DRF-304
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RESEARCH ARTICLE Asian Journal of Dairy and Food Research

Prevalence and Risk Factors of Sub-clinical Mastitis in Lactating Dairy Cows with Special Emphasis on Antibiogram of the Causative Bacteria in Bangladesh

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10.18805/ajdfr.DRF-304

ABSTRACT

Background: Mastitis is one of the most devastating conditions for the dairy farms because of its alarming impact on production. Khulna is one of the dairy intensive regions of Bangladesh but comprehensive epidemiological studies regarding prevalence and risk factors of sub-clinical mastitis with antibiogram of the causative bacteria are scant. Therefore, an epidemiological study was conducted in Khulna district to investigate the prevalence and risk factor of sub-clinical mastitis in lactating dairy cows with antibiogram of the causative bacteria.

Methods: Five dairy farms were selected and a total of 400 quarter milk samples of 100 dairy cows were subjected to California Mastitis Test to detect sub-clinical mastitis. Antibiogram study was performed to determine sensitivity and resistant pattern of the isolated bacteria.

Results: An overall cow level prevalence of sub-clinical mastitis were recorded as 28%. Risk factors like type of breed, body condition score, milk yield, grass feeding, udder washing before milking, drying of the udder after washing, production system, dry cow therapy, stimuli before milking and milking techniques were statistically significant for the occurrence of sub-clinical mastitis. Antibiogram study revealed that most of the isolated 33.03% *E. coli and* 16.96% *Staphylococcus* sp. were sensitive to amoxicillin, gentamicin and trimethoprim/sulphamethoxazole.

Key words: Antibiogram, Bacteria, Epidemiological, Mastitis, Prevalence.

INTRODUCTION

In Bangladesh, dairy farming is getting popularity day by day where a major part of the national milk supply is produced by cross-bred cows using a national AI program since 1959 (Datta *et al*., 2019). However, these cross-bred cows are more susceptible to production diseases like mastitis (Curone *et al*., 2018). Bangladesh is currently ranked among the 25 largest milk producing countries in the world. Mastitis is a global production problem to the livestock industry since it adversely affects the welfare of dairy animals with their production, milk quality and the economics of milk production, affecting many countries, including developed countries and causes significant financial losses (Sharma *et al*., 2012). The term ‘‘Mastitis” derives from Greek word “Mastos” which means breast (mammary gland). It is a multi-etiological and a very complex disease and defined as the inflammation of parenchyma of mammary glands regardless of the cause and it is characterized by a range of physical, chemical and microbiological changes in the milk and pathological changes in the glandular tissues (Radostits *et al*., 2007). Discoloration, the presence of clots and the abnormally large number of leukocytes are the most important changes in the milk during mastitis. Mastitis can be classified into three major types like clinical mastitis (CM), sub-clinical mastitis (SCM) and chronic mastitis (ChM) (Taponen *et al*., 2017).

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How to cite this article: Sohidullah, M., Hossain, M.J., Alam, M.A., Rahman, N., Salauddin, M. and Matubber, B. (2023). Prevalence and Risk Factors of Sub-clinical Mastitis in Lactating Dairy Cows with Special Emphasis on Antibiogram of the Causative Bacteria in Bangladesh. Asian Journal of Dairy and Food Research. doi:10.18805/ajdfr.DRF-304.

Submitted: 16-12-2023 **Accepted:** 10-05-2023 **Online:** 09-06-2023

Sub-clinical mastitis is more common and characterized by the presence of inflammation that is high somatic cell count in the milk without any observable clinical signs or abnormalities in the udder or milk and reduced milk production (Ndahetuye *et al*., 2019). It is also said to be important because of its 15-40 times more prevalent nature than the clinical form, character to precede the clinical form usually, long durability, difficulty to be detectable and capability to reduce milk production, affect milk quality adversely and remain as a continuing source of infection for herd mates (Islam *et al*., 2011). The source of mastitis is contagious pathogens like *Streptococcus agalactiae*,

Staphylococcus aureus and *Mycoplasma bovis*, environmental pathogens like *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Escherichia coli*, *Klebsiella* sp. and *Pasteurella* sp. and other pathogens such as *Actinomyces pyogenes*, *Pseudomonas aeruginosa*, *Nocardia asteroides*, *Clostridium perfringens*, *Mycobacterium* sp., *Prototheca* sp. and yeasts (Khan and Muhammad, 2005; Radostits *et al*., 2000). There are so many risk factors that influence the occurrence of SCM like age, parity, lactation stage, milk yield, breed, previous mastitis record, floor type, disinfection of fingers and teat dipping, *etc*. (Madut *et al*., 2009). Other factors like lack of awareness; delay in disease detection in the absence of visible signs of abnormal milk; unhygienic milking practices; and delayed and incomplete treatment of clinical and chronic mastitis also contribute (Sharma *et al*., 2012). Diagnosis of SCM is not so easy because the milk appears normal but usually has an elevated somatic cell count (Islam *et al*., 2010). It can be detected by screening tests like California Mastitis Test (CMT), White Side Test (WST), Surf Field Mastitis Test (SFMT) (Madut *et al*., 2009). In detecting SCM, CMT was better (37.58%) than WST (36.67%) and SFMT (35.15%) (Islam *et al*., 2010). Under field conditions, CMT is done for the determination of somatic cell count (SCC) in milk where CMT scores are directly related to average SCC (Pyorala, 2003). Intramammary antibiotic therapy is effective for Gram-positive organisms and coagulase-negative staphylococci, but ineffective for Gram-negative organisms (Roberson *et al*., 2004). If teats are dipped after milking and cows are treated with penicillin-dihydrostreptomycin at dry-off, intramammary infection caused by major mastitis pathogens may be decreased by 75% and 45% respectively. For controlling sub-clinical mastitis, there is no easy solution. However, the easiest and most economical way to control intramammary infections is to maintain proper milking procedure and hygiene (Hutton *et al*., 1990). Our present study was conducted to know the prevalence and risk factors of SCM in the lactating dairy cows of Bangladesh. Moreover, we performed antibiotic sensitivity assay to know the antibiotic sensitivity status of the isolated bacteria.

MATERIALS AND METHODS

Study area and duration

The field investigation by CMT test was done at five dairy farms of Khulna district of Bangladesh during the period from October 2021 to April 2022. The study area of tropical wet and dry climate with an annual average temperature and rainfall of 26.3°C and 1,878.4 mm respectively is geographically located in between 21°41' and 23°00' north latitudes and in between 89°14' and 89°45' east longitudes. It is bounded by Jessore and Narail districts on the north, Bay of Bengal on the south, Bagerhat district on the east, Satkhira district on the west. All the laboratory tests were performed at the Bacteriology laboratory of Khulna Agricultural University.

Questionnaire preparation and data collection

During the farm visits, a pretested and structured questionnaire was used for data collection from each animal and herd. Both dichotomous and polychotomous questions were asked and the responses were coded for statistical analysis. Each questionnaire asking time was about 10-15 minutes. Though questionnaire was prepared in English, questions were asked in Bangla during data collection. Before collecting data, farmer's consent was taken. The information related with farm biodata and farm management practices were production system (intensive/semi-intensive), floor type (concrete/earthen), bedding (yes/no), floor cleaning frequency (daily/weekly), milking techniques (hand milking/machine milking), milking mastitic cow last (yes/no), washing of the udder before milking (yes/no), drying of the udder after washing (yes/no), type of stimuli before milking (calf/mustard oil/water), routine testing for mastitis (yes/no), use of teat dips (yes/no), dry therapy (yes/no), culling (yes/no), grass feeding (yes/no) and hand washing before milking (yes/no). The information regarding cow factors were age (2-4, >4-8 and >8 years), breed (cross/indigenous), body condition score (BCS) (2-2.5, >2.5-3 and >3), teat type, udder type, pregnancy, parity (1-2 calves, 3-4 calves and ≥5 calves), lactation stage (15-60, >60-120 and >120 days), milk yield (0.5-3, >3-5 and >5 L), history of previous clinical mastitis (yes/no) and history of reproductive diseases (yes/no). Data collection was done by interviewing the farm owners and in some cases abstracting the farm records. Data on cow factors like BCS, teat type, udder type, *etc*. were recorded by examining the cows. The collected risk factors were compared to the occurrence of sub-clinical mastitis based on isolation and identification of bacteria.

California mastitis test (CMT) and collection of milk samples

The procedure of CMT was followed in this study as per manufacturer's instruction (Leukocyst®, Synbiotics Corporation, France). All quarter milk samples (n=400) were collected conveniently from 100 cows were screened for subclinical mastitis using CMT either before or after the collection of data after wiping the teat by cotton soaked with 70% ethanol where there were 54 cross-bred and 46 indigenous cows. In brief, about 2 ml milk was collected in individual cups by hand milking when the paddle held nearly horizontal position and an estimated equal volume of CMT reagent which is composed of Alkyl Aryl Sulfonate (3%), Sodium hydroxide (1.5%) and bromocresol purple (1:10,000) was squirted from a polyethylene wash bottle. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane for few seconds. The reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of reaction was obtained within 10 seconds and scored. The paddle was rinsed properly with water before being used for the next test. The CMT test results were classified as either negative or positive depending on the intensity of reaction. The result of the CMT

was scored and recorded based on gel formation. A cow was defined as CMT positive if it had at least one quarter with positive CMT reaction (Doherr *et al*., 2007). Grades of the CMT were evaluated and the results scored as 1 for negative and 2, 3 and 4 for positive (Table 1). Identification of SCM was made based on physical examination of udder, nature and appearances of milk secretion and reaction to CMT (Radostits *et al*., 2007). Accordingly, a CMT positive cow with no visible abnormality of the milk and udder was defined as SCM positive (Radostits *et al*., 2007). In total, 112 quarter milk samples from 28 CMT positive cows of five dairy farms were collected aseptically into the sterile plastic tubes and transported to the laboratory using ice-box.

Inclusion and exclusion criteria

Cows with no visible abnormality of the milk and udder were included but the cows that were in the first 14 days of lactation or experienced with clinical mastitis during study were excluded.

Data analysis

All the collected data were entered into MS excel worksheet and transferred into statistical package for social science (SPSS version 26) for statistical analysis to estimate the strength and statistical significance of associations between risk factors and SCM.

Enrichment and isolation of *E. coli* and *staphylococcus* sp.

Enrichment and isolation of *E. coli* and *Staphylococcus* sp. from the milk samples were performed according to standard methods with slight modification (Laboratory Handbook on Bovine Mastitis; Halder *et al*., 2022). 500 µl of the collected milk sample was inoculated into 4.5 ml Nutrient broth (HiMedia, India) followed by incubation overnight at 37°C. 100 µl of the enriched culture was streaked onto Eosin Methylene Blue (EMB) Levine agar (Liofilchem, Italy) and Mannitol Salt (MS) agar (Liofilchem, Italy) and incubated overnight at 37°C. After overnight incubation, colonies specific for *E. coli* and *Staphylococcus* sp. were picked and purified colonies were isolated by subsequent streaking onto EMB and MS agar plates (Liofilchem, Italy).

Identification of *E. coli* and *staphylococcus* sp. by conventional methods

For identification of isolated *E. coli* and *Staphylococcus* sp., Gram staining and biochemical tests were performed. In case of Gram staining, the method described by Merchant and Packer, (1967) and Sohidullah *et al*. (2016) was performed where all the reagents like crystal violet, Gram's iodine, safranin, acetone alcohol, immersion oil were brought from the German company, Merck. Biochemical tests such as sugar fermentation test, MR-VP reaction, indole reaction and catalase test were performed according to the methods described by OIE, (2000) where all the reagents were brought from the German company, Merck.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of the isolated *E. coli* and *Staphylococcus* sp. was determined by disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI, 2007, 2018) and interpreted as susceptible, intermediate and resistant. A total of 8 antimicrobials comprising five different antimicrobial classes commonly used in the dairy farms and human clinical cases in Bangladesh were selected in this study. Commercially available 8 antibiotic discs (Bioanalyse, Turkey) which belonged to 5 different antimicrobial classes namely aminoglycosides (Gentamicin-CN), cephalosporins (Ceftriaxone-CRO, Cephradine-CE), fluoroquinolones (Ciprofloxacin-CIP), penicillins (Amoxicillin-AX, Penicillin G-P, Cloxacillin-CX) and sulfonamides (Trimethoprim/ sulphamethoxazole-SXT) were used in this study.

RESULTS AND DISCUSSION

Risk factors

Out of 400 samples collected from 5 dairy farms of Khulna district, we recorded an overall cow level prevalence of SCM as 28% (n=112) through examination by CMT. Although the present study reported SCM prevalence was lower than the study conducted by Abrahmsén *et al*. (2014) in Uganda (86.2%) and Mekonnen *et al*. (2017) in Ethiopia (62%) but within the range (19.9% - 44.8%) stated by some researchers (Rabbani and Samad, 2010; Islam *et al*., 2012).Out of 26 variables, 10 variables like type of breed, body condition score (BCS), milk yield in litter, grass feeding, udder washing before milking, drying of the udder after washing, production system, dry cow therapy, stimuli of cow before milking and milking techniques were documented as statistically significant for the occurrence of sub-clinical mastitis in dairy cows (Table 2 and 3). We recorded higher prevalence in the cows of 2-4 years and >8 years of age compared to the cows of >4-8 years of age (Table 2). Our findings were

Table 1: Scoring of california mastitis test (CMT) results.

Reading	Interpretation	Score
Mixture remains liquid, no slime or gel formation	Negative	1
Mixture becomes slimy or gel like. It seems to best advantage by tipping the CMT paddle back and forth, while observing mixture as it flows over the bottom of the cup	Suspicious (mild)	2
Mixture distinctly forms a gel	Positive (moderate)	3
Mixture thickens immediately tends to form jelly. Spinning the cup moves the mixture in towards the centre exposing the outer edges of the cup	Positive (severe)	4

supported by Barua *et al*. (2014) where they commented that the teat canal of older animals become more dilated which persists permanently due to years of repeated milking and leads to long time exposure to SCM causing microorganisms. In case of breed type, we recorded higher prevalence of SCM in cross-breed dairy cows and our findings were almost similar to the findings of Almaw *et al*. (2008) (Table 2). Sarker *et al*. (2013) reported that animals with higher BCS might produce more milk which makes them prone to SCM. We also recorded higher prevalence of SCM in the cows of higher BCS (Table 2). Our findings of increased prevalence of SCM with the advancement of parity are in line with the findings of Rabbani and Samad, (2010) (Table 2). A strong association of the occurrence of SCM with pendulous udder and cylindrical teats was reported earlier (Uddin *et al*., 2009). We also recorded higher prevalence of SCM in the cows with pendulous udders and cylindrical teats (Table 2). Cows with history of previous clinical mastitis are at greater risk of being re-infected, because repeated infections of the mammary tissues with microorganisms along with other stress factors could put the mammary glands at greater risks of re-infection and the treatment of clinical mastitis may suppress the clinical signs, but infective agents are not completely eliminated and infection may remain in subclinical form (Biffa *et al*., 2005). We recorded almost similar findings in our study (Table 2). Another important risk factor was the feeding of no green grass to the dairy cows where they contain different vitamins and trace minerals that have the influence on the sound udder health and are related with the increased resistant to intramammary infection (Warly *et al*., 2010). In this study, we recorded 50% of the SCM affected cows that were exposed to no grass feeding (Table 3). Most of the farm owners of our country have tendency to milk the cows for a very long periods; sometimes more than a year, which can increase the risk of SCM (Abrahmsén *et al*., 2014). Our findings also supported this study where we recorded higher prevalence of SCM in the cows with >120 days of lactation (Table 2). A significant risk factor for SCM is floor type and its cleanliness where cemented floor and cleanliness of it was documented previously as an important factor for increasing the risk of SCM occurrence elsewhere (Mekonnen

Table 2: Distribution of SCM in relation to different cow related risk factors.

Risk factors	Categories	Sub-clinical Mastitis			
		Present (n=28) No. (%)	Absent (n=72) No. (%)	Chi-Square (χ^2) value	*p*-value
Age (years)	2-4	18 (64.28%)	37 (51.38%)	6.559	0.585
	>4-8	9 (32.14%)	32 (44.44%)		
	>8	1 (3.57%)	3 (4.16%)		
Breed	Cross	21 (75%)	33 (45.83%)	6.904	0.009*
	Indigenous	7 (25%)	39 (54.16%)		
BCS	2-2.5	0 (0.00%)	11 (15.27%)	15.475	0.004*
	>2.5-3	3 (10.71%)	27 (37.5%)		
	>3	25 (89.28%)	34 (47.22%)		
Parity	1-2	1 (3.57%)	10 (13.88%)	8.151	0.227
	3-4	11 (39.28%)	24 (33.33%)		
	≥5	16 (57.14%)	38 (52.77%)		
Stage of lactation (days)	15-60	1 (3.57%)	4 (5.55%)	39.815	0.848
	>60-120	10 (35.71%)	29 (40.27%)		
	>120	17 (60.71%)	39 (54.16%)		
Milk yield (L)	0.5-3	26 (92.85%)	49 (68.05%)	16.788	0.032**
	>3-5	2 (7.14%)	22 (30.55%)		
	>5	0 (0.00%)	1 (1.38%)		
Pregnancy	Yes	11 (39.28%	32 (44.44%)	0.219	0.640
	No	17 (60.71%)	40 (55.55%)		
Udder type (pendulous)	Yes	26 (92.85%)	57 (79.16%)	2.678	0.102
	No	2 (7.14%)	15 (20.83%)		
Teat type (cylindrical)	Yes	24 (85.71%)	56 (77.77%)	0.794	0.373
	No	4 (14.28%)	16 (22.22%)		
History of previous clinical mastitis	Yes	17 (60.71%)	45 (62.5%)	0.027	0.869
	No	11 (39.28%)	27 (37.5%)		
History of reproductive disease	Yes	17 (60.71%)	45 (62.5%)	0.027	0.869
	No	11 (39.28%)	27 (37.5%)		

*, ** indicate 1% and 5% levels of significance respectively

et al., 2017). We also recorded almost similar associations in our study (Table 3). This study revealed that farms which did not practice milking mastitic cows last were more likely to have mastitis than the farms where it was practiced (Table 3). This finding was in agreement with the findings of Abebe *et al*. (2016) and Nielsen and Emanuelson, (2013). Farms that did not use an udder towel for each cow had significantly higher mastitis than farms that used it (Table 3) and it was supported by the findings of Abebe *et al*. (2016) and Mekonnen *et al*. (2017) who documented that the practice of the same drying towel was responsible for spreading mastitis pathogens. It is reported that, the practice of dry cow therapy is necessary to achieve an efficient control of SCM (Hashemi *et al*., 2011). We also recorded almost similar finding (Table 3). We recorded that the cows which were under the regular practice of teat dipping were less prone to SCM (Table 3) which was supported by Kivaria *et al*. (2004) who documented almost similar finding. The prevalence of SCM was higher in cows given stimuli by the calves, compared to other methods of stimuli (Table 3). Barua *et al*. (2014) also documented almost similar findings.

Isolation and cultural characterization of *E. coli* and *staphylococcus* sp.

The growth of *E. coli* and *Staphylococcus* sp. was indicated by the presence of turbidity in the nutrient broth after overnight incubation at 37°C. Following streaking, *E. coli* produced greenish-black colonies with metallic sheen on EMB agar and *Staphylococcus* sp. produced two types of colonies on MS agar where one type was yellow colonies with yellow zones and the other type was colorless colonies with light pink colored media. Halder *et al*. (2022) and Hasan *et al*. (2016) also reported almost similar findings in their study. Out of 112 collected milk samples, 37 (33.03%) were

Table 3: Distribution of SCM in relation to different production and management related risk factors.

Risk factors	Categories	Sub-clinical mastitis			
		Present (n=28) No. (%)	Absent (n=72) No. (%)	Chi-Square (χ^2) value	*p*-value
Floor type	Earthen	25 (89.28%)	55 (76.38%)	2.096	0.148
	Concrete	3 (10.71%)	17 (23.61%)		
Grass feeding	Yes	14 (50%)	19 (26.38%)	5.083	0.024**
	No	14 (50%)	53 (73.61%)		
Teat dipping before and after milking	Yes	3 (10.71%)	17 (23.61%)	2.096	0.148
	No	25 (89.28%)	55 (76.38%)		
Udder washing before milking	Yes	25 (89.28%)	38 (52.77%)	11.527	0.001*
	No	3 (10.71%)	34 (47.22%)		
Drying of the udder after washing	Yes	26 (92.85%)	38 (52.77%)	14.056	0.0001*
	No	2 (7.14%)	34 (47.22%)		
Hand washing before milking	Yes	3 (10.71%)	13 (18.05%)	0.808	0.369
	No	25 (89.28%)	59 (81.94%)		
Milking of mastitic cow last	Yes	6 (21.42%)	27 (37.5%)	2.355	0.125
	No	22 (78.57%)	45 (62.5%)		
Floor cleaning frequency	Daily	7 (25%)	9 (12.5%)	2.344	0.126
	Weekly	21 (75%)	63 (87.5%)		
Bedding	Yes	26 (92.85%)	64 (88.88%)	0.353	0.553
	No	2 (7.14%)	8 (11.11%)		
Production system	Intensive	20 (71.42%)	38 (52.77%)	2.879	0.090***
	Semi-intensive	8 (28.57%)	34 (47.22%)		
Dry cow therapy	Yes	9 (32.14%)	9 (12.5%)	5.270	0.022**
	No	19 (67.85%)	63 (87.5%)		
Stimuli of cow before milking	Clean water	1 (3.57%)	0 (0.00%)	9.120	0.010*
	Mustard oil	12 (42.85%)	52 (72.22%)		
	Calf	15 (53.57%)	20 (27.77%)		
Routine testing for mastitis	Yes	15 (53.57%)	41 (56.94%)	0.093	0.760
	No	13 (46.42%)	31 (43.05%)		
Udder towel for each cow	Yes	0 (0.00%)	3 (4.16%)	1.203	0.273
	No	28 (100%)	69 (95.83%)		
Milking techniques	Hand milking	20 (71.42%)	62 (86.11%)	2.944	0.086***
	Machine milking	8 (28.57%)	10 (13.88%)		

*, **, *** indicate 1%, 5% and 10% levels of significance respectively.

detected as positive for *E. coli*, 19 (16.96%) for *Staphylococcus* sp. (Fig 1). Hasan *et al*. (2016) reported 23.0% isolates as Staphylococci and 3.0% isolates as *E. coli* among 42 positive cases of SCM.

Identification of *E. coli* and *staphylococcus* sp. by conventional methods

Halder *et al*. (2022) reported that in Gram staining, *E. coli* revealed as Gram negative, pink colored, short plump rod-shaped appearance arranged as single, paired or in short chain. Our findings were almost similar with those findings. Hasan *et al*. (2016) reported *Staphylococcus* sp. as Gram positive, violet colored, spherical shaped appearance arranged as clusters resembling bunch of grapes in Gram staining. We also recorded similar findings. Begum *et al*. (2016) reported *E. coli* as positive to fermentation of five basic sugars such as glucose, fructose, lactose, sucrose and mannitol with the production of acid and gas. In our study we also recorded similar findings. Hasan *et al.* (2016) documented *Staphylococcus* sp. as fermenter of all the sugars only with the production of acid. Our findings were also in line with those findings. *E. coli* were found as indole positive where *Staphylococcus* sp. were found as indole negative. *E. coli* were detected as MR test positive, VP test negative and catalase test positive while *Staphylococcus* sp. were detected as positive for MR test, VP test and

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

Name of the bacteria	Resistant		Intermediate		Sensitive	
	Antibiotic	No. (%)	Antibiotic	No. (%)	Antibiotic	No. (%)
E. coli (n=37)	AX	0 (0%)	AX	14 (37.8%)	AX	23 (62.1%)
	P	34 (91.8%)	P	3 (8.1%)	P	0 (0%)
	CX	37 (100%)	CX	0 (0%)	CX	0 (0%)
	CIP	0 (0%)	CIP	0 (0%)	CIP	37 (100%)
	CRO	6 (16.2%)	CRO	12 (32.4%)	CRO	19 (51.3%)
	CE	11 (29.7%)	CE	21 (56.7%)	CE	5 (13.5%)
	CN	6 (16.2%)	CN	3 (8.1%)	CN	28 (75.6%)
	SXT	0 (0%)	SXT	7 (18.9%)	SXT	30 (81.0%)
Staphylococcus sp.	AX	0 (0%)	AX	5 (26.3%)	AX	14 (73.6%)
(n=19)	P	0 (0%)	P	3 (15.7%)	P	16 (84.2%)
	CX	3 (15.7%)	CX	7 (36.8%)	CX	9 (47.3%)
	CIP	0 (0%)	CIP	2 (10.5%)	CIP	17 (89.4%)
	CRO	11 (57.8%)	CRO	5 (26.3%)	CRO	3 (15.7%)
	CE	3 (15.7%)	CE	6 (31.5%)	CE	10 (52.6%)
	CN	0 (0%)	CN	4 (21.0%)	CN	15 (78.9%)
	SXT	1 (5.2%)	SXT	5 (26.3%)	SXT	13 (68.4%)

AX- Amoxicillin; P- Penicillin G; CX- Cloxacillin; CIP- Ciprofloxacin; CRO- Ceftriaxone; CE- Cephradine; CN- Gentamicin; SXT- Trimethoprim/ sulphamethoxazole.

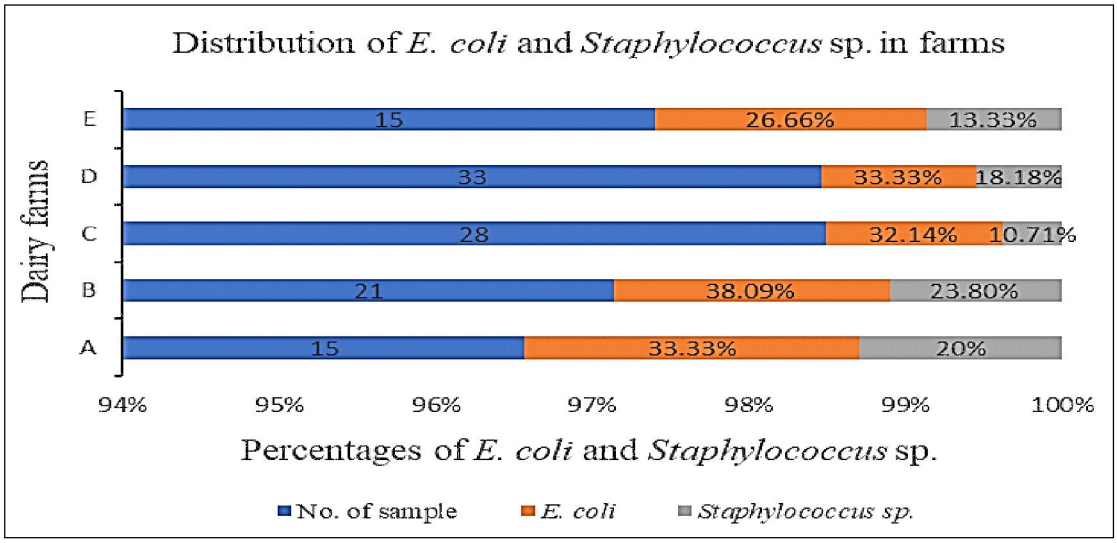

Fig 1: Distribution of *E. coli* and *Staphylococcus* sp. in five dairy farms.

catalase test. Begum *et al*. (2016) and Singha *et al*. (2021) also reported more or less similar findings in their study.

Antimicrobial susceptibility testing

All the isolates of *E. coli* and *Staphylococcus* sp. were subjected to the antibiogram study. The results of antibiogram study are presented in Table 4. All the *E. coli* isolates were resistant to cloxacillin, but sensitive to ciprofloxacin (Table 4). Most of the *E. coli* isolates were resistant to penicillin, intermediately resistant to cephradine, but sensitive to amoxicillin, ceftriaxone, gentamicin and trimethoprim/sulphamethoxazole where their percentages were 91.8%, 56.7%, 62.1%, 51.3% 75.6% and 81.0% respectively (Table 4). Hasan *et al*. (2016) reported almost similar findings. 73.6%, 84.2%, 47.3%, 89.4%, 52.6%, 78.9% and 68.4% *Staphylococcus* sp. isolates were sensitive to amoxicillin, penicillin, cloxacillin, ciprofloxacin, cephradine, gentamicin and trimethoprim/sulphamethoxazole respectively where 57.8% were resistant to ceftriaxone (Table 4). Hasan *et al*. (2016) documented more or less similar findings in their study.

CONCLUSION

From the results of the present study, it may be concluded that different factors are significantly associated with the occurrence of subclinical mastitis in the lactating dairy cows like type of breed, BCS, milk yield in litter, grass feeding, udder washing before milking, drying of the udder after washing, production system, dry cow therapy, stimuli of cow before milking and milking techniques which should be considered for the control of SCM. *E. coli* and *Staphylococcus* spp. were recorded as the major causal agents of SCM in cows from the present study. Antibiogram study indicated that gentamicin, ciprofloxacin, amoxicillin and trimethoprim/sulphamethoxazole in optimum doses would be the drug of choice to resolve the most cases of sub-clinical mastitis. By maintaining the proper hygienic condition in the dairy farm along with their milk production procedures, it may be possible to reduce SCM in the study area. Since SCM is clinically an undetectable problem, awareness building among the farm owners through workshop and training may also prevent the considerable economic losses.

Conflict of interest: None.

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