TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURE

LIST OF ABBREVIATIONS AND SYMBOLS USED

ABBREVIATIONS AND SYMBOLS	ELABORATIONS
UVH	Upazilla Veterinary Hospital
SAQTVH	S. A Quaderi Teaching Veterinary Hospital
CVASU	Chittagong Veterinary and Animal Sciences University
BPW	Buffer Peptone Water
UK	United Kingdom
CS	Cultural Sensitivity
AML	Amoxicillin
CRO	Ceftriaxone
CTX	Cefotaxime
CN	Gentamycin
CT	Colistin Sulphate
S	Streptomycin
CIP	Ciprofloxacin
CI	Confidence Interval
MIC	Minimum Inhibitory Concentration
EMB	Eosin Methylene Blue
MH	Mueller Hinton
CAEV	Canine Arthritis Encephalitis Virus
CLSI	Clinical and Lab Standard Institute

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ABSTRACT

Rearing of goat has an important impact in improving social-economic status of rural people of the country. Coliform mastitis caused by *Escherichia coli* is one the predominant constraints of this livestock production in this country. This study was designed to investigate the situation of coliform mastitis by *E. coli* in clinically affected goat in Chittagong. A total of 29 milk samples of mastitic doe were collected from upazilla veterinary hospital of Hathazari, Rangunia and SAQ Teaching Veterinary Hospital, CVASU during internship placements. Confirmation of coliform mastitis by *E. coli* was done following culture (on MacConkey and EMB agars), biochemical and staining techniques. Later, isolates were tested against 7 antimicrobials of 5 different groups using disc-diffusion technique. Epidemiological data were analyzed using STATA software to reveal their association with occurrence of *E. coli* coliform mastitis. Out of 29 samples 8 were confirmed as *E. coli* (prevalence 27.6%; 95% confidence interval (CI) 14.5%-45.9%). In relation to different host factors such as breed, age, body weight and parity the highest occurrence was observed in cross breed, doe of ≤ 2 years, doe having body weight of > 30 kg, and in doe of 1st parity which was 50%, (95% CI 2.5%-78.4%), 28.6% (95% CI 7.6-64.8), 36.4% (95% CI 15%-64.8%), and 50% (95% CI 21.5%-78.45) respectively. *E. coli* isolates were highly sensitive to Gentamycin (75%) and Colisitn sulfate (75%) followed by Streptomycin (37.5%) and Ceftriaxone (37.5%). Highest resistance was recorded against Amoxicillin (100%) and Ciprofloxacin (100%) followed by Cefotaxime (62.5%), Ceftriaxone (50%). Statistical analysis using chi^2 test showed that there is no significant relationship between host factors with the occurrence of *E. coli* coliform mastitis in doe. Study suggests use of Gentamycin and Colistin sulfate for the treatment of coliform mastitis in doe caused by *E. coli*.

Key words: Coliform mastitis, *E. coli*, doe

CHAPTER-1

INTRODUCTION

Bangladesh is an agricultural country containing large number of domestic animals. Goat is one of them. Currently estimated goat population in Bangladesh is about 14.8 million (Banglapedia). This density has been increasing every year in the country. In agriculture-based country like Bangladesh goat is more familiar as poor man's cow. Initial investment for starting goat farming is lesser than dairy, piggery, poultry and consumes less feed which is about one fifth of the consumption in cattle and buffalo (Das, 2001). In Bangladesh, goats are reared in only backyard farming system. Low income people are like to rear goat in their household for extra income. They prefer goat rearing than cattle because, it needs small scale space, low feeding cost, high litter size, low manpower for maintenance and meat has good market demand.

Goat farming in Bangladesh is very challenging due to many problems. Lack of financial and technical support, inadequate veterinary services are most crucial. Infectious diseases make the condition worse for goat rearing mainly viral diseases like PPR, goat pox, contagious ecthyma and viral pneumonia, and bacterial diseases include enterotoxaemia, tetanus, brucellosis, mastitis and metritis whereas main fungal diseases are ring worm infection, and rickettial infections like conjunctivitis are common causes for goat mortality in rural areas. Gastro-intestinal nematodiasis, fascioliasis and tape worm infestations cause less mortality but cause severe depression in the growth and reproductive rate of the goats. Production disease such as mastitis, pregnancy toxemia, mineral deficiency also decreases production (Kashem et al., 2012).

Common goat breeds reared in Bangladesh are: Black Bengal, JamunaPari, Crossbred- Black Bengal JamunaPari. More than 90% of the goats of the country is Black Bengal breed.

The advancement of goat farming in Bangladesh is interrupted by a number of constraints of which major one is mastitis. Mastitis is an inflammation of the parenchyma of mammary gland (udder). It is characterized by physical, chemical and usually, bacteriological changes in milk and pathological changes in glandular tissues (Radostis et al., 2000).

The bedding used to house in animal is the primary source of environmental pathogens, but contaminated teat dips, intramammary infusions, water used for udder preparation before milking, water ponds or mud holes, skin lesions, teat trauma, and flies have all been incriminated as sources of infection (Matofari et al., 2003; Kivaria and Noordhuizen, 2007). Majority of coliform isolates from a raw milk there were *E. coli* 32%, *Enterobacter* spp. 29.2%, *Klebsiella* spp. 19.4%, *Serratia*spp. 11.1% and Citrobacter 1.0% (Salman and Hamad, 2011).Coliform bacteria causes as many as 30-40% of the clinical mastitis in farm. *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiellapneumoniae*and *Serratiamarcesans* are four common coliform bacteria that causes mastitis. Among the coliform mastitis only *E. coli* cause 5.719% mastitis in goat.

Depending on the severity of the disease, mastitis is classified as clinical and sub-clinical mastitis. Clinical mastitis results in alterations of milk composition and appearance, decreased milk production, and the presence of the cardinals signs of inflammation (pain, swelling and redness, with or without heat in infected mammary quarters). It is readily apparent and easily detected. In contrast, detection of mammary quarters with sub-clinical mastitis is more difficult because signs are not readily apparent (Kivaria, 2006) and because of the lack of any overt manifestation, its diagnosis is a challenge in dairy animal management and in veterinary practice. The sub-clinical form is 15 to 40 times more prevalent than the clinical form, and usually precedes the clinical form and is of long duration (Seeger et al., 2003).

The most common bacteria that causes mastitis in goats are: *Staphylococcus aureus,* coagulase-negative *Staphylococci*spp., *Streptococcus agalactiae, Streptococcusuberi, Streptococcus. dysgalactia, Streptococcus caprae, Mycoplasma capricolum, Enterobacteria*spp, *Escherichia coli, Pseudomonas aeruginosa*, *Clostridium* sppetc.Thecaprine arthritis-encephalitis virus (CAEV) causes mastitis in goats. In addition, mastitis can result from yeast infection, and it appears to be associated with the frequent use of penicillin, along with the prolonged and repetitive use of systemic and intra-mammary infusions.

Escherichia coli is a gram negative, non-spore forming rod. It may or may not be mobile. (Some rods are flagellated and some are not.) The organism is a facultative anaerobe and ferments simple sugars such as glucose to form lactic, acetic, and formic acids.The optimal conditions for growth are a temperature of 98.6°F, with a range of 45° to 114°F. The optimum pH for growth is 6.0 to 8.0. However, growth can occur as low as pH 4.3 and as high as 9 to 10 pH (Banwart, 1983; Mitscherlich and Marth, 1984).

Bacteriological culturing of the milk can be used to determine if mastitis is caused by *E. coli causing* coliform bacteria. Amoxicillim, Ceftiofur, Penicilln, Cephapirim are those types of systemic antibiotic which can be used in coliform mastitis. In Bangladesh numerous antibiotics are used in field condition for treating the mastitis in goat.According to (Gerrit et al., 2015) commonly ued antimicrobilals are Gentamycin, Gentamycin+Amoxicillin, Amoxicillin, Benzyl-Penicillin, Streptomycin+Procaine Penicillin, Ceftriaxone, Sulphadimidine, Gentamycin+Sulphadimidine+Trimethoprim, Gentamycin+Amoxicillin+Sulphadimidine, Tetracycline.

There is very little or no published information regarding the occurrence of clinical mastitis in goat caused by *E. coli* in Chittagong, Bangladesh. Considering the social and economic importance of goat rearing in Bangladesh present study was undertaken to reveal the present scenario in Chittagong.

The objectives of the present study are:

1. To know the prevalence of coliform mastitis caused by *E. coli* in clinically affected goat.
2. To investigate the antimicrobials sensitivity pattern of *E. coli* that causing mastitis in goat.
3. To reveal the association of risk factors with the occurrence of coliform mastitis.

CHAPTER-2

MATERIALS AND METHODS

2.1 Study area and duration:

The study was conducted during the period of 22nd January to 6th April; 2017.About 29 milk samples were collected from the doe which were suffering from clinical mastitis. Most of the samples were collected during Upazilla Veterinary Hospital (UVH) placement of internship.Eighten samples from UVH, Hathazari, seven sample from S. A Quaderi Teaching Veterinary Hospital (SAQTVH), CVASU during the period of lab rotation and another four samples from Rangunia Veterinary Hospital, Rangunia.

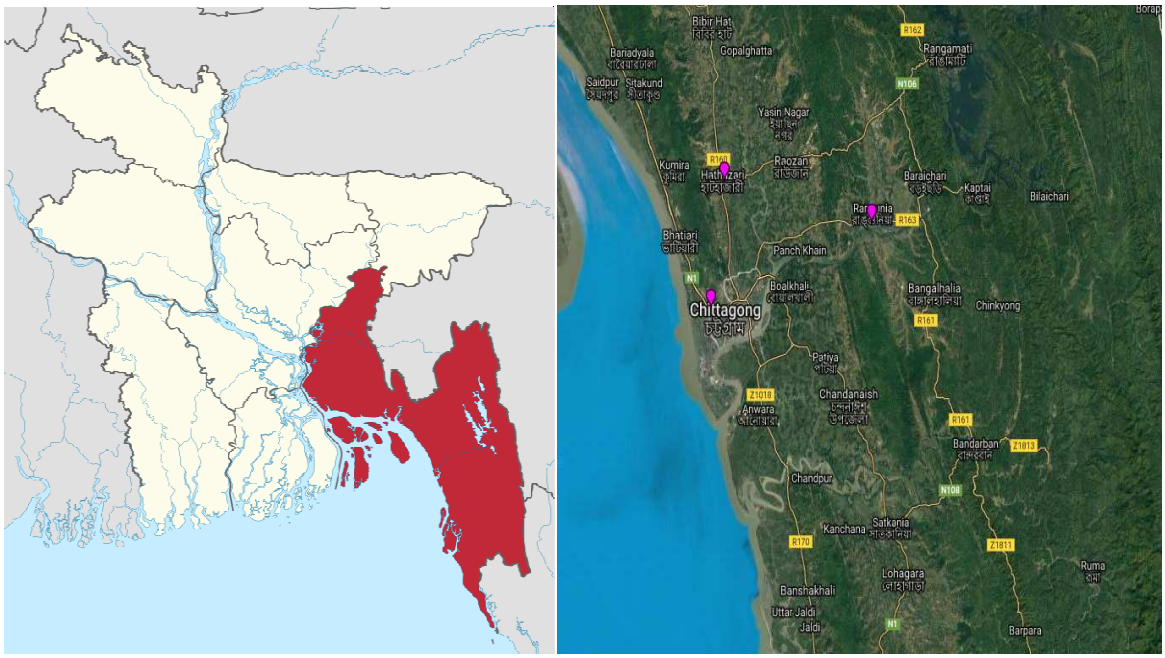

Fig: Geographical location of sampling area

2.2 Sample collection:

The samples were collected from mastitis infected quarter(s) of the mammary gland through hand milking following aseptic procedure and immediately transferred into eppendorf tube. Samples were transported from the collection site to Microbiology Laboratory, CVASU maintaining cool chain for detailed analysis. Collected samples were preserved in a refrigerator at 4ºC until screening out the bacteria.

2.3 Data collection:

Data were collected during sample collection using a pre-designed questionnaire. Collected data include basic information regarding the animals (breed, age, parity, lactation period, body weight, litter size, previous mastitis history etc) and records on treatment of mastitis. Owner's contact details were collected to follow up the cases.

2.4 Isolation and identification of *E. coli*:

For the isolation of *E. coli* from each collected milk samples at first 1ml milk sample was inoculated into the test tube containing buffer peptone water (BPW) (Oxoid Ltd, P^H:6.2±0.0, Basingstoke, Hampshire, UK) and incubated at 37°C overnight for primary enrichment. After primary enrichment the culture was streaked on MacConkey agar medium (Oxoid Ltd, P^H: 7.4±0.2, Basingstoke, Hampshire, UK) and incubated at 37°C for 24 hours. Culture of the sample produced bright pink colored, large and non-mucoid colonies on MacConkey agar. The organism was suspected as *E. coli* based on colony morphology. Later individual colony from MacConkey agar was streaked on EMB agar plate (Merck, P^H: 7.1±0.2) and incubated at 37°C for 24 hours. Based on "green metallic sheen" colony morphology the organism was confirmed as *E. coli* along with following biochemical and Gram's staining properties.

2.5 Preservation of isolates:

All *E. coli* isolates were cultured in brain heart infusion (BHI) broth, incubated overnight at 37°C. For each isolate 700 µl BHI broth culture was add to 300 µl 15% glycerol in an eppendorf tube. Tubes were properly leveled and stored at -80°C for further investigation.

2.6 Screening of the antimicrobial sensitivity pattern of the isolates using a panel of antimicrobials:

The antimicrobials commonly used for treatment of clinical cases of goat in field condition especially for mastitis were included in the cultural sensitivity (CS) test. Details about the antimicrobials along with their interpretation are summarized in Table: 2.1

Table 2.1: Concentrations and diffusion zone breakpoints for resistance against anti-microbials' standard for *E. coli* isolates (CLSI, 2011):

Microbial group	Anti-microbial agent	Disc potency	Zone Diameter (mm)		
			Sensitive	Intermediate	Resistant
B-lactams antibiotics	Amoxicillin (AML)	10 µg	≥17	14-16	≤13
	Ceftriaxone (3rd Generation cephalosporin) (CRO)	30 µg	≥18	15- 18	≤14
	Cefotaxime (3rd Generation cephalosporin) (CTX)	30 µg	≥ 18	15-18	≤14
Macrolides	Gentamycin (CN)	10 µg	≥15	13-14	≤12
Polymixin	Colistin Sulphate (CT)	8 µg	≥11		≤10
Aminoglycosides	Streptomycin(S)	10 µg	≥19	15-18	≤14
Quinolones	Ciprofloxacin (CIP)	5µg	≥21	17-20	≤16

2.6. Procedure of cultural sensitivity (CS) test:

At first sub-culturing of the preserved organism was done on blood agar and incubated at 37° for 24 hours to obtain a pure growth. Using sterile inoculating loop 3 or 4 individual colonies from the blood agar were transferred into a tube containing 3ml of sterile phosphate buffer saline solution (0.85% w/v NaCl solution). Emulsification of the inoculums was done to avoid clumping of the cells inside test tube using vortex machine. Then the bacterial suspension was adjusted to the turbidity of 0.5 McFarland standard (equivalent to growth of $1\text{-}2\times10^{8}$CFU/ml). Within 15 minutes of preparing the inoculums, a pre-sterile cotton swab was dipped into the Inoculums and rotated against the side of the tube with firm pressure to remove excess fluid. Then the swab was streaked over the entire dry surface of Mueller Hinton agar for three times rotating the plate approximately at 60 degrees. After 15 minutes of inoculation the discs were placed on the agar surface using a sterile forceps. After dispensing all the discs the agar plates were incubated at 37°C for 18 hours. After incubation the size of zone of inhibition (in mm) around a disc including the diameter of the disc was measured using a ruler and the result was interpreted according to CLSI, 2011.

2.7 Data analysis:

The antimicrobial susceptibility data are expressed as percentages or frequency of the *E. coli* isolated from mastitis effected goat milk. During conducting the study epidemiological data were collected to explore their association with occurrence of coliform mastitis in goat. The epidemiological data included breed, age, parity, lactation period, body weight, litter size, previous mastitis history. All data were inputted into a spreadsheet (Microsoft Office Excel 2010) and transferred to STATA-11 for statistical analysis. The tests were conducted at 95%level of confidence and 5% level of significance. The p value less than 0.05 were considered statistically significant.

CHAPTER-3

RESULTS

3.1 Prevalence of *E. coli* coliform mastitis:

Out of 29 samples collected from mastitis affected goat, 8 samples were found positive for *E. coli*. **Table 3.1** shows the overall prevalence of E. coli in mastitis affected doe.

Table 3.1 Prevalence of coliform mastitis caused by *E. coli*:

Total samples no	*E. coli* positive samples no	Prevalence (%)
29	8	27.6 (95% CI 14.5% - 45.9%)

3.2 Prevalence of *E. coli* coliform mastitis relation to the breed of doe:

The results of occurrence of *E. coli* causing coliform mastitis in relation to breed of the animals are shown in Table 3.2. Here, it's seen that highest (50%; 95% CI 21.5%-78.4%) occurrence was recorded in cross breed goats.

Table 3.2: Occurrence of *E. coli* coliform mastitis in doe relation to the breed of animal:

Name of the breed	**No of mastitis effected doe**	**Percentage (%)**
Cross breed	4	50% (95% CI 21.5%-78.4%)
Jamunapari	1	12.5% (95% CI 1.1%-49.2%)
Black Bengal	3	37.5% (95% CI 13.4%-69.6%)
Total	8	100

3.3 Occurrence of *E. coli* coliform mastitis in relation to age:

The results of occurrence of *E. coli* causing coliform mastitis in relation to age of the animals are shown in Table 3.3. Here it is seen that, highest (28.6 %, CI 7.6% – 64.8%) occurrence was recorded in age of two years or less than two years.

Table 3.3 Occurrence of *E. coli* coliform mastitis in relation to age:

Categories	No of samples (positive sample no)	Prevalence (%) (95% CI)
≤ 2 years	7 (2)	28.6 (7.6 – 64.8)
> 2 years	22 (6)	27.8(10.7 – 50.2)

3.4 Occurrence of *E. coli* coliform mastitis in relation to body weight:

The results of occurrence of *E. coli* causing coliform mastitis in relation to weight of the animals are shown in Table 3.4. Here it is seen that, highest (36.4%, CI 15% – 64.8%) occurrence was recorded in the doe which weight over 30 kg.

Table 3.4 Occurrence of *E. coli* coliform mastitis in relation to body weight:

Categories	No of samples (positive sample no)	Prevalence (%) (95% CI)
≤ 30 kg	18 (4)	22.2 (8.5 – 45.8)
> 30 kg	11 (4)	36.4 (15 – 64.8)

3.5 Occurrence of coliform mastitis according to the parity of doe:

The results of occurrence of *E. coli* causing coliform mastitis in relation to parity of the animals are shown in Table 3.3. Here, it's seen that highest (50%; 95% CI 21.5%-78.4%) occurrence was recorded in 1st parity goats.

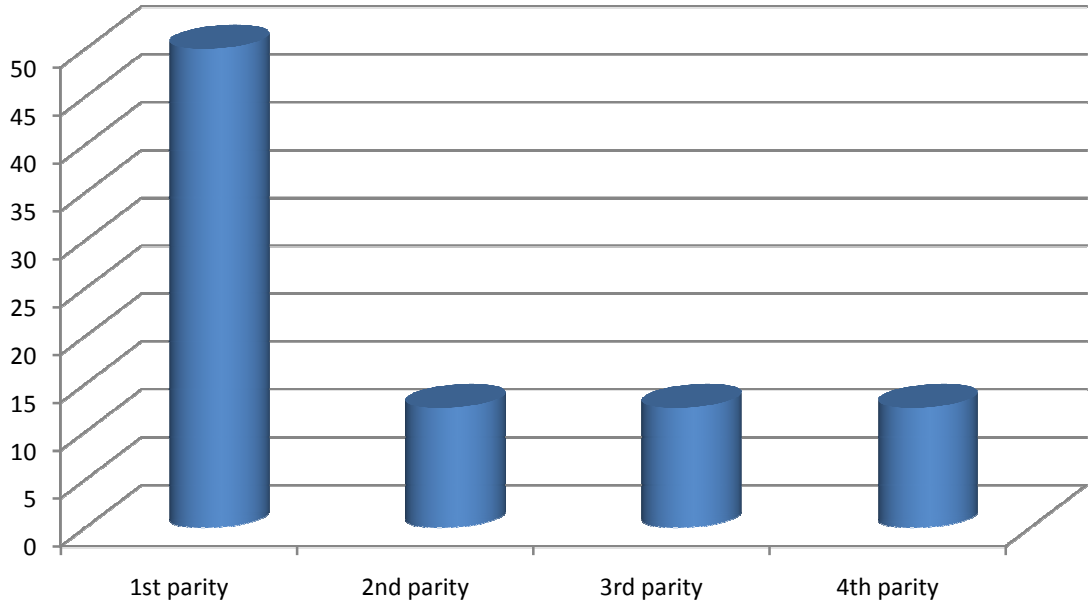

Figure 3.1: Occurrence of coliform mastitis in doe relation to parity

3.6 Antimicrobial sensitivity profiles of bacterial isolates:

The results of antimicrobial sensitivity pattern of the *E. coli* isolates against seven antimicrobials tested are shown in **Table 3.6** and **Table 3.7.**

Table 3.6 Results of Cultural Sensitivity (CS) Test:

SL no	Sample ID	Antimicrobial resistance pattern						
		AML	**CN**	**CT**	**S**	**CIP**	**CRT**	**CTX**
01	05	R	S	S	S	R	S	S
02	07	R	S	S	I	R	I	R
03	11	R	I	S	R	R	R	R
04	17	R	R	S	R	R	S	S
05	18	R	S	S	S	R	R	R
06	22	R	S	I	R	R	R	R
07	23	R	S	I	R	R	R	R
08	24	R	S	S	S	R	S	S

Table 3.7 Antimicrobial resistance profiles of the *E. coli* isolates:

Antimicrobials	Antimicrobial resistance pattern (95% Confidence Interval, CI)		
	Sensitive (%)	**Intermediate (%)**	**Resistant (%)**
AML	0%	0%	100 %(62.8%-100%)
CN	75% (40%-93.7%)	12.5% (0.1%-49.2%)	12.5 %(0.1%-49.2%)
CT	75 %(40%-93.7%)	0%	25 %(6.3%-59.9%)
S	37.5 %(30.5%-69.6%)	12.5 %(0.1%-49.2%)	50 %(21.5%-78.5%)
CIP	0%	0%	100 %(62.8%-100%)
CRO	37.5 %(13.5%-69.6%)	12.5 %(0.1%-49.2%)	50 %(21.5%-78.5%)
CTX	37.5 %(13.5%-69.6%)	0%	62.5 %(3.4%-86.5%)

3.7 Association of factors with the occurrence of *E. coli* coliform mastitis in doe:

Statistical analysis of the epidemiological data of host factors and the treatment strategies followed to treat the clinical cases showed no statistical significant association with the *E. coli* coliform mastitis. Results of the statistical analysis are summarized in **Table 3.7**.

Table 3.7: Statistical analysis of the host factors with the occurrence of coliform mastitis of doe by *E. coli*:

Variables	No of samples	No of positive sample for coliform mastitis	Chi square value	P value
Breed:				
Cross breed	12	4	1.13	0.567
Jamunapari	2	1		
Black bengal	15	3		
Age group				
≤ 2 years	7	2	0.005	0.947
> 2 years	22	6		
Body weight				
≤ 30 kg	18	4	0.684	0.408
> 30 kg	11	4		
Parity				
1st	10	5	4.118	0.249
2nd	17	3		
3rd	1	0		
4th	1	0		
Treatment				
No antibiotics	2	0	4.738	0.094
One antibiotic	16	7		
Combined antibiotics	11	1		
Recovery status				
Yes	9	3	0.514	0.773
No	6	2		
Unknown	14	3		

CHAPTER-4

DISCUSSION

The aim of this study was to focus on isolation and identification of the *E. coli* from clinically affected mastitic doe and to know the antimicrobial pattern of the *E. coli* isolates to find out most successful antimicrobials' against *E. coli*. Eight (27.6%) milk samples were positive for *E. coli* in this study and are in close agreement with that of Abdurahman (2006) and Kalla et al. (2008) who found almost similar prevalence in Eastern Ethiopia and Kano State, Nigeria respectively. However, higher prevalence of coliform mastitis were reported by Sena et al. (2001) in India, Woubit et al. (2001) in Southwestern Ethiopia and in Jordan by Hawari and Hassawi (2008). The isolated genus of coliform bacteria in this study only was *E. coli*. This is in close agreement with Matofari et al. (2003), Abdurrahman (2006), Kalla et al. (2008), Giannino et al. (2009), Abera et al. (2010) and Garedew et al. (2012), who publicized *Escherichia coli is a* major mastitogens along with *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp. and *Proteus* spp. Cross breed goats produce more milk than Black Bengal goat and cross breed were more susceptible to *E. coli* coliform mastitis than Black Bengal which is agreed with Radostits et al. (2005) who stated that high yielding animals are more susceptible to mastitis than low-yielding ones. We found age, breed, parity, body weight, lactation period were not significantly associated ($p > 0.05$) with the prevalence of *E. coli* causing mastitis in does though Mubak et al. (2016) stated that age, parity number, stage of lactation, management system, hygiene of milking process, and presence of lesion on udder/teat have statistically significant association ($p<0.05$) with the prevalence of mastitis in cow. This variation may be due to differences in species, geographical location and sample size.

Antimicrobial susceptibility testing of *E. coli* isolated from those clinically mastitis affected does showed that, all *E. coli* isolates showed highest sensitivity to Gentamycin (75%) and Colistin Sulphate (75%) which is supported by the findings of Islam et al. (2016) who found *E. coli* highly sensitive to Gentamycin (58.9%) and Colistin Sulphate (84.1%) isolated from goat. Isolates showed decreasing susceptibilities to Ciprofloxacin and Amoxicillin (0%), Streptomycin (37.5 %) followed by Ceftriaxone (37.5%), Cefotaxime (37.5%). This study

demonstrates increasing resistance of the *E. coli* against different important groups of antimicrobials which may be due to prolonged and indiscriminate usage of those drugs.

CHAPTER-5

CONCLUSIONS

Mastitis is one of the most important constrains in goat farming in Bangladesh. *E. coli* is found to be the main organism that causes the coliform mastitis in clinically affected mastitic doe. Gentamycin and Colistin Sulphate are the two most effective antimicrobials for clinical management of the clinical mastitis in goat.

CHAPTER-6

LIMITATIONS AND RECOMMENDATIONS

Limitations:

- Further studies should be conducted in large number of population size.
- Antimicrobial pattern should be investigated by MIC value.
- Study should be conducted wide geographical location.
- Temporal pattern of the disease should be investigated.

CHAPTER-7

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CHAPTER-8

APPENDIX-I

8.1: Peptone water

Composition	gm/liter
Peptone	10.0
Sodium chloride	5.0
Disodium phosphate	3.5
Potassium dihydrogen phosphate	1.5

8.2: Nutrient broth

Composition	gm/liter
'Lab-Lemco' powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0

8.3: MacConkey agar

Composition	gm/liter
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075
Agar	12.0

8.4: EMB (Eosin Methylene Blue) agar

Composition	gm/liter
Peptone	10.0
Lactose	10.0
Dipotassium hydrogen phosphate	2.0
Eosin Y	0.4
Methylene blue	0.065
Agar	15.0

8.5: MH (Mueller Hinton) Agar

Composition	gm/liter
Beef Extract	2.00
Acid Hydrolysate of casein	17.50
Starch	1.50
Agar	17.00

APPENDIX-II

QUESTIONNAIRE USED FOR THE STUDY

Case No: **Date:**

Species: **Breed:**

Age: **Parity:**

Body weight: **Litter size:**

Clinical symptoms of mastitis:

Antibiotics used for treatment:

Sample collected (milk):
