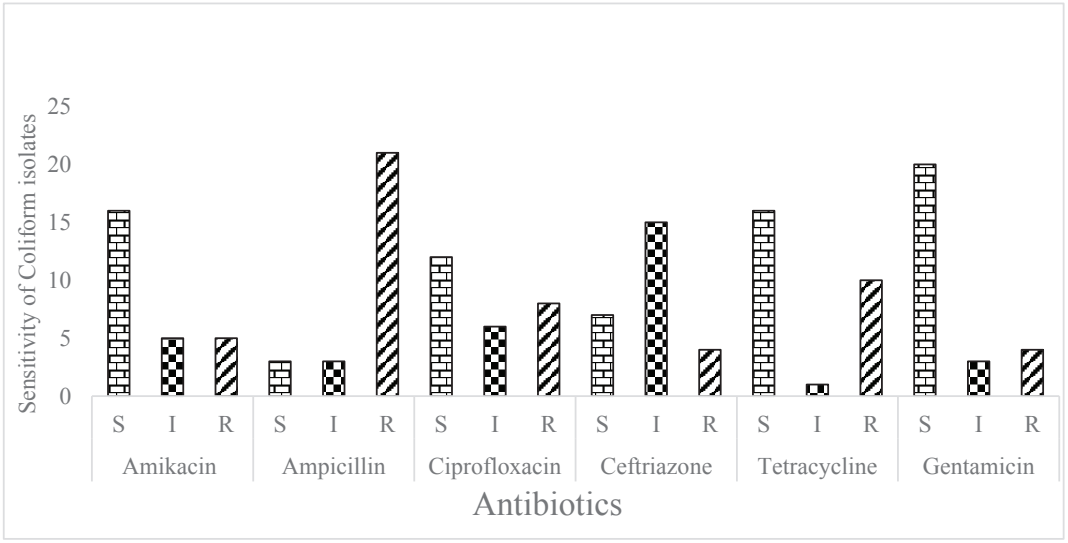

Fig 2 Antibiotic sensitivity Test (AST) of Coliform isolates (Abbr.: S, Sensitive; I, Intermediate; R, Resistant)

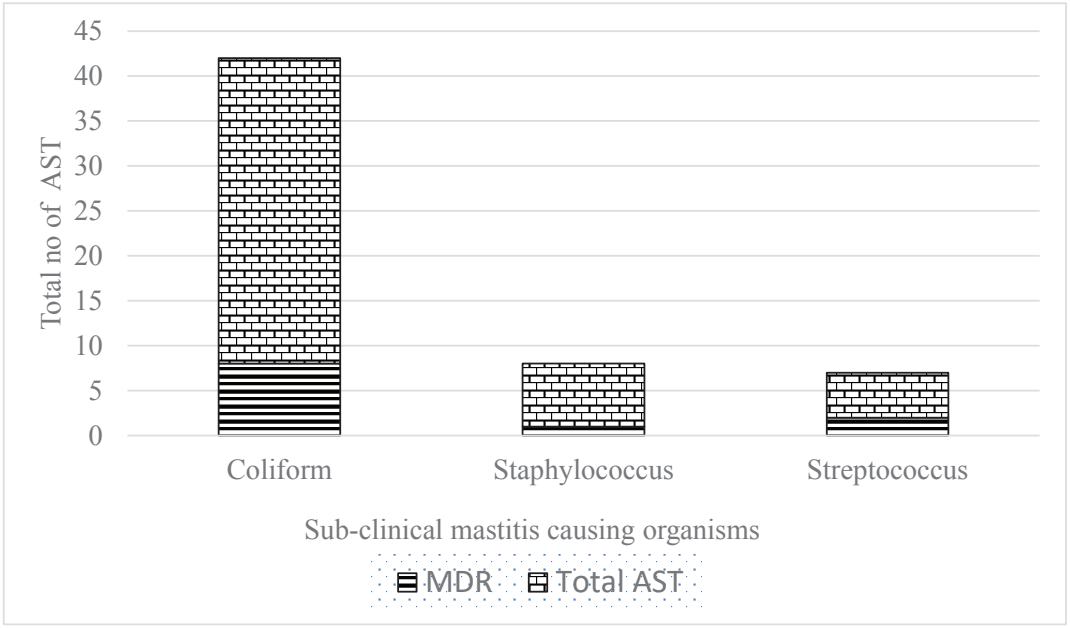

Fig 3 Multidrug resistance pattern of sub-clinical mastitis causing organisms

CONCLUSION

The prevalence of SCM was found 62.77% in the commercial cattle of Rupandehi district. This higher positivity of SCM certainly indicates that the animals are underdiagnosed and the treatment has not been initiated timely. Most of the causative organisms (23.9%) were found multidrug resistant. This shows that the dairy farmers are losing significant amount of money from this

disease. Therefore, preventive measures must be strictly followed in the farms. There is a need of regular screening of milking animals for the presence of sub-clinical mastitis. Either antimicrobial susceptibility test or at least recent knowledge of drug susceptibility ensures the right selection of antimicrobials for the successful treatment.

RECOMMENDATION

Adoption of preventive measures like milking order, teat dipping, farm biosecurity as well as early detection and treatment with judicious use of antimicrobials, will ensure the sub-clinical mastitis to control successfully.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this study.

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