PREVALENCE OF MASTITIS IN GOATS, ISOLATION OF ETIOLOGICAL BACTERIA AND IDENTIFICATION OF THEIR ANTIBIOTIC RESISTANCE PROPERTIES

A THESIS

BY

JANNATUL FERDOUS

REGISTRATION NO.: 1605182

SEMESTER: JULY-DECEMBER, 2017

SESSION: 2016-2017

MASTER OF SCIENCE (M.S.)

IN

MEDICINE

DEPARTMENT OF MEDICINE, SURGERY AND OBSTETRICS

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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DECEMBER, 2017

DEDICATED
TO MY
BELOVED PARENTS

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The Author *June, 2017*

ABSTRACT

Mastitis is one of the multifactorial and expensive diseases of the dairy animals as it not only reduces the milk production but also leads to detrimental changes in the milk composition. The aim of this work was to determine the prevalence of clinical and sub-clinical caprine mastitis with their associated bacterial pathogens and antibiotic sensitivity patterns during the period from January to June, 2017. A total of 120 lactating goats in Dinajpur district were randomly selected for this study and 240 milk samples from each udder half were screened for evidence of clinical mastitis by clinical examination of udder and subclinical mastitis by CMT (California Mastitis Test). The overall prevalence of clinical and subclinical mastitis was found to be 11.67% and 38.75%, respectively. Bacteriological examination of mastitic milk samples revealed that *Staphylococcus spp.* (32.5%) was the major etiological agent of caprine mastitis followed by *Escherichia coli* (22.5%), *Pseudomonas spp.* (12.5%), *Klebsiella spp.* (12.5%), *Bacillus spp.* (5%). Statistical results of the study showed that parameters like age, parity, litter size and teat lesions had significant effect on caprine mastitis ($p< 0.05$) but lactation stage had insignificant effect on mastitis ($p> 0.05$). Antibiotic sensitivity test studies of mastitic pathogens from goat milk revealed sensitivity to Levofloxacin, Gentamicin, Ciprofloxacin and Chloramphenicol. Antibiotic resistance was seen against Penicillin, Amoxicillin, Azithromycin, Cloxacillin, Erythromycin, Vancomycin, Amikacin, Cefixime and Cephradine at various degree; the antibiotic resistance me be due to indiscriminate use of antibiotics in diseases control. It may be concluded that consumption of infected goat milk by children may host antibiotic resistance bacteria, the antibiotic resistance gene may transfer to the normal micro-flora that may lead super infection.

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LIST OF ACRONYMS

%	= Percent
°C	= Degree of Celsius
<	= Less than
>	= Greater than
AK	= Amikacin
AMX	= Amoxycillin
AZM	= Azithromycin
C	= Chloramphenicol
CFM	= Cefixime
CH	= Cephradine
CIP	= Ciprofloxacin
CM	= Clinical Mastitis
CMT	= California Mastitis Test
CN	= Cephalexin
COX	= Cloxacillin
EMB	= Eosin Methylene Blue
ERY	= Erythomycin
et al.	= Associates
etc	= Etcetera
Fig	= Figure
gm	= Gram
GN	= Gentamicin
HSTU	= Hajee Mohammad Danesh Science and Technology University
i.e.	= That is
IMI	= Intramammary Infection
LE	= Levofloxacin
LQ	= Left Quarter
Ltd.	= Limited
MA	= MacConkey Agar
ml	= Mililitre
mm	= Milimeter
MR	= Methyl Red

MR-VP = Methyl Red Voges- Proskauer

NA = Nutrient Agar

NB = Nutrient Broth

No. = Number

P = Penicillin

PBS = Phosphate Buffered Saline

RQ = Right Quarter

SCM = Subclinical Mastitis

spp = Species

SSC = Somatic Cell Count

TE = Tetracycline

Va = Vankomycin

CHAPTER 1

INTRODUCTION

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INTRODUCTION

Small ruminants especially goat plays a pivotal role for poverty alleviation in rural areas of Bangladesh (Kashem *et al*., 2011). Bangladesh possesses about 34 million head goats and more than 90% of the goats of the country are Black Bengal breed and it provides about 127,000 MT meat per year and accounts for 25% of total red meat source (FAO, 2007). The goat ranked second in terms of meat, milk and skin production, representing about 28, 23 and 28% respectively of the total livestock contribution in Bangladesh (Sarker and Samad, 2011).

Goats are significant for subsistence of a large population in the developing countries, and especially to weaker and downtrodden sections, who are the most vulnerable members of society in terms of undernourishment and poverty. The dairy goat industry is rapidly gaining importance throughout the world in recent years (Kumar *et al.*, 2016). Goat milk's has got tremendous nutritional values in human and given uncountable impetus to the consumption of goat milk and its products (Silanikove *et al.,* 2010). According to FAOSTAT (2016), Bangladesh remained in the 2nd position for producing goat milk globally as it reached 17,65,973 tons/ year.

Goat milk is cheaper, wholesome, easily digestible and nutritious (Panicker *et al.,* 2015) and is universally recognized as a complete diet (Javaid *et al*., 2009; Mbindyo *et al*., 2014). It is recommended for use in dyspepsia, peptic ulcer and pyloric stenosis. Therapeutic potential of goat milk for liver dysfunction, jaundice, biliary disorders, acidosis and insomnia have also been reported (Miranda *et al.,* 2010; Pirjada *et al.*, 2016). It has a similar nutritional profile to those of human's breast milk. But milk quality may be affected by bacterial contamination of mammary gland, which causes clinical and subclinical mastitis (Boscos *et al.,* 1996). It results in severe economic losses from reduced milk production, treatment cost, increased labor, milk withheld following treatment and premature culling (Sharif *et al.,* 2009).

However, any factor such as mastitis that adversely affects the quantity and quality of goat milk is of great financial interest in the goat rearing countries of the world including Bangladesh (Razi *et al.,* 2012). Mastitis has been recognized as the important hurdle on

rural and global economy (Ali *et al.,* 2011). Goats with mastitis lost its production potentials, act as carriers and transmit mastitis-inducing bacteria to healthy animals (Tanja and Karen, 2010). Mastitis in goat is mainly of sub-clinical type which reduced milk yield, kid mortality and is responsible for major economic losses due to damage of udder tissue (Contreras *et al.,* 2003). However, gangrenous mastitis occurred as common clinical form of mastitis in goats (Samad, 2008).

Mastitis is an inflammatory condition, characterized by changes in the physical properties of the udder and physical and chemical changes in milk (Nazifi *et al*., 2011). Mastitis is diagnosed based on clinical signs, an increase in the SCC (Somatic cell count) and identification of bacteria in the milk (Droke *et al*., 1993). Several causal agents and predisposing factors have been attributed to dairy goat mastitis with *Staphylococcus* spp. as the main etiological agent (Ibrahim *et al.,* 2009). *Staphylococcus aureus* is the most important pathogen of caprine mastitis worldwide (Aras *et al.*, 2012) and presence of *S. aureus* in milk has public health significance (Fagundes *et al.*, 2010; Razi *et al.*, 2012). Even if milk is pasteurized, the risk of cross contamination during production and handling, as well as the risk from thermostable staphylococcal enterotoxins which can persist in the processed products (Contreras *et al.,* 2003; Gelasakis *et al.,* 2016).

Mastitis can be manifested in two forms: subclinical form and clinical and may be acute or chronic (Raikwar and Shukla, 2015). In the clinical mastitis all the five cardinal signs of udder inflammation (redness, heat, swelling, pain and loss of milk production) are present, while the sub-clinical form is bereft of any obvious manifestation of inflammation (Sarker and Samad, 2011). Subclinical mastitis is the most common in goats and is mainly caused by contagious bacteria (Persson *et al.,* 2011). This form of the disease is important for the following reasons: It is 15 to 40 times more prevalent than the clinical form, usually precede towards clinical form, it is of long duration, difficult to detect. Subclinical mastitis reduced milk production and adversely affects milk quality. This form of mastitis is also important because it constituted a reservoir of microorganisms that lead infection to other animals within the herd (Shearer *et al.*, 2003).

The microorganisms associated with mastitis have been widely studied but they are still focused of research, as the isolated species of etiological agents change over time. Over 100 species of microorganisms are involved in mammary gland inflammatory condition

(Hristov *et al.,* 2016). A large number of microbiological studies report staphylococci as the most prevalent mastitis pathogens in goats (Islam *et al.,* 2012; Marogna *et al.,* 2012). Though *Staphylococcus spp*. is the main etiological agent, different bacteria can be also frequently isolated from mastitic goats including *Streptococcus spp*., *Pasteurella* spp. and *E. coli* (Contreras *et al*., 2007; Mughabe *et al.,* 2017). *Proteus* spp., *Salmonella* spp. and *Bacillus* spp. have been isolated from milk samples collected from goats suffering from mastitis (Iqbal *et al.,* 2004). Other than bacteria several fungi, yeasts and viruses were also causative factors (Schalm *et al.*, 1971). Several authors also reported Coagulase Negative Staphylococci as the most common pathogen isolated from mastitic goat milk (McDougall *et al.*, 2002; Contreras *et al.*, 2003; Najeeb *et al.,* 2013). In addition, several risk factors such as milking hygiene, management practice, stage of lactation, parity etc. had influence on the occurrence of mastitis in goats (East *et al.,* 1987; Boscos *et al.,* 1996; Razi *et al.,* 2012).

Diagnosis of clinical as well as sub-clinical cases of mastitis largely depends onto the presence of significantly higher leukocytes count in the milk from affected glands. In context of milk, these leukocytes are called somatic cells (Najeeb *et al.*, 2013). In dairy animals, the SCC is directly correlated to the severity of the mastitis (Pantoja *et al*., 2009). A somatic cell count less than1,000 means that the goat's glands are healthy; 2,000 to 500,000 indicates an infection by weak pathogens (easy to treat), over a million consider a problem and over 1,500,000 count definitely have an infection (Scott and Haskell, 2005). Among the indirect tests, CMT, WST, SFMT are commonly used for indirect somatic cell count as indicator of subclinical mastitis both in cows and does (Islam *et al.,* 2012).

The greatest problem in the treatment and control of mastitis is the emergence of drug resistance mastitic bacteria (Jha *et al.,* 1994). Antibiotics are used to treat diseases of various animals as well as preservatives for milk (Deveriese *et al.,* 1997). Currently, the other major concern to human health is the issue of antimicrobial resistance due to indiscriminate use of antibiotics in livestock production in developing countries (Haftay *et al.,* 2016). This indiscriminate use of antibiotics has led to the development of multiple antibiotic resistances thereby rendering the antibiotic treatment ineffective (Alian *et al.,* 2012). This situation needs new therapeutic agent with novel mechanism of action to circumvent these infections (Benton *et al.,* 2004).

Literature available indicated that there are works onto the goat mastitis in Bangladesh but the information of mastitis in goat of northerm district of Bangladesh is mostly scanty. Management of mastitis is particularly recognized as one of the most expensive diseases in the goat farming (Islam *et al*., 2011). Hence the topic deserves most attention with the objectives to;

1. study the prevalence of mastitis in goat
2. isolate and characterize the causative bacteria involved in clinical and subclinical mastitis in goats
3. identify the antibiotic sensitivity properties of the isolated mastitic bacteria

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1 Prevalence of caprine mastitis

The negative impacts of mastitis on milk production pose a challenge to the profitability and sustainability of small scale dairy enterprises, thereby affecting the social welfare of farmers. The prevalence and epidemiological profile of Caprine mastitis in Botswana remains undocumented. **Mugabe *et al.* (2017)** conducted a cross-sectional study in which a total of 163 lactating goats from 17 flocks were purposefully selected based on the use of goat milk and stage of lactation (45 to 60 days post-partum). All goats were subjected to clinical examination and Somatic Cell Count Test (SCC). Samples with SCC above the threshold of 0.5×106 cells/ml were classified to be infected with sub-clinical mastitis. Each milk sample (0.5 ml) declared positive from Nucleo-counter SCC was further subjected to bacteriological tests to isolate for bacteria. The overall prevalence rate of mastitis was 17.8% (95% CI), with a significantly ($P<0.05$) higher prevalence rate for sub-clinical (13.5%) than clinical mastitis (4.29%). **Kumar *et al.* (2016)** examined 397 quarter foremilk samples from 200 apparently healthy randomly selected lactating beetal crossbred goats. The average prevalence of sub-clinical .mastitis was found 55.50 % at animal basis with 22.50% and 23.50% quarters showing specific and non-specific mastitis respectively in which 32 udder halves were unilaterally affected and 152 udder halves had bilateral affection. Subclinical mastitis is a disease of the udder that passes mostly unnoticed. A study was conducted to determine the prevalence of subclinical mastitis in local goats in Laghouat region (Algeria) based on the detection of sub-clinical mastitis by Califirnian Mastitis Test (CMT) in 60 goats aged 1 to 9 years and weighing 18 to 45 kg of live weight and whose lactation number varies from 2 to 8 and found a prevalence of subclinical mastitis was 46.6%. The highest rate is recorded especially in goats aged between 3 to 7 years with 62% of detected subclinical mastitis, followed by the older goats (over 7 years) with a prevalence of 27%, and at the end the youngest goats (1-3 years) with a prevalence of 11%. The goats having the highest rank of lactation were most affected by subclinical mastitis. Prevalence of 46%, 31%, 11% and 8% respectively are found for the 6th rank of lactation and more, 4 lactations, 5 lactations and 1 lactation **(Yahia *et al.*, 2016).** Mastitis is common

managemental problem of dairy goats. Prevalence of mastitis in Dera Din Pannah, Beetal, Teddy, Desi and non-descriptive dairy goat breeds was determined. 235 milk samples from lactating goats of different breeds were tested in which 51 (21.70%) were found positive for subclinical (18.29%) and clinical mastitis (3.4%) **(Rizwan *et al.,* 2016). Priya *et al.* (2016)** carried out a study to determine the age, breed, season, lactation and udder half wise prevalence of clinical mastitis in goats and recorded highest prevalence in 3-4 years (50%), in non-descript goats (50%). Seasonal prevalence was highest during monsoon (58.33%) and in the month of July (33.33%). Goats in first lactation (50%) showed highest prevalence and the right quarters (55.56%) were more affected than the left quarters (44.44%). Single quarter involvement was noticed in 50% of the goats and remaining 50% showed double quarter infection. To estimate the prevalence of subclinical mastitis in goats using California Mastitis Test (CMT), a total of 228 lactating goats from Aba'lla district, Afar region were examined. The prevalence of subclinical mastitis was 20.6% (47/228). Age showed statistically significant association with the prevalence of subclinical mastitis ($p = 0.009$). However, parity number and length of lactation did not show statistically significant relation with the prevalence of subclinical mastitis in goats **(Haftay *et al.,* 2016).** 2120 milk samples were taken from each udder half, for the mastitis test and the bacteriological test in Croatia. Subclinical mastitis was diagnosed in 211 out of 1060 goats which was 20% of the studied population. Mastitis of one udder half was diagnosed in 84% of the affected population, while mastitis of both udder halves was diagnosed in 16% of the affected goats. A positive mastitis test reaction was identified in 605 samples (28%), and the pathogenic bacterium was isolated from 244 of these samples (36%) **(Kostelic *et al.,* 2009).** Humeimeh research station for Damascus goats, General commission for scientific agricultural research (GCSAR) carried out a study on dairy goats aged 2 to 6 years. Milk samples were taken from 134 dairy goats during morning milking according to habitual procedure and screened for evidence of subclinical mastitis by California Mastitis Test (CMT), electrical conductivity measurement (EC) and lactose content (LC) of milk. Positive (+1 to +3) CMT and/or high electrical conductivity milk samples were subjected to bacteriological examination to distinguish between healthy (absence of mastitis agents) and infected (presence of contagious or environmental mastitis agents). The overall prevalence of subclinical mastitis was found to be 24.6%. Percentage of positive (+1 to +3), suspected and negative CMT reactions were 76.85, 8.95 and 14.2%, respectively **(Roukbi *et al.,* 2015).** 26 animals of the breed Bulgarian white dairy goat

and its crosses, first or second lactation found in early lactation period - 15-20 days after birth to investigate the effectiveness of direct and indirect methods for diagnosis of subclinical mastitis in goats and prevalence of the disease in early lactation. The results showed 42.31% prevalence of subclinical mastitis in the early stages of lactation. Final analysis showed that the self-administration of the indirect tests for the diagnosis of subclinical mastitis in goats is ineffective in early lactation, but can be used for selection of animals which produce a sterile milk samples for laboratory analysis **(Khristov *et al.*, 2014).** The overall prevalence of subclinical mastitis was 18.03% and 28.14% in sheeps and goats by screening 390 lactating animals comprising 255 goats and 135 sheep at Kafta Humera and Tanqua Abergelle Districts of Ethiopia **(Gebrewahid *et al.*, 2012). Islam *et al*. (2012)** conducted a study to for subclinical mastitis (SCM) in goats and sheep and found that 4% and 36% sheep and goats suffered from SCM, respectively. The prevalence of clinical mastitis (CM) was 4 and 6% in sheep and goats, respectively. The prevalence of caprine subclinical mastitis in Mymensingh area was assessed by California Mastitis Test (CMT). Milk samples were collected from 59 goats (113 udder halves). The overall prevalence of caprine subclinical mastitis as determined by CMT was 18.64% and udder half basis prevalence was 15.04%. Certain risk factors associated with caprine subclinical mastitis were identified. The prevalence was higher in older animals, with greater parity and longer lactation period. The prevalence was also higher in farms where goats were raised under traditional farming system with earthen floors. The highest prevalence (66.66%) was detected both at 6th and 5th parity, whereas lowest prevalence (4.76%) was found at 2nd parity. The highest prevalence of subclinical mastitis was detected in animals with a lactation period of 3-4 months which gradually decreased as the length of the lactation period shortened **(Razi *et al*., 2012)**. Goats are the major parts of subsidiary economy of the rural people in Bangladesh. Dairy animals including lactating goats are prone to the intramammary infection (IMI). The teat and udder of a lactating population of 1025 Black Bengal goats maintained under rural (village) condition in two different districts (Joypurhat and Mymensingh) of Bangladesh were physically examined, of which 54 (5.27%) goats had clinical mastitis. Of the 54 selected goats, 59 (54.63%) udder-halves were affected with clinical mastitis whereas the remaining 49 (45.37%) udder-halves of the selected goats were found physically normal. Out of 59 udder-halves, 49 (90.74%) were unilaterally and 5 (9.26%) were bilaterally affected with clinical mastitis. The prevalence of clinical mastitis was found significantly ($p < 0.01$) higher in left udder-haves (H = 47; 79.66%) in comparison to the right (H =

12; 20.34%) udder-halves. Milk samples collected from all the 108 udder-halves were examined bacteriologically, of which 102 (94.44%) udder-halves had bacterial infection. No significant differences was observed on the status of bacterial pathogens between clinically (H = 55; 93.22%) and sub-clinically (H = 47; 95.92%) affected udder-halves, and between the single (CH = 45; 76.27% and SCH = 35; 71.43%) and mixed (CH = 10; 16.95% and SCH = 12; 24.49%) bacterial infections in both the clinical and sub-clinical mastitis udder-halves **(Sarker and Samad, 2011). Contreras *et al.* (2007)** found that the prevalence of subclinical mastitis was 5-30% in small ruminants but the annual incidence of clinical mastitis is generally lower than 5%. 543 goats in Kohat (Pakistan) were examined to find the prevalence of subclinical mastitis by Surf Field Mastitis Test and California Mastitis test. Result showed that 62 (11.41%) and 71 (13%) were positive for mastitis on SFMT and CMT basis respectively. Prevalence of mastitis was higher in goat having age more than 4 years and had completed 3 or more than 3 lactations. On the basis of milking technique, prevalence of mastitis was higher in folded thumb method as compared to whole hand method. Similarly, prevalence was higher where the sanitary conditions were poor and in those cases where teat injury was present **(Ali *et al.,* 2010). Macesic *et al.* (2008)** revealed 20.1% prevalence of intramammary infection in all udder halves by examining 606 milk samples of 303 Alpine goats reared under an intensive system and machine milking in regions of North Croatia, French. **Swai *et al.* (2008)** reported the prevalence of subclinical mastitis based on CMT at flock, doe and udder halves level were 51.5%, 38.92% and 28.9% respectively by examining 99 smallholder fams comprising 149 pure and cross bred lactating does. The overall prevalence of subclinical mastitis was 76.6% by screening a total of 43 lactating goats comprising 26 does from Sokonie University of Agriculture and 17 does from smallholder farmers **(Mbilu, 2007).** 274 milk samples from both udder halves of 137 clinically healthy, local breed does were collected from Al-Diwanyia province and examined by california mastitis test , somatic cells counting and bacteriological culture on Staphylococcal selective media.The prevalence of subclinical mastitis in does was 29.92% ,the percentage infection in one udder half was 21.29% and 38.54% in both udder halves. The percentage of affection of left half was 48.2% while the right halves give the percentage of 51.8%, with no significant difference **(Al-Ramahi and Al-Nassrawi, 2007). Schaeren and Maurer (2006)** reported that about 40% of mammary halves and 30% of goats were infected in the 3 herds examined in their study. In most cases infections were caused by CNS or corynebacterium. Over 20% of the udder halves infected with CNS

showed negative CMT reaction. On the other hand, 25% of samples from mammary halves without a proven infection reacted positively. The prevalence of clinical mastitis was 2.4% by screening 680 milk samples from 340 lactating goats to estimate the prevalence of mastitis in the Southern Rift Valley Region of Ethiopia **(Assefa *et al.*, 2006). Sharma *et al.* (2005)** reported the prevalence of SCM from 100 udder halves of 50 healthy goats in Bikaner, India and found the prevalence of subclinical mastitis was 35% 0n quarter basis and 56% on animal basis. The highest prevalence was found in third lactation. The prevalence of bacterial isolates and the somatic cell count of the milk from goats and sheep at parturition and approximately 40 days later between February and April 1999 were examined. In the goats, 27.3% and 25.5% were infected at parturition and 40 days later while 15% and 9.1% of sheep were infected at parturition and 40 days later, respectively **(McDougall *et al.*, 2002).** The occurance of subclinical and clinical mastitis was 26.8% and 2.4% respectively **(Khan and Jafari, 2000). Ndegwa *et al*. (2000)** screened 630 milk samples and found the prevalence of subclinical mastitis was 9.8, 9.7 and 28.7 % according to the CMT (California Mastitis Test), DLC (Direct Leucocyte Count) and bacterial isolation, respectively. The prevalence of subclinical mastitis in goat was found 40.9% on quarter basis and 65.9% on animal basis in an organized farm **(Bhujbal *et al.*, 1999)**. **White *et al.* (1999)** performed a routine examination on 2911 udder halves over 8 years in Connecticut and Rhode Island. Result showed that the prevalence of mastitis, including both clinical and subclinical mastitis, was 36.4% (n = 1061) of all samples taken.

2.2 Etiology of caprine mastitis

Rashid *et al.* (2017) conducted a study on 25 Beetal Faisalabadi goat farms and revealed *Staphylococcus spp.*as the main etiological agent. A cross-sectional study on 163 lactating goats was carried out and reported highest prevalence of *Staphylococcus aureus* (83%) followed by Streptococcus species (62%), *Escherichia coli* (48%), Bacillus species (41%) and unidentified gram negative (51%) bacteria **(Mugabe *et al.*, 2017).** A total of 229 milk samples were analysed to isolate and identify microorganisms causing subclinical mastitis in goats and showed that *Staphylococcus spp.* (52.75%) is the highest prevalence bacteria **(Hristov *et al.*, 2016). Kumar *et al.* (2016)** isolated Coagulase Negative Staphylococci as the chief pathogen (75.65%) from infected udder halves by examining 200 apparently healthy randomly selected lactating beetal crossbred

goats. In Greece, 755 milk samples were subjected to microbiological examination, resulting in 661 positive cultures. Coagulase-negative and coagulase-positive staphylococci were isolated from 50.2 and 34.5% of the positive cultures, respectively. The incidence of infections (new infections per 1,000 goat-months) for the first and the second year of the study were 34 and 53 for coagulase-negative staphylococci, 23 and 28 for coagulase-positive staphylococci, 3 and 5 for *Streptococcus/Enterococcus spp.,* and 5.5 and 9.1 for gram-negative bacteria **(Gelasakis *et al.,* 2016).** Subclinical mastitis is well known to cause huge economic losses in the dairy production. In the Afar region of Ethiopia goats are among the most important animals used for milk and meat production. A study was conducted from February to June, 2014 to estimate prevalence of subclinical mastitis in goats using California Mastitis Test (CMT), to isolate major bacterial pathogens causing mastitis and to establish antimicrobial sensitivity for bacterial isolates. A total of 228 lactating goats from Aba'lla district, Afar region were examined. The prevalence of subclinical mastitis was 20.6% and the prevalence of mastitic pathogens were as follows; *S. aureus* (23.4%), *E. coli* (21.3%), *Streptococcus spp* (19.1%), *Salmonella spp* (17.0%), *Staphylococcus spp* (10.6%) and others (8.5%) **(Haftay *et al.,* 2016). Kostelic *et al.* (2009)** conducted a study on 1060 French alpine goat in Croatia and found a positive mastitis test reaction in 605 samples (28%), and the pathogenic bacterium was isolated from 244 of these samples (36%). From 22 samples (1.5%) which were negative to mastitis test, pathogenic bacteria, namely *S. aureus* (21 samples) and *Streptococcus* D (1 sample), were isolated. *Staphylococcus aureus* was isolated from 72% mastitis test positive samples, coagulase-negative staphylococci in 16%, other bacteria were *Streptococcus* D (6%), *Bacillus* spp. (2%), and *E. coli* (2%). Subclinical mastitis is a highly prevalent disease in dairy goats in China, which is usually caused by intramammary bacterial infection. 683 dairy goats in the main breeding areas of China were selected, and milk samples were collected. Out of these, 313 (45.82 %) goats were detected distinct or strong positive for subclinical mastitis by using California mastitis test. Among these positive goats, 209 milk samples were used to identify the causing agents by a multiplex PCR assay, and results were listed as follows: coagulase-negative staphylococci (59.52 %), *Staphylococcus aureus* (15.24 %), *Escherichia coli* (11.43 %), and *Streptococcus spp.* (10.95 %) **(Zhao *et al.,* 2015).** Prevalence of mastitis in dairy goats ranges between 5% and 30%, with *Staphylococcus spp.,* and coagulase-negative staphylococci which were identified as the most frequent cause of infection **(Nickerson *et al.,* 2015). Silanikove *et al.* (2014)** revealed that 15–40% of the udders in

most herds were infected by different bacteria species, mainly Coagulase Negative Staphylococci. A study was carried out in 310 lactating dairy goats under zero grazing system in Mount Kenya region from January 2012 to December 2012. Among 620 samples collected from the 310 goats of Meru Nyeri and Embu counties, Coagulase Negative *Staphylococcus* was - at 28.3% (176/620), *Staphylococcus aureus* - at 13.5% (84/620), *Streptococcus - at* 8.8% (46/620), *Escherichia coli* - at 3% (19/620), *Micrococcus* - at 4% (24/620), *Corynebacterium* - at 1% (7/620), *Pseudomonas* - at 0.1% (1/620), *Streptococcus agalactiae- at* 1.5% (9/620) **(Mbindyo *et al*., 2014).** Jamunapari goat was brought to the Campus Veterinary Hospital, Teaching Veterinary Clinical Complex, College of Veterinary Science, Rajendranagar with history of enlargement of udder. The confirmatory diagnosis was done by cultural examination of milk samples from mastitis goat revealed mixed infection of *Staphylococcus spp., E. coli* and *Klebsiella spp.* **(Ayodhya *et al.,* 2013).** *Staphylococcus aureus* infects animals and humans as normal flora or pathogens. **Nathawat *et al.* (2013)** collected a total of 71 milk samples from mastitic goats from Bikaner city (Rajasthan, India) for bacterial isolation and determine the prevalence of *S.aureus* in clinically infected mastitic goats. Result revealed that the prevalence of *S. aureus* in the milk sample was 38.03% (27/71). 200 milk samples of dairy goats from D. G. Khan and Lahore districts, Punjab, Pakistan were screened with Whiteside Test (WST) for sub-clinical mastitis. Samples positive for mastitis were cultured for bacterial growth on blood agar. Bacterial growth was obtained in 45 % milk samples. From WST positive milk samples, 146 bacterial isolates were identified on the basis of colonial, microscopic and biochemical profiles. Highest prevalence was of *Staphylococcus aureus* (61.64 %) followed by *Escherichia coli* (10.96 %), *Streptococcus spp.* (9.59 %), *Pseudomonas spp., Bacillus spp.* (6.85 %, each) and *Corynebacterium spp.* (4.11 %) **(Najeeb *et al*., 2013). Bourabah *et al.* (2013)** investigated the etiology of subclinical mastitis in goats in Tiaret region (Western Algeria), milk samples were collected from 298 lactating goats for bacteriological and fungal analysis. *Enterobacteria* ceae were the predominant bacterial isolated (54.02%) and *Aspergillus niger* was the predominant fungi. CNS have emerged to be pathogens causing intramammary infections in Egyptian dairy herds. The CNS as mastitis-causing agents could not be neglected as they can cause substantial economic losses. **El-Jakee *et al.* (2013)** conducted a study on 884 quarter milk sample to determine the prevalence of CNS among mastitic and subclinically mastitic cows, buffalo, ewes and does in Egypt with percentages of 16.6%, 59.4%, 50% and 55.6%, respectively. A total of 1388 goats

from 31 farms distributed throughout Sardinia, Italy, were collected, a detailed clinical examination of the udder of lactating animals was carried out, and microbiological examination of milk was performed. Results were then subjected to statistical analysis in order to highlight possible correlations among all findings. Significant results were obtained by comparing clinical findings with bacteriological positivity of milk. Intra-mammary infections were detected in 22.7% of all goats examined. *Staphylococcus* spp., *Streptococcus* spp., and *Mycoplasma* spp. were detected in 73.5%, 9.7%, and 4.7% of positive milk cultures, respectively. Other bacterial genera showed a prevalence lower than 3%. Milk positivity to *Staphylococcus aureus* was significantly associated to presence in the udder of pustules, ulcers, nodules, and *rubor* ($p < 0.05$). *Staphylococcus caprae* was associated to manual *vs* mechanical milking, age, udder edema, and absence of mammary secretions ($p < 0.05$). *Staphylococcus epidermidis* was positively associated to age ($p < 0.05$). *Streptococcus uberis* was associated to mechanical milking, atrophic udder texture, and reactive mammary lymph nodes ($p < 0.05$) **(Marogna *et al.*, 2012).** Mastitis is a significant disease in small ruminants that affects their productivity and measures need to be taken to control the disease. A study was conducted at Kafta Humera and Tanqua Abergelle Districts to assess the prevalence of subclinical mastitis in lactating small ruminants and identify bacterial causative agents. A total of 390 lactating animals comprising 255 goats and 135 sheep were randomly selected from population and screened for evidence of subclinical mastitis. California mastitis test (CMT) positive milk samples were subjected to bacteriological examination and the following bacteria were isolated; coagulase negative Staphylococcus (44.7%), Staphylococcus aureus (27.7%), Escherchia coli (17.0%) and streptococci (10.63%) **(Gebrewahid *et al.*, 2012).** Intramammary infections (IMI) in goats constitute an enormous animal health problem, alter milk composition, lower the hygienic value of milk, impair the processing properties of milk and cause high economical losses in dairy farming today. Staphylococci are certainly the most common type of pathogens in goat milk, but the frequency of occurrence of this pathogenic group varies greatly **(Stuhr *et al.*, 2010). Ibrahim *et al.* (2009)** conducted a study on 700 milk samples of clinically healthy hair goat in Turkey and isolated CNS as the predominant organism followed by *Streptococcus spp.* (15%), *S. aureus* (11.7%) and other pathogens (23.3%). **Byeng *et al.* (2007)** isolated bacteria from 110 samples (26.2%) by examining 420 samples. The prevalence of CNS was 43.7%, *Staphylococcus aureus* 35.4%, *Pseudomonas aeroginosa* 12.4%. Small ruminant mastitis is generally a chronic and contagious infection. The

primary sources are mammary and cutaneous carriages, and spreading mainly occurs during milking. Staphylococci are the main aetiological agents of small ruminants intramammary infections (IMI), the more frequent isolates being *S. aureus* in clinical cases and coagulase negative species in subclinical IMI **(Bergonier *et al.*, 2003). Dominique *et al.* (2003)** found the high prevalence of *Staphylococcus aureus* in sporadic cases of clinical mastitis in sheep and goat. The other bacteria isolated were Coagulase Negative Staphylococci, Streptococci, Enterobacteria, *Arcanobacterium pyogenes*, Corynebactria, Pasteurellaceae, *Pseudomonas spp.* 5 herds of Alpine goats in Northern Italy were screened by using bacteriological and cytological method and found that, a high prevalence of infections (39.2% overall, range 9-57% in the 5 herds), of which 83% were caused by Coagulase Negative Staphylococci **(Pisoni *et al.*, 2003). White *et al.* (1999)** performed a routine examination on 2911 udder halves over 8 years in Connecticut and Rhode Island. Result showed that the most prevalent mastitis agents were non-hemolytic *Staphylococcus* spp., 38.2%; *Staphylococcus aureus*, 11.0% and *Streptococcus spp*., 4.1%. Other than *Streptococcus agalactiae*, other isolates included *Escherichia coli*, 1.6% and *Pseudomonas* spp., 1.2%. Coagulage Negative Staphylococci were 80%, Coagulase Positive Staphylococci were 16%, Alpha hemolytic streptococci were 2% and *Pasteurella hemolytica* were 2% by examining 170 milk samples taken from 5 goat herds by **Manser *et al.* (1986).**

2.3 Antibiotic resistance of mastitic bacteria

Over the years the extensive use of antimicrobials has led to increase antimicrobial resistance. Early and specific antibiotic based treatment reduces the severity of the disease. **Ceniti *et al.* (2017)** collected 282 milk samples from sheep, cows and goats with clinical mastitis to isolate the organisms associated with it and to investigate their antimicrobial resistance. Result showed that *Streptococcus spp*. represented the most frequently isolated genus (33.84%) in cow saples, while *Staphylococcus* spp. was the most prevalent genus in sheep and goat samples (44.4 and 73.86%, respectively). Gentamicin and chloramphenicol were found as the most effective drugs against the tested isolates, while the highest resistance rates were observed for amoxicillin, ampicillin, tetracycline, trimethoprim-sulfamethoxazole. High resistance of *Staphylococcus* spp. to penicillin G followed by ampicillin was observed. Streptococci were highly resistant to ampicillin, norfloxacin, ciprofloxacin and clindamycin. *E.coli*

were resistant to clindamycin, whereas Salmonella were found to be highly resistant to kanamycin, ampicillin and erythromycin **(Haftay *et al.,* 2016). Rizwan *et al.* (2016)** tested 235 milk samples from lactating goats of different breed and found *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysagalactiae* and *Bacillus cereus*. Enrofloaxin, Ciprofloaxin, Ampicillin, Gentamycin, Oxytetracycline, Penicillin, Amoxicillin, Norfloxacin, Chloramphenicol and Streptomycin were used for antibiogram profiling against bacterial isolates. Chloramphenical, norfloxacin, gentamicin, penicillin and ciprofloxacin were found effective.204 raw milk samples were collected from randomly selected lactating camels (n=62) and lactating goats (n=142) in live-stock producing pastoralists' areas of Somali region for isolation and identification of *S. Aureus* and determine their antimicrobial susceptibility pattern. Antibiotic susceptibility tests were performed on all *S. aureus* isolates to 16 antibiotics by Kirby-Bauer disk diffusion method. *S. aureus* isolates showed resistance to Nalidixic acid, Polymixin B, and Penicillin G. *S. aureus* isolates were sensitive to vancomycin, ciprofloxacin, cefoxitin, cephalotin, gentamycin, Doxycycline, kanamycin, trimethoprim-sulfamethoxazole, chloramphenicol, norfloxacin, and erythromycin. Multi drug resistance was detected in 69.2% of the isolates. The present study has demonstrated the existence of alarmingly high level of multiple antimicrobial resistances of *S. aureus* among camel and goat milks **(Teshome *et al.,* 2016)**. **Mbindyo *et al*. (2014)** performed California Mastitis Test (CMT) of 620 samples collected from the 310 goats of Meru Nyeri and Embu counties. Among 620 milk samples to isolate the mastitis causing pathogens and to identify their antibiotic resistance pattern. Coagulase Negative *Staphylococcus* was - at 28.3% (176/620), *Staphylococcus aureus* - at 13.5% (84/620), *Streptococcus - at* 8.8% (46/620), *Escherichia coli* - at 3% (19/620), *Micrococcus* - at 4% (24/620), *Corynebacterium* - at 1% (7/620), *Pseudomonas* - at 0.1% (1/620), *Streptococcus agalactiae- at* 1.5% (9/620). The organisms were most sensitive to Norfloxacin and gentamycin while the organisms were least sensitive to kanamycin and amoxicillin. *Staphylococcus aureus* isolates were subjected to antibiogram studies using 32 antibiotics belonging to different categories and generations. The analysis of antibiogram obtained revealed that the most effective antibiotics were Gentamicin and Tobramycin (100%) followed by Netillin, Methicillin, Cefalexin, Chloramphenicol, Enrofloxacin, Azithromycin and Nitrofurazone in descending order of their efficacy. All the isolates were resistant to Cefixime and Metronidazole **(Nathawat *et al.,* 2013).** Antibiotic susceptibility of *Staphylococcus aureus, E. coli* and *Streptococcus spp*. were

tested against ten selected antibiotics used for treatment of mastitis in field. Highest resistance (58.69%) was recorded against Penicillin. Resistance of bacterial isolates to more than two antibiotic classes was 44.44%, declared as multiple drug resistance (MDR). Most of the MDR isolates were sensitive (83.33%) to Amoxicillin and Clavulanic acid combination out of the four tested **(Najeeb *et al.,* 2013). Franca *et al.* (2012)** isolated *Staphylococcus* spp.(n=210) from small animal mastitis in Brazil. Antibiotic resistance pattern showed that the isolates were most resistant to amoxicillin (50.0%), streptomycin (42.8%), tetracycline (40.4%), lincomycin (39.0%) and erythromycin (33.8%). *Staphylococcus aureus* isolates were resistant to ampicillin (54.3%), followed by resistant to oxacillin (28.3%), tetracycline (26.1%), penicillin G (23.9%), erythromycin (23.9%), trimethoprim-sulfamethoxazole (17.4%) and cephalotin (2.2%). All isolates tested for antibiotic sensitivity were susceptible to methicillin, vancomycin, chloramphenicol and ciprofloxacin **(Alian *et al.,* 2012). Sarker and Samad (2011)** isolated *Staphylococcus* spp. as the major mastitic pathogen (38.98%), followed by *Escherichia coli* (27.12%) and *Bacillus* spp. (10.17%). Antibiotic sensitivity results showed a relatively high level of resistance to ampicillin, amoxycillin and streptomycin, whereas gentamicin and ciprofloxacin were found to be the most effective drugs. There is a need for the prudent use of antibacterial in animal health and production through bacteriological and antibiogram studies in Bangladesh. A study was designed on 543 goats in Kohat (Pakistan) to isolate the mastitis causing pathogen and to identify their antimicrobial susceptibility against different commercially available antibiotics where *Staphylococcus aureus* was in the highest percentage while gentamicin was more effective against mastitic bacteria **(Ali *et al.,* 2010).** The sensitivity of *Staphylococcus spp.* to 10 chemotherapeutics from 115 cases of subclinical mastitis was tested through the disk diffusion method, 6 strains (26.08%) were determined as methicillin-resistant in which 4 isolates were *S. aureus* and 2 were *S.epidermidis. S. aureus* and *S. epidermidis* isolates were 100% resistant to Cloxaciline and Kanamycin while the resistance to Penicillin was 100% in *S. aureus* and 33.33% in *S. epidermidis* isolates **(Ebrahimi *et al.,* 2009). Virdis *et al.* (2010)** determined antibiotic resistance pattern for 25 *Staphylococcus aureus* and 75 Coagulase Negative Staphylococci from milk samples of goat with subclinical mastitis and found that fourteen (56.0%) *S. aureus* and thirty-one (41.3%) CNS isolates were resistant to one or more antimicrobial agents. *Staphylococcus aureus* showed the highest resistance rate against kanamycin (28.0%), oxytetracycline (16.0%), and ampicillin (12.0%). The CNS tested were more frequently

resistant to ampicillin (36.0%) and kanamycin (6.7%). 700 milk samples from clinically healthy udder halves of 350 lactating hair goats were examined to isolate the pathogen responsible for subclinical mastitis and determine their susceptibility to antimicrobial drugs. CNS was resistant to lactam antibiotics, while *S. aureus* and *Streptococcus* sp. were susceptible to lactams. Although, CNS and *S. aureus* were susceptible to aminoglycosides, fluoroquinolones, erythromycin, sulphamethoxazole/trimethoprim, lincomycin, oxytetracycline and florfenicol, *Streptococcus* sp. were susceptible to lactams, aminoglycosides, sulphamethoxazole/trimethoprim, erythromycin, oxytetracycline and florfenicol **(Aydin *et al.,* 2009).** 156 goats from 2 commercial dairy goat farms were tested and 97 isolates of CNS were investigated by **Moroni *et al.* (2007)** for in vitro susceptibility to several antimicrobial agents and discovered that Benzylpenicillin was the most effective antimicrobial agent against *Staphylococcus caprae* and *Staphylococcus epidermidis.* Amoxicillin and the combination of amoxicillin and clavulanic acid were slightly less effective. **Ebrahimi *et al.* (2007)** examined 400 milk samples half udders of 20 flocks of native breed goats to evaluate the resistance of isolated bacteria against different antibiotics by disc diffusion method. CNS resistance to amikacin was 78.5%, to penicillin was 50%, to tetracycline 50%, to ampicillin 42.8%, and to doxycycline was 28.5%. *S. aureus* and *E. coli* isolates showed susceptible to ampicillin (10µg), enrofloxacin (5µg) and gentamicin (10µg) by using *in vitro* drugs susceptibility test using disk diffusion method **(Ribeiro *et al.,* 2007**). **Adwan *et al.* (2005)** determined antimicrobial resistance of 132 staphylococcal isolates from subclinical mastitis of Awassi ewes, local goats and Fresian cows to 10 antibiotics. Results indicated that among all the antimicrobial agents tested the highest resistance of staphylococcal isolates was to ampicillin. The frequency of resistance to ampicillin was 75.8 and 66.7% against *S. aureus* and *S. epidermidis* isolates, respectively. Resistance to amikacin, cefepime, vancomycin, tobramycin or chloramphenicol was rare. Fourty strains of Staphylococci from clinical and subclinical goat mastitis in Brazillian dairy herds were submitted to antimicrobial susceptibility tests and demonstrated that Penicillin G was the drug which had the highest in vitro resistance rates when tested against both *S. aureus* and CNS **(Silva *et al.,* 2004).**

CHAPTER 3

METHODS AND MATERIALS

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Study population

3.1.1.1 Study area and period

The research work was carried out on Black Bengal goats of different places of Dinajpur town and adjacent villages of HSTU Campus. The laboratory works were performed at the Department of Medicine, Surgery and Obstetrics and Department of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during the period of July 2016 to December 2016.

3.1.1.2 Husbandry and management practices

The goats are reared under extensive and semi extensive husbandry system with ground muddy floor and are kept together in common shed. They are often provided with concentrated feed in addition to natural pasture. Does are not milking; only sucked by their kids. Bucks are also kept in same shed with does.

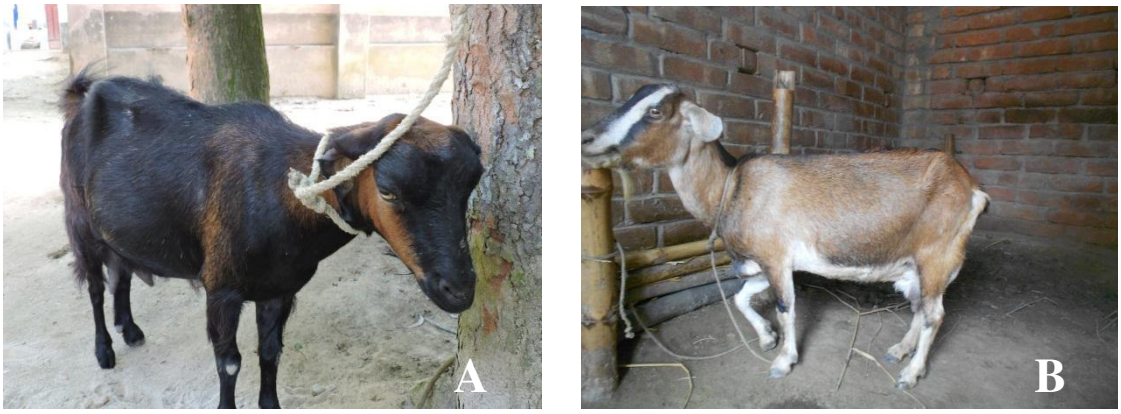
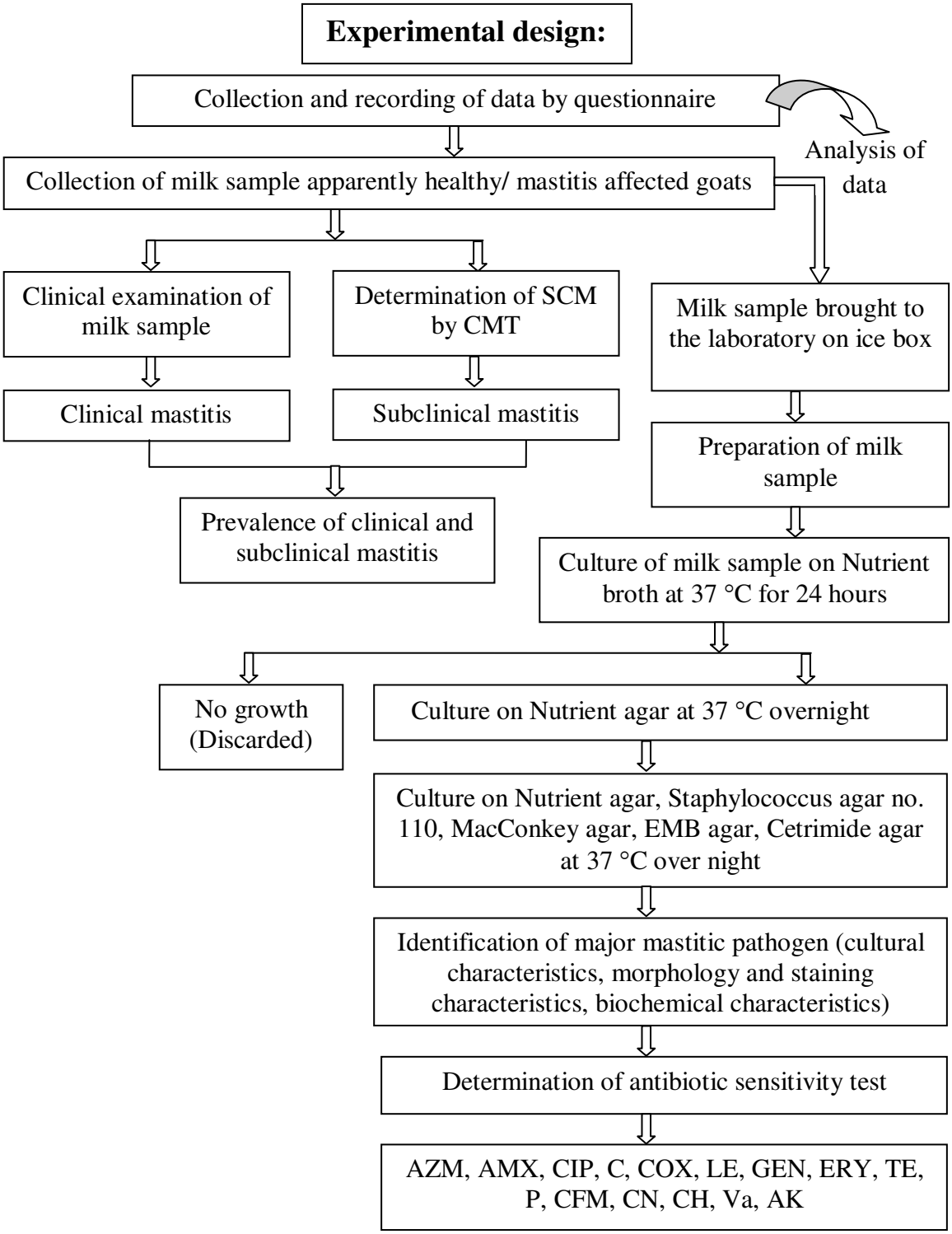


Fig. 1: Rearing system of Black Bengal goats; A: Extensive rearing system maintained at rural area adjacent to HSTU campus, Dinajpur and B: Semi extensive rearing system maintained in different places of Dinajpur Town.



Legends:

AZM = Azithromycin, AMX = Amoxycillin, CIP = Ciprofloxacin, C = Chloramphenicol, COX = Cloxacillin, LE = Levofloxacin, GN = Gentamicin, ERY = Erythomycin, TE = Tetracycline, P = Penicillin, CFM = Cefixime, CN = Cephalexin, CH = Cephradine, Va = Vankomycin, AK = Amikacin

Fig.2: The schematic illustration of the experimental design

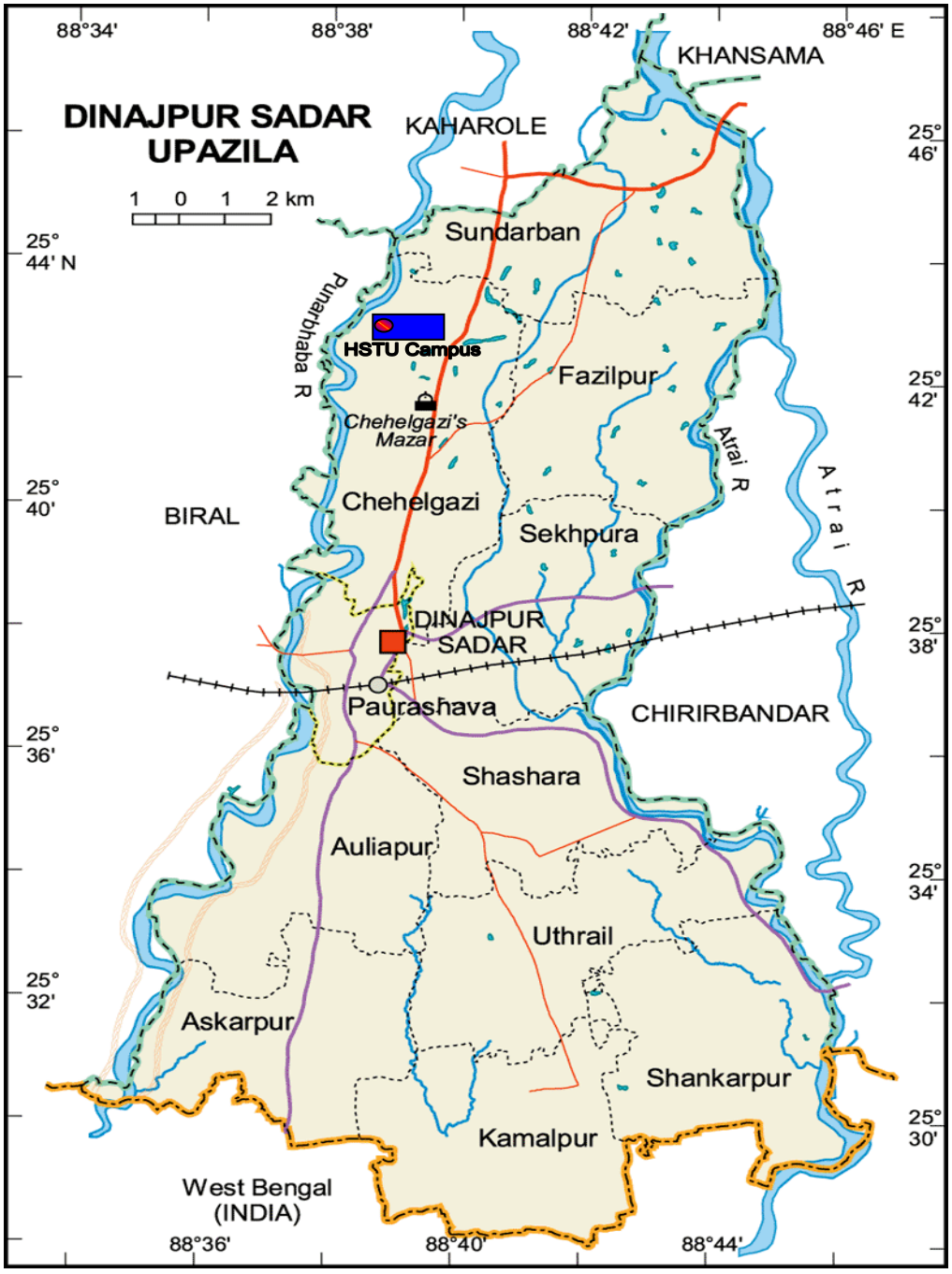


Fig. 3: Location of the experimental site (map of Dinajpur Sadar Upazila showing the research plot)

3.1.2 Survey design and sampling

A cross sectional observational study was conducted in different places of Dinajpur town and adjacent villages of HSTU campus, Dinajpur. A total of 240 milk samples from 60 lactating does of Dinajpur town and 60 lactating does of adjacent villages of HSTU campus were randomly selected for milk sampling.

Table 1: Details of study population and sampling

Collection areas	No. of goats	No. of quarter milk sample collected
Dinajpur town	60	120
Adjacent villages of HSTU campus	60	120
Total	**120**	**240**

3.1.3 Laboratory preparation

All items of glassware including test tubes, pipettes, cylinder, flasks, conical flasks, glass plate, slides, vials and agglutination test tubes soaked in a household dishwashing detergent solution ('Trix', Reckit and Colman Bangladesh Ltd.) for overnight, contaminated glassware were disinfected in 2% sodium hypochlorite solution prior to cleaning. The glassware were then cleaned by brushing, washed thoroughly and finally sterilized either by dry, heat at 160^0C for two hours or by autoclaving for 15 minutes at 121^0C under 15 lbs pressure per square inch. Autoclaved items were dried in a hot air oven over 50^0C. Disposable plastic were (micropipette tips) was sterilized by autoclaving. All the glassware was kept in oven at 50^0C for future use.

3.1.4 Instruments and Appliances

The different kinds of glassware and appliances used during the course of the experiment were as follows:

- Test tubes (with or without Durham's fermentation tube and stopper)
- Conical flasks
- Petridishes
- Pipettes
- Slides
- Coverslips

- Glass rod spreader
- Test tube stand
- Inoculating loops
- Water bath
- Centrifuge tube
- Spirit lamp
- Ice box
- Autoclave
- Refrigerator
- Hot air oven
- Compound Microscope
- Micropipette

3.1.5 Media

3.1.5.1. Solid Media

- Nutrient agar base (Himedia, India)
- Eosin methylene Blue (EMB) Agar(Himedia, India)
- MacConkry agar media (Himedia, India)
- Cetrimide agar media (Himedia, India)
- Staphylococcus no.110 agar media (Himedia, India)
- Simmon's Citrate agar media

3.1.5.2 Liquid Media

- Nutrient broth
- Lactose broth (Himedia, India)
- Tetrathionate broth (Himedia, India)
- Peptone broth (Himedia, India)
- 1% peptone water (Himedia, India)

3.1.6 Reagents

The chemicals and reagents used during the study were-

- Gram's staining reagents (Crystal violet, Gram's iodine, Acetone alcohol, Safranin)
- Methylene blue stain

- Dehydrated sodium citrate
- Phosphate Buffered Saline(PBS)
- Physiological Saline Solution(PSS)
- Methyl Red(MR) solution
- Voges-Proskauer (VP) solution
- Kovac's reagent
- Alpha naphthol solution
- Potassium hydroxide solution
- Hydrogen per oxide solution

3.1.7 Antimicrobial sensitivity discs

Biochemically characterized bacterial isolates were tested against different types of commercially available antimicrobial discs (Himedia, India). The methods allowed for the rapid determination of the efficacy of the drug by measuring the diameter of the zone of inhibition that result from different of the agent into the medium surrounding the disc. The following are the antibiotics that were tested against the selected organism with their concentration.

Table 2: Antimicrobial agents with their disc concentration

S/N	Name of antibiotics	Disc concentration (µ/disc)	S/N	Name of antibiotics	Disc concentration (µ/disc)	S/N	Name of antibiotics	Disc concentration (µ/disc)
01	Azithromycin (AZM)	15 µg	06	Levofloxacin (LE)	5 µg	11	Cefixime (CFM)	5 µg
02	Amoxycillin (AMX)	30 µg	07	Gentamicin (GEN)	10 µg	12	Cephalexin (CN)	30 µg
03	Ciprofloxacin (CIP)	05 µg	08	Erythomycin (ERY)	15 µg	13	Cephradine (CH)	25 µg
04	Chloramphenicol (C)	30 µg	09	Tetracycline (TE)	30 µg	14	Vankomycin (Va)	30 µg
05	Cloxacillin (COX)	1 µg	10	Penicillin (P)	10 µg	15	Amikacin (AK)	30 µg

Legend: µg=Microgram

3.2 Methods

The following methods were used to know the prevalence of caprine mastitis and for the isolation and identification of causative bacteria

3.2.1 Questionnaire based data collection and processing

Data from selected goats were collected using a questionnaire. The animal level variables were age, parity, lactation stage, number of kids and teat lesion. The data were entered in Microsoft Excel 2003 and transferred to SPSS, Version 22.0 for statistical analysis.

3.2.2 Clinical examination for the detection of mastitis

A thorough clinical examination of the udder and its secretions were done for the detection clinical mastitis. A clinical examination of the lactating does consisted of detailed visual inspection and systemic palpation of udder and teats for symmetry and size, indurations and fibrosis. Teats ends were observed for alterations such as wounds, scars, markers, vesicles, warts, patent orifices and ease of milking. Inspection of udder included visual examination posteriorly to ascertain size and disproportional symmetry. Right and left quarters were expressed relative to the examiner position. All individual quarters were observed for the abnormal consistency like firmness, presence of super-numerary or multiple teats, fibrosis, oedema, warmth and other physical defects. Hard, swollen with painful udder indicates clinical mastitis. Visible abnormalities of udder secretions i.e. milk with pus, blood, clot, flakes, watery etc. were indicator for clinical mastitis.

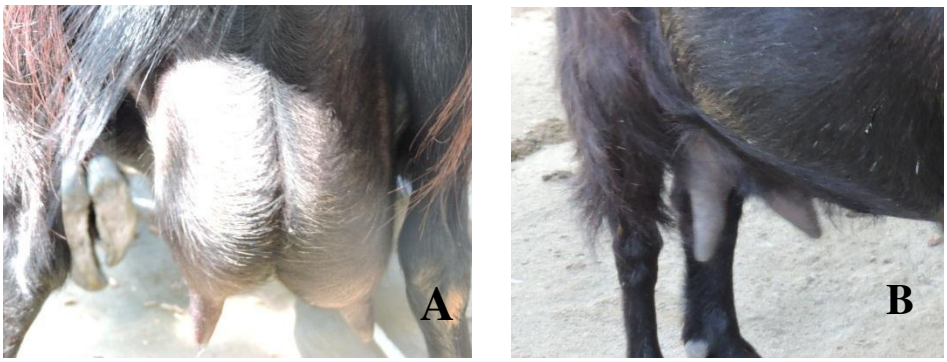


Fig. 4: A: Black Bengal goat with clinical mastitis and B: An apparently healthy Black Bengal goat showing normal udder and teat but affecting with subclinical mastitis.

3.2.3 Collection of sample

3.2.3.1 Collection of milk sample

Milk sample were collected by the owners, who were instructed to soak the teat with 70% ethanol and drying off by tissue paper, one to two drops of milks was discarded and then 10 ml of milk were taken from each quarter into labeled L or R (L for left and R for right udder halves) sterilized test tubes with rubber cap. The milk sample was taken first from the teats nearest the operator before proceeding to those on the off side.

Fig. 5: Collection of milk sample

3.2.3.2 Preparation of sample

Firstly, prior to taking samples, the screw-capped vial in which initially sampling milk was collected was shaken thoroughly for proper mixing of the milk sample. After proper shaking, 1ml milk of sample was pipetted out from the screw capped vial by sterile pipette. It was transferred to 9 ml diluent kept in a test tube. The test tube was shaken electrically in a whirling mixing machine. Decimal dilutions as necessary were prepared in the usual way as per instruction of Milk and Dairy Byproduct (Harrigan and Cancell, 1976).

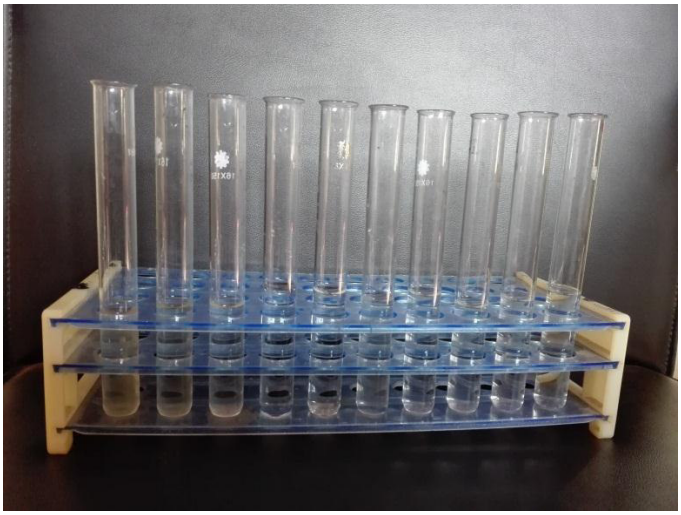


Fig. 6: Preparation of milk sample by decimal dilution method

3.2.4 Physical examination of milk sample

Immediately after collection, milk sample were subjected to physical examination with naked eyes to detect any abnormalities in color, odor, consistency, presence of blood and clot, flakes and any other visible abnormalities.

3.2.5 California Mastitis Test (CMT)

The procedure of CMT in this study was followed as per manufacturer's instruction as follows:

A shallow half black paddle having four cups supplied with kit was used and was rinsed after each use. About 2 ml milk was drawn from bottle into the cup and an estimated equal volume of CMT reagent 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 CMT reaction/ results were read immediately as per manufacturer's recommendation and were scored for each half gland (teat) depending on the amount and thickness of gel formed as described by Shearer *et al.* (2003) as shown in Table 3 and Table 4.In this study both the CMT score of o and Trace were considered as negative or normal while CMT scores of 1+(weak positive), 2+(distinct positive), 3+(strong positive) were taken as indicators of subclinical mastitis.

Fig. 7: California Mastitis test kit (Other products by ImmuCell Corp., PBS Animal Health, USA)

Table 3: Interpretation of CMT on goat milk (Shearer *et al.,* 2003)

CMT score	Reaction	Mean No. of neutrophils/ ml
0	No reaction	68,000
Trace	Slight slime, tends to disappear with continued swirling	268,000
1+	Distinct slime but without gel	800,000
2+	Immediate gel formation, moves as a mass during swirling	2,560,000
3+	Gel develops a convex surface and adheres to the bottom of the cup	≥10,000,000

Table 4: Interpretation of somatic cell count (SCC) from individual goat milk samples (Shearer *et al.,* 2003)

SCC per ml of milk	**Interpretation**
Less than 1000	Healthy glands
2000-500,000	Infection by weak pathogens
Over 1,500,000	Signals of infection

Fig. 8: Results of California Mastitis Test with caprine milk sample showing color change and gel formation in positive case (lower right corner) and in negative cases (upper right and left corner) with no gel formation

3.2.6 Preparation of culture media and broth

All the media, broth and reagents used in this experiment were prepared according to instruction of the manufacturer.

3.2.6.1 Nutrient Broth

Thirteen grams of dehydrated nutrient broth (HI-MEDIA, India) was dissolved into 1000 ml distilled water and heated to boiling to dissolve it completely. The solution was then distributed in tubes, stopper with cotton plugs and sterilized in autoclaves at 121^0C and 1.2kg/cm^2 pressure for 15minutes. The sterility of the medium was checked by incubating at 37^0C for overnight and stored at 4^0 C in refrigerator for the future use.

3.2.6.2 Nutrient agar medium

Twenty eight grams of nutrient agar powder (HI-MEDIA) was suspended in 1000 ml of cold distilled water in a flask and heated to boiling for dissolving the medium completely. The medium was then sterilized by autoclaving. Then the medium was poured into each sterile petridish and allowed to solidify. After solidification of the medium in the petridishes, these were incubated at 37^0 C for overnight to check their sterility and used for cultural characterization or stored at 4^0 C in refrigerator for future use.

3.2.6.3 Eosin Methylene blue agar

Thirty six grams of EMB agar base (HI-MEDIA, India) was added to 1000 ml of water in a flask and boil to dissolve the medium completely. The medium was sterilized by autoclaving at 1.2 kg/cm^2 pressure and 121^0C for 15 minutes and shake the medium in order to oxidize the methylene blue (i.e. to restore its blue color). Then the medium was poured into each sterile petridish (medium sized) and allowed to solidify. After solidification of the medium in the petridishes, these were incubated at 37^0 C for overnight to check their sterility and petridishes without contamination were used for cultural characterization or stored at 4^0C in refrigerator for future use.

3.2.6.4 MacConkey agar medium

51.5 grams MacConkey agar base (HI-MEDIA, India) powder was added in 1000 ml of distilled water in a flask and boil to dissolve the medium completely. The medium was

then sterilized by autoclaving at 1.2 kg/cm^2 pressure and 121^0 C for 15 minutes.Then the medium was poured into sterile glass petridishes. The sterility of the medium was checked by incubating at 37^0 C for overnight. Then the medium was used for cultural characterization or stored at 4^{0}C in refrigerator for future use.

3.2.6.5 Staphylococcus agar no. 110

149.5 grams Staphylococcus agar no. 110 (HI-MEDIA, India) powder was suspended in 1000 ml distilled water. Mix thoroughly and heat to boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at 1.2 kg/cm^2 pressure and 121^0 C for 15 minutes. Then the medium was poured into sterile glass petridishes. The sterility of the medium was checked by incubating at 37^0 C for overnight. The sterile medium was used for cultural characterization or stored at 4^{0}C in refrigerator for future use.

3.2.6.6 Cetrimide agar base

46.7 grams Cetrimide agar base (HI-MEDIA, India) powder was added in 1000 ml distilled water containing 10ml glycerol. Then heated to boiling for dissolving the medium completely. The medium was then sterilized by autoclaving. After autoclaving the medium was poured into each sterile petridishes and allowed to solidify.

3.2.6.7 Simmon's Citrate agar media

24.28 grams of Simmon's Citrate agar base (HI-MEDIA, India) was added to 1000 ml of distilled water in a conical flask and heated until boiling to dissolve the medium completely. On sterilization by autoclaving, the medium was poured in 10 ml quantities in sterile test tubes to prepare Simmon's Citrate agar slant. The medium was allowed to solidify for about 2 hours. The sterility of the medium was judged and used to store at 4^{0}C in refrigerator for further use.

3.2.7 Preparation of reagents

3.2.7.1 MR-VP test

3.2.7.1.1 Methyl Red- Voges Proskauer broth

17 gms of MR-VP medium (HI-MEDIA) was dissolved in 1000 ml of distilled water, dispensed in each sterile tube and the tubes were autoclaved. After autoclaving the tubes

containing medium were incubated at 37^0 C for overnight o check their sterility and then in refrigerator for future use.

3.2.7.1.2 Methyl-Red solution

The indicator MR solution was prepared by dissolving 0.1 gm of Bactro methyl-red in 300 ml of 95% alcohol and diluted to 500 ml with the addition of distilled water.

3.2.7.1.3 Voges –Proskauer solution

Alpha-naphthol solution was prepared by dissolving 5 gm of Alpha-naphthol in 100 ml of 95% ethyl alcohol.

Potassium hydroxide (KOH) solution was prepared by adding 40 grams of Potassium hydroxide crystals in100 ml of cooled water.

3.2.7.2 Indole test

3.2.7.2.1 Kovac's reagent

This solution was prepared by dissolving 25ml of concentrated Hydrochloride acid in 75 ml of amyl alcohol and to the mixture 5 grams of paradimethyl-amino-benzyldehyde crystals were added. This was then kept in a flask equipped with rubber cork for future use.

3.2.7.3 Phosphate buffered saline (PBS) solution

Eight grams of sodium chloride (NaCl), 2.89 grams of di-sodium hydrogen phosphate Na_2HPO_4,$12H_2O$), 0.2 grams of potassium chloride (KCl) and 0.2 gram of potassium hydrogen phosphate (KH_2PO_4) were suspended in 1000 ml of distilled for the preparation of phosphate buffered saline solution. The solution was heated to dissolve completely. Then the solution was sterilized by autoclaving at 1.2 kg/cm^2 pressure and 121^0 C for 15 minutes and stored for future use.

3.2.7.4 Buffered peptone water

20 grams Buffered peptone water (HI-MEDIA) powder was suspended in 1000 ml distilled water and dispensed in 50 ml amount. Then the solution was sterilized by autoclaving at 1.2 kg/cm^2 pressure and 121^0 C for 15 minutes and stored for future use.

3.2.8 Culture of milk sample

About 40 sample positive for mastitis were randomly selected for bacteriological examination. Each sample of milk was inoculated separately in Nutrient agar (NA) and Nutrient broth to promote growth of bacteria. These media were incubated at 37^0C for overnight. The colonies on primary culture were repeatedly sub-culture by streak plate methods (Cheesbrough, 1985) until the pure culture with homogenous colonies was obtained. Media such as Nutrient agar, MacConkey agar, Eosin Methylene Blue agar were used for sub-cultures and were incubated at 37^0C for 24 hours for growth.

3.2.9 Staining Methods

Gram's staining method was done to study the morphological and staining properties of the bacteria and to provide information about the presumptive bacterial identification as per recommendation of Merchant and Packer (1967). The procedure was as follows:

A small colony was picked up from pure culture plates with a bacteriological loop and smeared on separate glass slide and fixed by gentle heating. Crystal violate was then applied on each smear to stain for two minutes and then washed with running water. Few drops of gram's iodine was then added for one minute and again washed with running water. Acetone alcohol was then added (acts as decolorizer) for few second. After washing with water safranin was added as counter stain and allowed to stain for two minutes. The slides were then washed with running water , blotted and dried in air then examined under microscope with high power objects (100x) using immersion oil.

3.2.10 Biochemical test

3.2.10.1 Catalase test

This test was performed by taking 2-3 drops of 3 percent H_2O_2 on clean grease free glass slide and single colony was mixed with the help of a wire loop. Immediate formation of gas bubbles was considered as positive test.

3.2.10.2 Indole test

Peptone water was inoculated with isolated colony of test organism and incubated at 37^0 C for 24 hours.0.5 ml kovac's reagent was added, shaked well and examined after 1

minute. A red color ring indicated production of indole. In negative case there was no development of red color (Cheesbrough, 1985).

3.2.10.3 Methyl-Red test

A colony of test organism was inoculated in MR-VP broth to perform this test and incubated at 37^0C overnight. After incubation a drops of methyl red solution was added. A red color indicated an acid P^H resulting from the formation of glucose and was considered as positive. A yellow color indicated negative result (Cheesbrough, 1985).

3.2.10.4 Voges-Proskauer test

A tube of MR-VP broth was inoculated with pure culture of test organisms. It was incubated at 37^0C for 24 hours. Then 0.6 ml of 5% alpha naphthole solution followed by 0.2 ml of 40% KOH solution was added. The tube was shaked gently and allowed to remain undisturbed for 10 to 15 minutes. In positive case, a red colour is produced. There is no development of red colour indicate negative result (Cheesbrough, 1985).

3.2.10.5 Simmon's Citrate test

A colony of test organism was inoculated on Simmon's Citrate agar slant. It was incubated at 37^0C for 24 hours. In positive case, green colour of the media turns to prussian blue colour. No colour change indicate negative result (Cheesbrough, 1985).

3.2.11 Techniques for the isolation and identification of *Staphylococcus spp.*

3.2.11.1 Culture into different media

Loopful aliquot was taken from the Nutrient broth culture and streaked on Nutrient agar, Staphylococcus 110 agar media to get isolates in pure culture. All inoculated media were kept at 37^0 C for overnight in an incubator.

3.2.11.2 Nutrient agar (NA)

Gray-white to yellowish colony was produced on Nutrient agar in case of staphylococcus spp.

3.2.11.3 Staphylococcus no. 110 agar

Staphylococcus spp. produced cream colour colonies on Staphylococcus no.110 agar.

3.2.11.4 Microscopic study for identification of *Staphylococcus spp*. Suspected colonies by Gram's staining methods

Gram's staining was performed to determine the size, shape and arrangement of bacteria. The organism found as Grams positive, cocci arranged in cluster indicating *staphylococcus spp.*

3.2.11.5 Identification of *Staphylococcus spp.* Isolated by biochemical test

Catalase test, Citrate, MR and Indole test were positive and VP test was negative if the isolates were *Staphylococcus spp.*

3.2.12 Techniques for the isolation and identification of *Escherichia coli*

3.2.12.1 Culture into different media

Sterilized platinum loop was used for streaking the nutrient broth culture on MacConkey agar and EMB agar to get isolates in pure culture. All inoculated media were kept at 37^0 C for overnight in an incubator.

3.2.12.2 MacConkey agar

Martials from nutrient broth tubes were inoculated into MacConkey agar plates. After incubation, *Escherichia coli* showed rose pink color colonies.

3.2.12.3 Eosin Methylene Blue (EMB) agar

Smooth, circular, small pink colonies with dark center and metallic sheen were produced in case of *Escherichia coli.*

3.2.12.4 Microscopic study for identification of *Escherichia coli* suspected colonies by Gram's staining methods

Gram's staining was performed to determine the size, shape and arrangement of bacteria. *Escherichia coli* revealed Gram negative, pink color large rod shape appearance, arranged in single or paired.

13.2.12.5 Identification of *Escherichia coli* isolates by biochemical test

Catalase, MR and Indole test were positive and Citrate and VP test were negative if the isolates were *Escherichia coli.*

3.2.13 Techniques for the isolation and identification of *Pseudomonas spp.*

3.2.13.1 Culture into different media

Sterilized platinum loop was used for streaking the nutrient broth culture on Nutrient agar and Cetrimide agar. The media containing streaked culture were kept at 37^0 C overnight in incubator.

3.2.13.2 Nutrient agar (NA)

On Nutrient agar *Pseudomonas spp.* produced greenish colony with putrefied odour.

3.2.13.3 Cetrimide agar

Pseudomonas spp. produced yellow-green to blue colony on cetrimide agar.

3.2.13.4 Microscopic study for identification of *Pseudomonas spp* suspected colonies by Gram's staining method

Gram's staining was performed to determine the size shape and arrangement of bacteria. *Pseudomonas spp*. found as gram negative, thin, rod shaped organism.

3.2.13.5 Identification of *Pseudomonas spp*. by biochemical test

Catalase and Citrate test were positive, Indole, MR, VP test were negative in case of *Pseudomonas spp.*

3.2.14 Techniques for the isolation and identification of *Klebsiella spp.*

3.2.14.1 Culture into different media

Sterilized platinum loop was used for streaking the nutrient broth culture on MacConkey agar and EMB agar. The media containing streaked culture were kept at 37^0 C overnight in incubator.

3.2.14.2 MacConkey agar

Mucoid, convex, lactose positive colonies in case of *Klebsiella spp.*

3.2.14.3 Eosin Methylene Blue (EMB) agar

Klebsiella spp. produced large, mucoid, pink to purple colour colonies with no metalic sheen on EMB agar.

3.2.14.4 Microscopic study for identification of *Klebsiella spp* suspected colonies by Gram's staining method

Microscopic study revealed gram negative, small, rod shaped organism.

3.2.14.5 Identification of *Klebsiella spp*. by biochemical test

Catalase, Citrate and VP tests were positive, Indole and MR tests were negative.

3.2.15 Techniques for the isolation and identification of *Bacillus spp.*

3.2.15.1 Culture into different media

Sterilized platinum loop was used for streaking the nutrient broth culture on Nutrient agar. The media containing streaked culture were kept at 37^0 C overnight in incubator.

3.2.15.2 Nutrient agar

Bacillus spp. produced thick, grayish white or cream coloured colony with uneven surface on Nutrient agar.

3.2.15.3 Microscopic study for identification of *Bacillus spp* suspected colonies by Gram's staining method

Gram positive, large rod shaped organism with spores, arranged in pair or long chain.

3.2.15.4 Identification of *Bacillus spp* by biochemical test

Catlase, Citrate and VP tests were positive, Indole and MR tests were negative.

3.2.16 Maintenance of stock culture

The stock culture was maintained following the procedure as described by Choudhury *et al.* (1987). Nutrient agar slants were used for the maintenance stock culture for each of the bacterial isolates. One slant was used for an individual isolate. After growth of the organism in the slant the sterile mineral oil was overloaded and the cultural was kept at 4ºc temperature for further use.

3.2.17 Antibiotic sensitivity test

Antimicrobial sensitivity test was carried out using the disc diffusion method (Ellner, 1978; Quinn *et al.,* 1999) to determine the sensitivity and resistance pattern of different types of bacteria isolated from milk. Sensitivity to antibiotic was studied on nutrient agar plates used in different types of commercial antibiotics discs. The antibacterial used were Azithromycin (AZM) 15µg, Cloxacillin (COX) 1 µg, Gentamicin (GN) 10µg, Erythomycin (ERY) 15µg, Tetracycline (OT) 30µg, Levofloxacin (LE) 5µg, Amoxycillin (AMX) 30µg, Ciprofloxacin (CIP) 05µg, Chloramphenicol (C) 30µg, Penicillin (P) 10µg, Cefixime (CFM) 5µg, Cephalexin (CN) 30µg, Cephradine (CH) 25µg, Vankomycin (Va) 30µg and Amikacin (AK) 30µg/ disk. The respective plates were seeded uniformly by rubbing the isolated organism against the entire agar surface. Each antibacterial impregnated disc was applied onto the surface of the inoculated plate by using sterile forceps. The plates were incubated at 37°C for 48hrs. The zone of inhibition around each disc was measured and the interpretation was made as per the zone size interpretation chart provided by the disc manufacturer. The results of sensitivity test was expressed as 3+, 2+, 1+ and R expressing highly sensitive, moderately sensitive, less sensitive and resistant, level of susceptibility respectively.

3.2.18 Statistical analysis

Chi square (χ^2) test was performed to observe the significant influence of risk factors (age, parity, litter size, lactation stage, teat lesion) onto the causs of mastitis of goats using Statistical Package for Social Science (SPSS) Version 22.0.

CHAPTER 4

RESULTS

CHAPTER 4

RESULTS

The present research was conducted in different areas of Dinajpur town and adjacent villages of HSTU campus to study the prevalence of clinical and subclinical mastitis of randomly selected 120 Black Bengal goats as well as to determine the etiological bacteria and to know the *in vitro* antibiotic sensitivity pattern of the bacterial isolates against the commonly used antibiotic. During collection of sample a semi structured questionnaire was duly furnished with age, parity, litter size, lactation stage and teat lesion of goats.

4.1 Overall prevalence of mastitis in goats

In this study, 240 milk samples from 120 goats were examined for mastitis by clinical examination and CMT (California Mastitis Test). The prevalence of clinical mastitis, subclinical mastitis and amastitis were 11.67%, 38.75% and 49.58% respectively are presented in Table 5 and Fig. 9.

Table 5. Overall prevalence of caprine mastitis

Total no. of goat	Total no. of sample	Clinical mastitis	Subclinical mastitis	Amastitis
120	240	28	93	119
Prevalence (%)		11.67	38.75	49.58

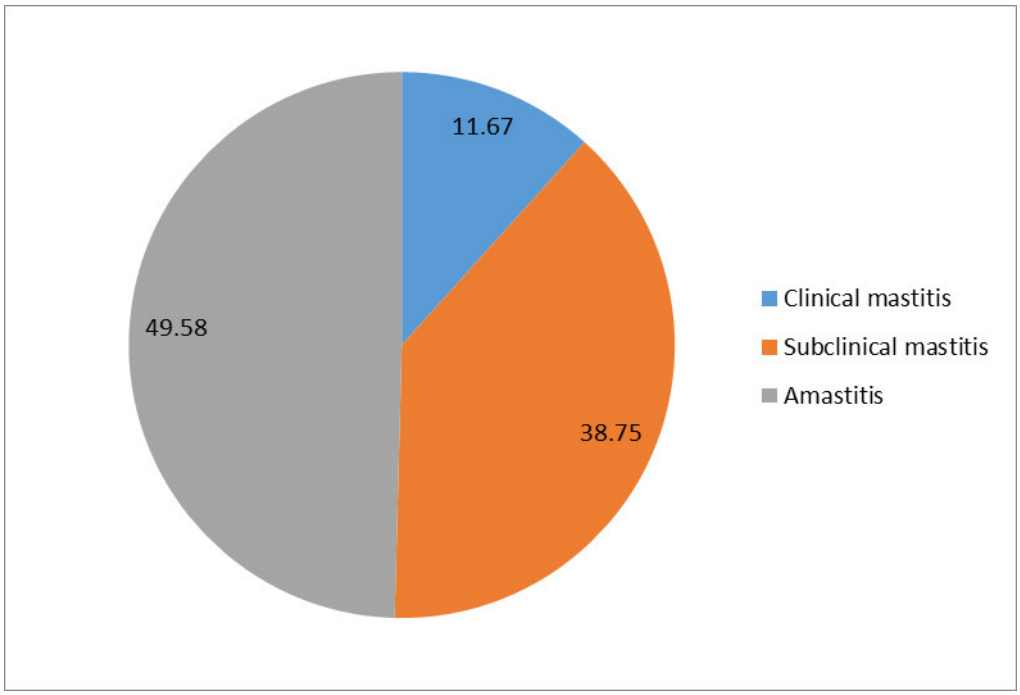


Fig. 9: Overall prevalence of mastitis in goat

4.2 Prevalence of clinical and subclinical caprine mastitis

The present research showed that 17 were positive for clinical mastitis and 52 were positive for subclinical mastitis among 120 lactating goats. The prevalence was varied depending on age, parity, litter size, lactation stage and teat lesion is presented in Table 6.

Table 6. Prevalence of clinical and subclinical mastitis in Black Bengal goats

Sl No.	Parameters	No of goats	Clinical mastitis No (%)	Subclinical Mastitis No (%)	Amastitis No (%)	p-value	Level of Significance
1.	**Age(Years)**						
	2-3	37	2(5.41)	13(35.14)	22(59.46)		
	3-4	57	9(15.79)	24(42.11)	24(42.11)	p = 0.02	*
	4-5	26	6(23.08)	15(57.69)	05(19.23)		
	Total	120	17(14.17)	52(43.33)	51(42.5)		
2.	**Parity**						
	1^{st}	11	00(00)	00(00)	11(100)		
	2^{nd}	22	02(9.09)	05(22.73)	15(68.18)		
	3^{rd}	19	02(10.53)	10(52.63)	07(36.84)	p = 0.00	**
	4^{th}	35	05(14.29)	17(48.57)	13(37.14)		
	5^{th}	33	08(24.24)	20(60.61)	05(15.15)		
	Total	120	17(14.17)	52(43.33)	51(42.5)		
3.	**Litter Size**						
	One	15	00(00)	02(13.33)	13(86.67)		
	Two	74	09(12.16)	30(40.54)	35(47.29)	p = 0.00	**
	Three	31	08(25.81)	20(64.52)	03(9.68)		
	Total	120	17(14.17)	52(43.33)	51(42.5)		
4.	**Lactation Stage**						
	Early	71	12(16.90)	32(45.07)	27(38.08)	p = 0.66	NS
	Mid	27	03(11.11)	12(44.44)	12(44.44)		
	Late	22	02(9.09)	08(36.36)	12(54.55)		
	Total	120	17(14.17)	52(43.33)	51(42.5)		
5.	**Teat Lesions**						
	Present	38	17(44.74)	18(47.39)	03(7.89)	p = 0.00	**
	Absent	82	00(00)	34(41.46)	48(58.54)		
	Total	120	17(14.17)	52(43.33)	51(42.5)		

Legends

*** means 5% level of significant ($p < 0.05$)**

**** means 1% level of significant ($p < 0.01$)**

NS means not significant ($p > 0.05$)

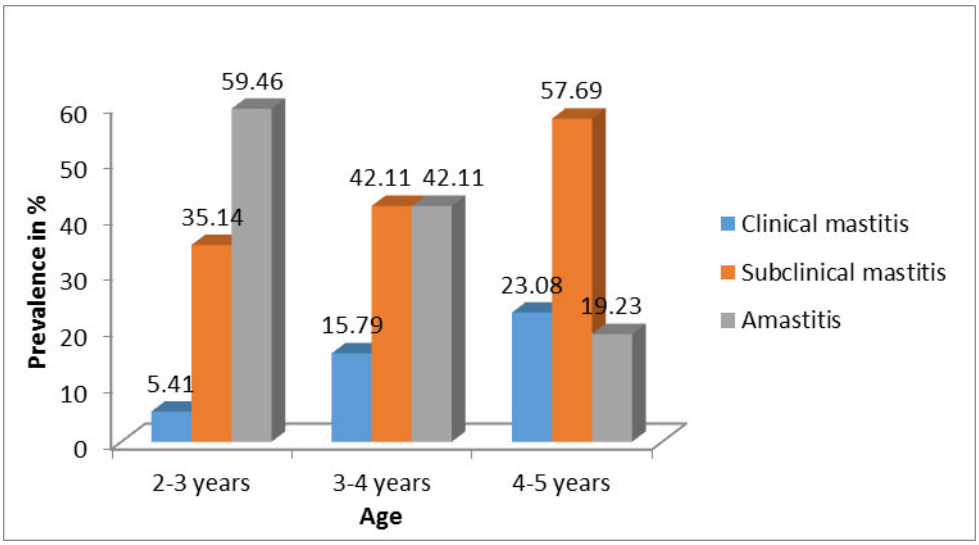


Fig. 10: Prevalence of mastitis in term of age

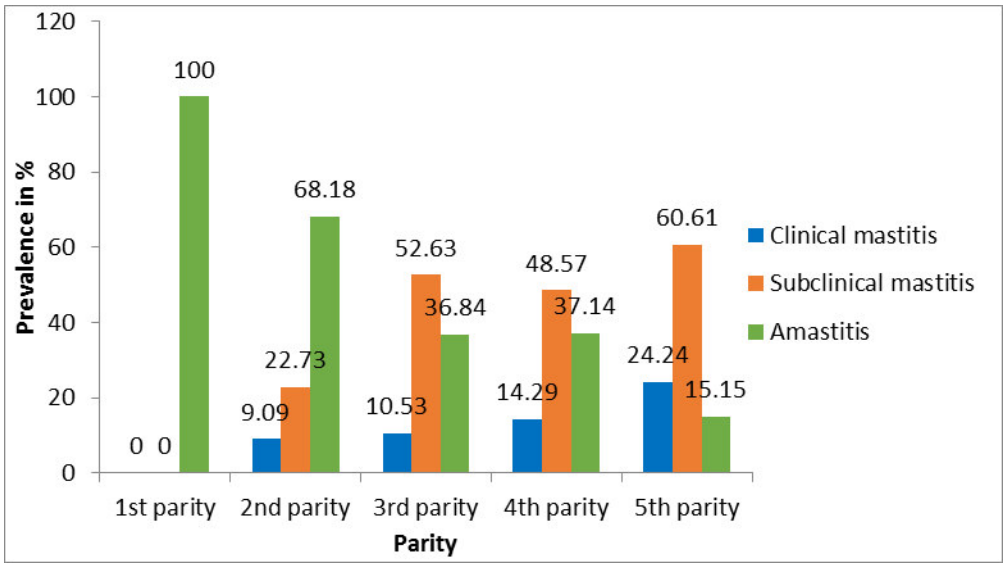


Fig. 11: Prevalence of mastitis in term of paritty

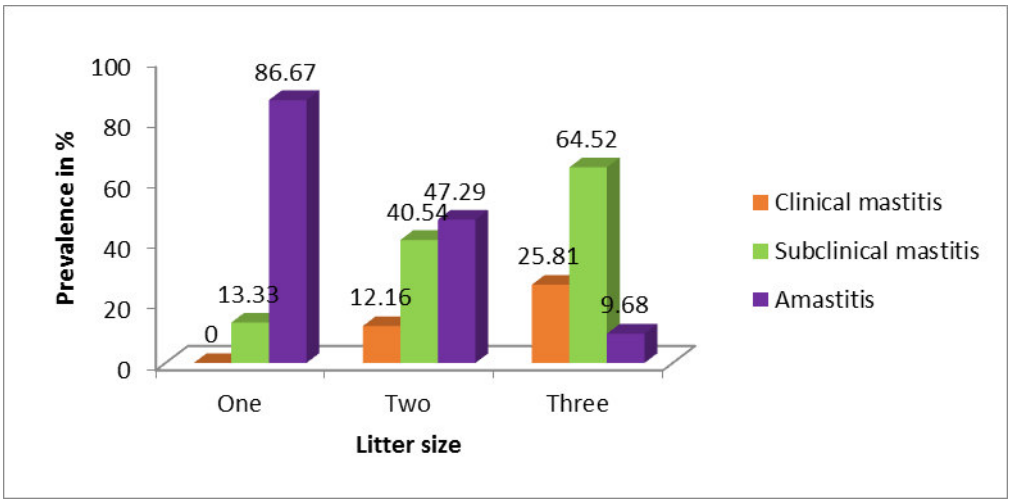


Fig.12:Prevalence of mastitis in term of litter size

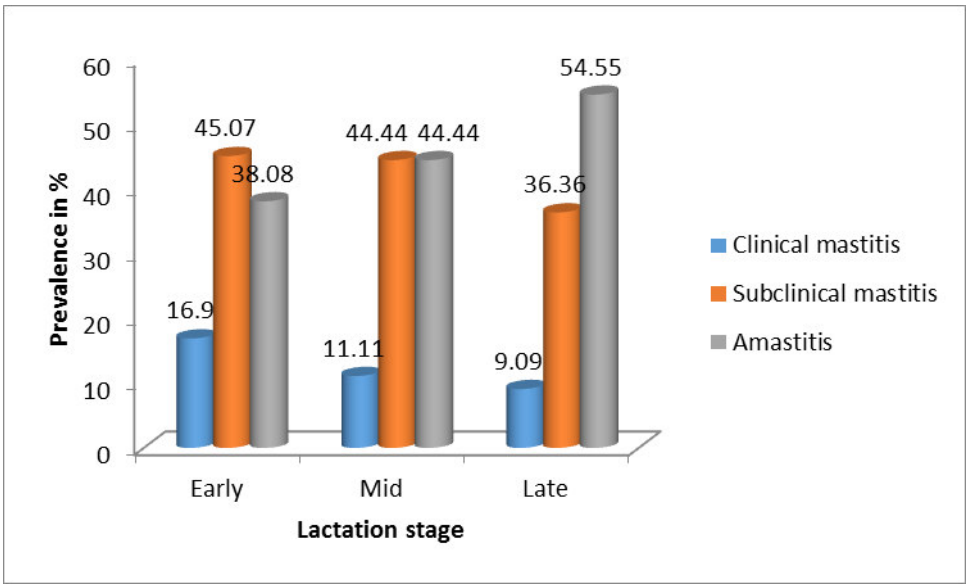


Fig. 13: Prevalence of mastitis in term of lactation stage

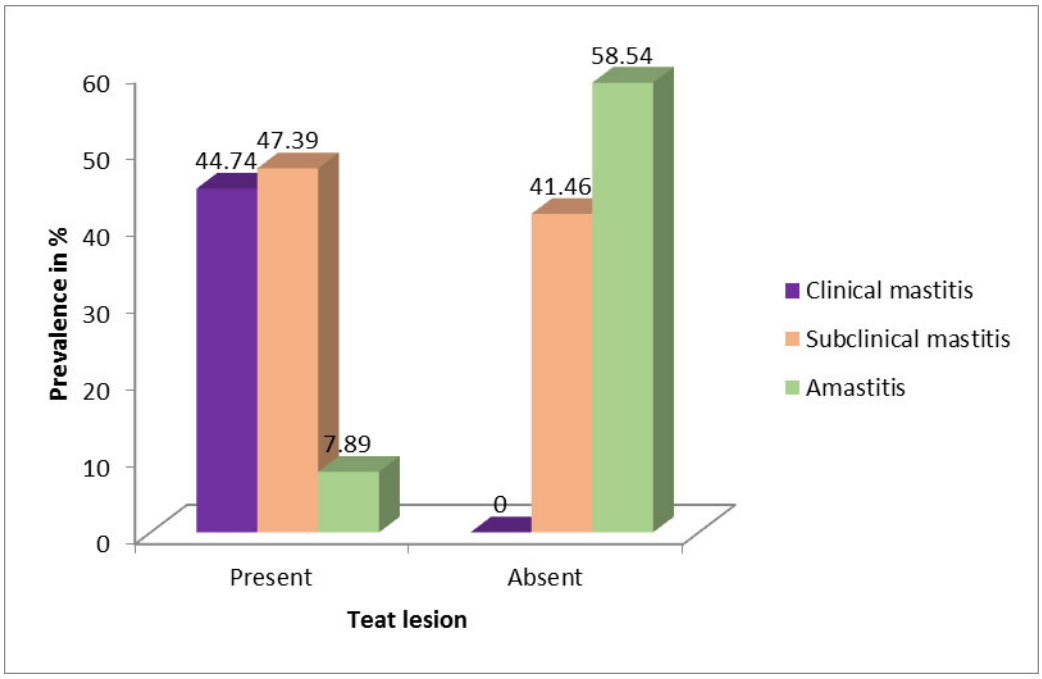


Fig. 14: Prevalence of mastitis in term of teat lesion

The highest prevalence was seen at the age 4 to 5 years which was 23.08% in case of clinical mastitis and 57.69% in case of subclinical mastitis while the lowest prevalence was seen at 2 to 3 years of age which was 5.41% in case of clinical mastitis and 35.14% in case of subclinical mastitis. The highest prevalence was seen at 5th parity which was 24.24% in case of clinical mastitis and 60.61% in case of subclinical mastitis while the lowest prevalence was seen at 2nd parity which was 9.09% in case of clinical mastitis and 22.73% in case of subclinical mastitis. However, 1st parity was not associated with clinical and subclinical mastitis. An increased prevalence of clinical and subclinical mastitis was observed with increased litter size in this study. Highest prevalence of clinical mastitis (25.81%) and subclinical mastitis (64.52%) was seen in does having 3 kids whereas lowest prevalence of clinical mastitis (12.16%) in does having 2 kids and subclinical mastitis (13.33%) in does having single kids. Case of clinical mastitis was not seen in does having single kids.

The highest prevalence of clinical (16.90%) and subclinical (45.07%) mastitis was detected in goats with an early lactation period, and the prevalence rate gradually decreased as the length of lactation period was decreased. In the presence of teat lesion, the prevalence of teat lesion was 44.74% in case of clinical mastitis and 47.37% in case of subclinical mastitis. Quarter having no lesions was shown 0% and 41.46% in case of clinical and subclinical mastitis respectively.

4.3 Udderhalve wise prevalence of clinical and subclinical mastitis

Clinical mastitis was detected as 11.67% (28/240) of the 28 halves (Left-13, Right-15) of 17 goats by clinical examination of udder and milk. Among the 17 goats, 11 had bilateral and 6 had unilateral mastitis.

Subclinical mastitis was detected as 38.75% (93/240) of the 93(Left-46, Right-47) halves of 52 goats by California Mastitis Test (CMT). Among the 52 goats, 41 had bilateral and 11 had unilateral mastitis. Different grades of subclinical mastitis of these 93 udderhalves are recorded in Table 8.

Side wise prevalence was 10.83% and 38.33% in left quarter and 12.5% and 39.17% in right quarter in case of clinical mastitis and subclinical mastitis respectively. There is no significant difference in the prevalence of mastitis in left and right quarter.

Table 7. Prevalence of clinical and subclinical mastitis in different quarters of udder

Types of mastitis	Quarter side	No of sample/ quarter	Positive quarter	Prevalence (%)
Clinical mastitis	**L**	120	13	10.83%
	R	120	15	12.5%
Total		240	28	11.66%
Subclinical mastitis	**L**	120	46	38.33%
	R	120	47	39.17%
Total		240	93	38.75%

Table 8. Prevalence of subclinical mastitis in Black Bengal goats by CMT test

Name of test	Total no of sample	Quarter side	No of sample tested	Positive results (%)		
				1+	2+	3+
CMT	240	**L**	120	25 (20.83)	18 (15)	03 (2.5)
		R	120	23 (19.17)	20 (16.67)	04 (3.33)

4.4 Isolation and identification of major mastitis causing pathogens

All the milk samples (40) were examined according to cultural, morphological and biochemical characteristics (Table 9). Among 40 suspected milk samples, 34 (85%) grew single type of bacterial colony and 6 (15%) grew mixed types. The highest prevalence of *Staphylococcus spp* (32.5%) was seen followed by *E. Coli* (22.5%), *Pseudomonas spp* (12.5%), *Klebsiella spp* (12.5%) and *Bacillus spp* (5%) in terms of growing single colony. The prevalence of *Staphylococcus spp + E. Coli, Klebsiella spp + E. Coli, Staphylococcus spp + Pseudomonas spp* were 10%, 2.5% and 2.5% respectively.

Table 9. Cultural, morphological and biochemical properties of isolated bacteria.

Name of the Bacteria	Cultural Characteristics		Biochemical Characteristics		Staining and Morphological Characteristics
Staphylococcus spp	Nutrient Agar	Gray white to yellowish colonies	Tests	Results	Gram positive cocci, arranged in cluster.
			Catalase test	+	
			Indole test	+	
	Staphylococcus no 110 Agar	Cream colour colonies	MR	+	
			VP	-	
			Citrate test	+	
Escherichia coli	MacConkey Agar	Rose pink colour colonies	Catalase test	+	Gram negative, pink color large rod shape appearance, arranged in single or paired
			Indole test	+	
	EMB Agar	Smooth, circular, small pink colonies with dark center and metallic sheen	MR	+	
			VP	-	
			Citrate test	-	
Pseudomonas spp	Nutrient Agar	Greenish colony with putrified odour	Catalase test	+	gram negative, thin, rod shaped organism
			Indole test	-	
			MR	-	
	Cetrimide Agar	yellow-green to blue colony	VP	-	
			Citrate test	+	
Klebsiella spp	MacConkey Agar	Mucoid, convex, lactose positive colonies	Catalase test	+	Gram negative, small, rod shaped organism
			Indole test	-	
	EMB Agar	Large, mucoid, pink to purple colour colonies with no metalic sheen	MR	-	
			VP	+	
			Citrate test	+	
Bacillus spp	Nutrient Agar	Thick, grayish white or cream coloured colony with uneven surface	Catalase test	**+**	Gram positive, large rod shaped organism with spores, arranged in pair or long chain.
			Indole test	**-**	
			MR	**-**	
			VP	**+**	
			Citrate test	**+**	

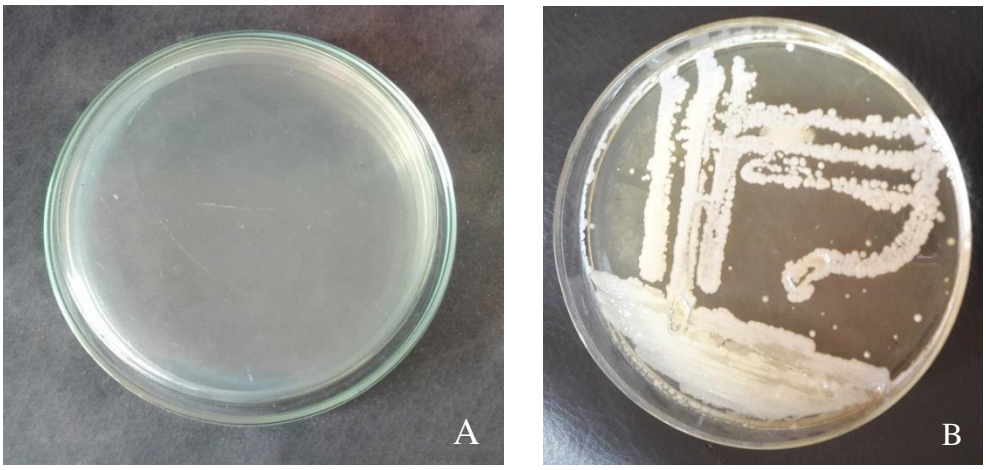


Plate 1: A: *Staphylococcus* no. 110 agar (control media) and B: Colony morphology of *Staphylococcus spp*. on *Staphylococcus* no. 110 agar

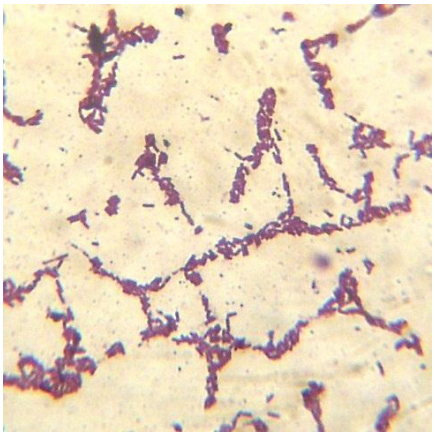


Fig. 15: Microscopic morphology of *Staphylococcus spp*. showing chain of small coccus strain with gram staining method

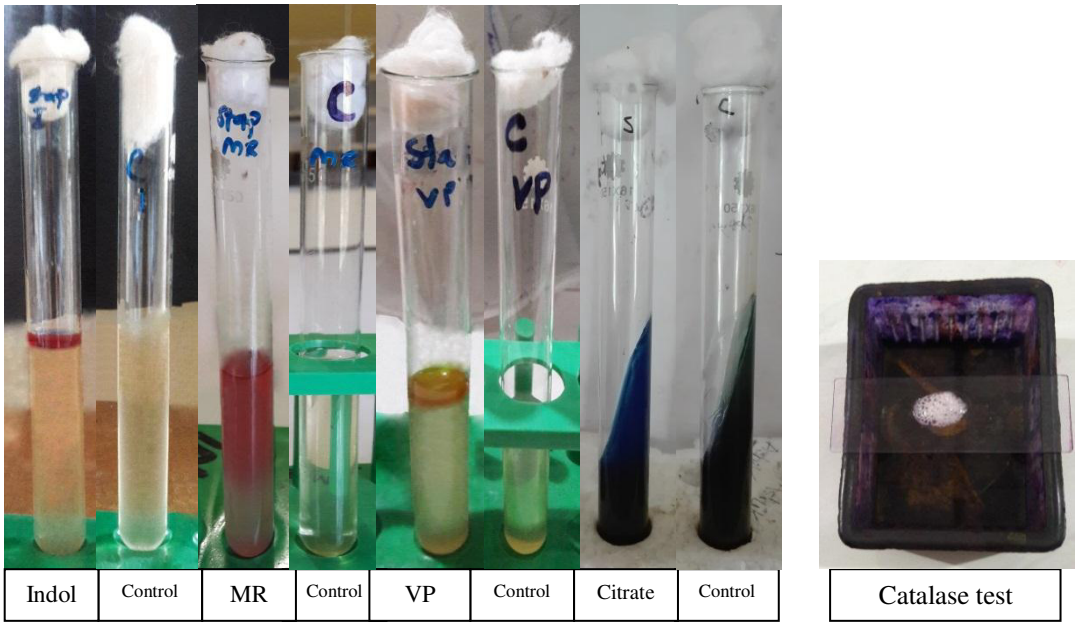


Fig. 16: Biochemical characteristics of *Staphylococcus spp*.

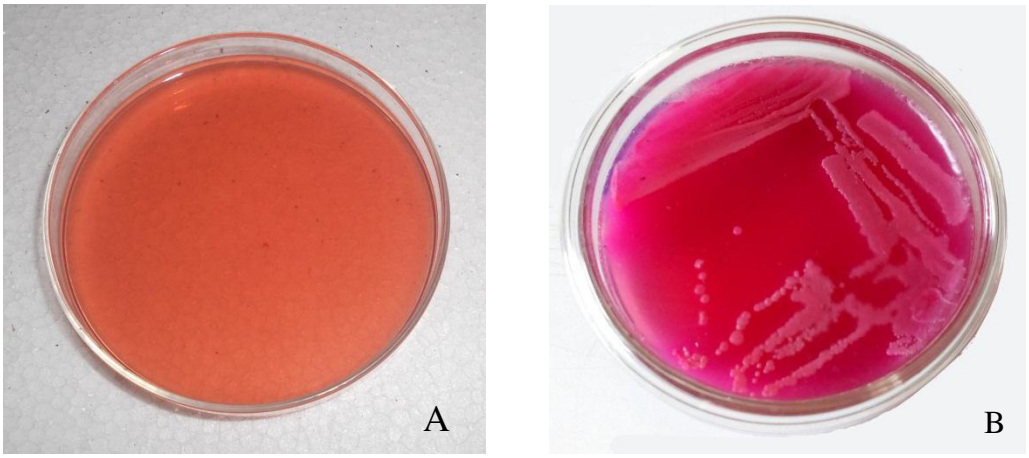


Plate 2: A: MacConkey agar (control media); B: Colony morphology of *Escherichia coli* on MacConkey agar

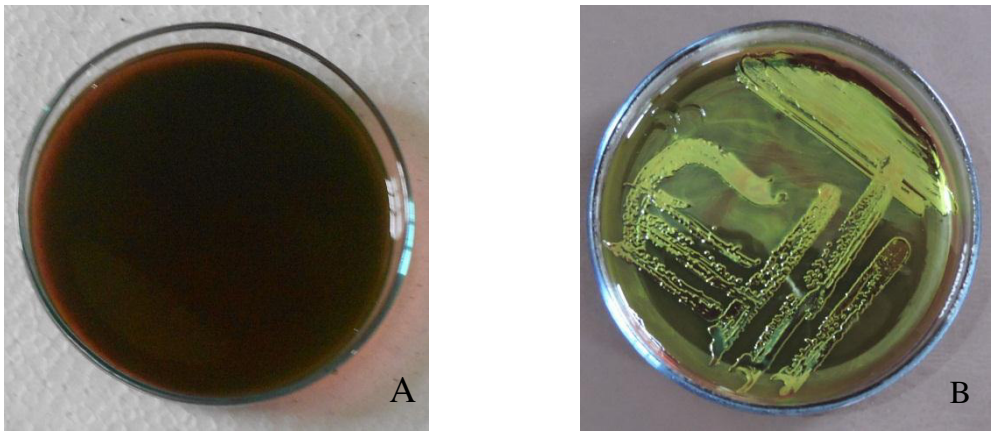


Plate 3: A: EMB agar (control media); B: Colony morphology of *Escherichia coli* on EMB agar

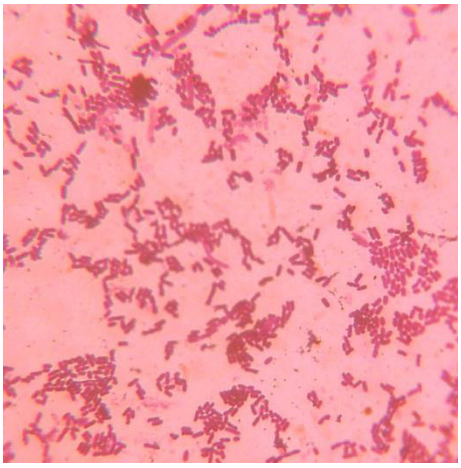


Fig. 17: Microscopic morphology of *Escherichia coli* showing pink color large rod shaped bacteria

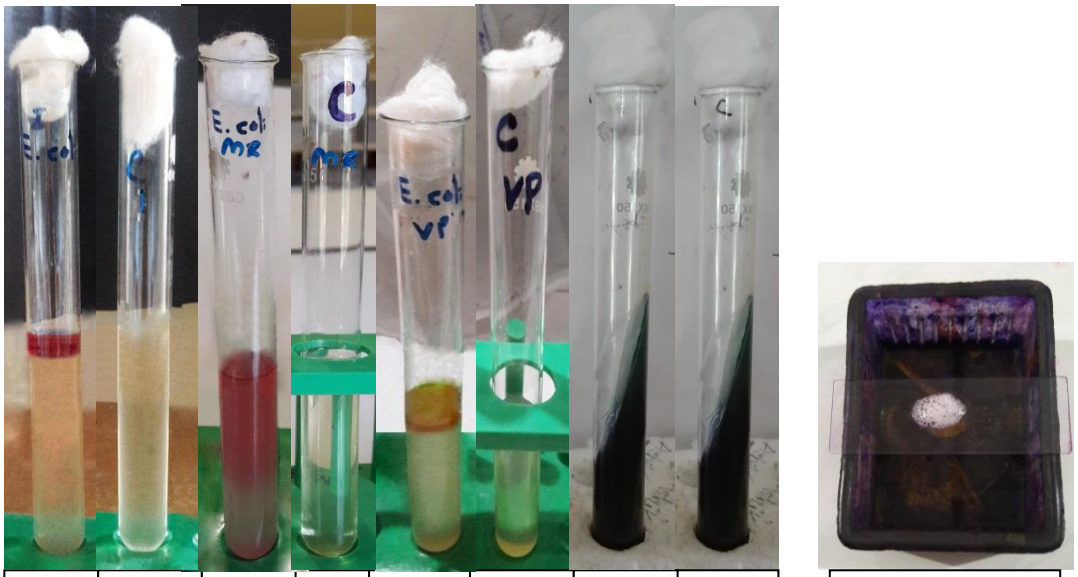


Fig. 18: Biochemical characteristics of *Escherichia coli*

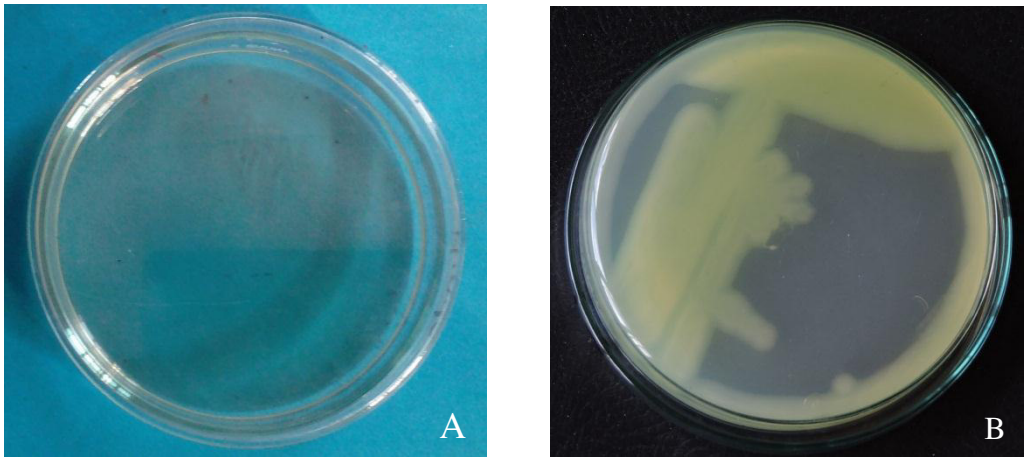


Plate 4: A: Cetrimide agar (control media); B: Colony morphology of *Pseudomonas spp.* on Cetrimide agar

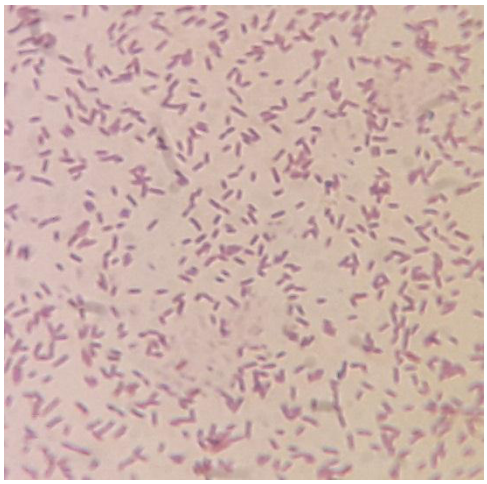


Fig. 19: Microscopic morphology of *Pseudomonas spp.*

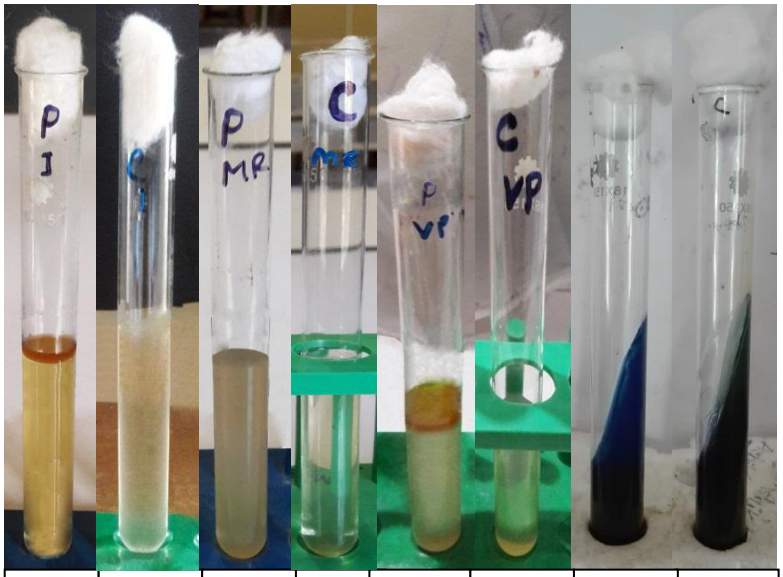


Fig. 20: Biochemical characteristics of *Pseudomonas spp.*

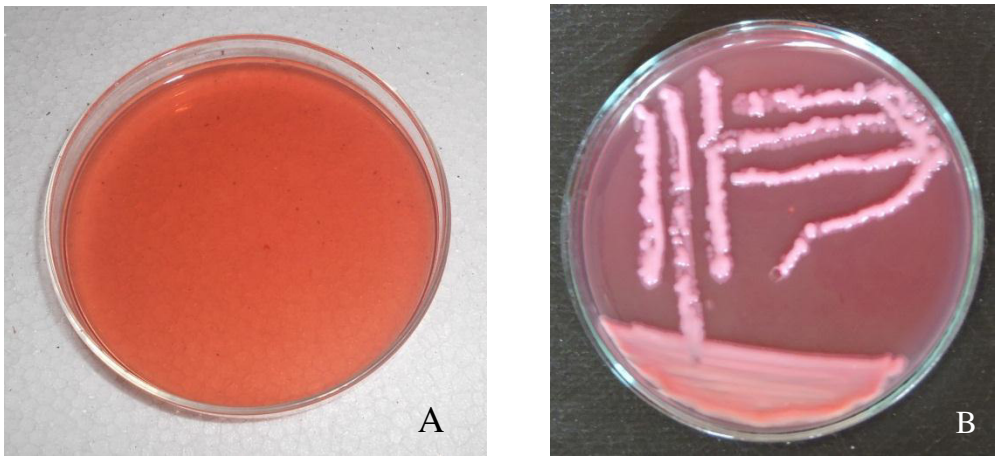


Plate 5: A: MacConkey agar (control media); B: Colony morphology of *Klebsiella spp.* on MacConkey agar

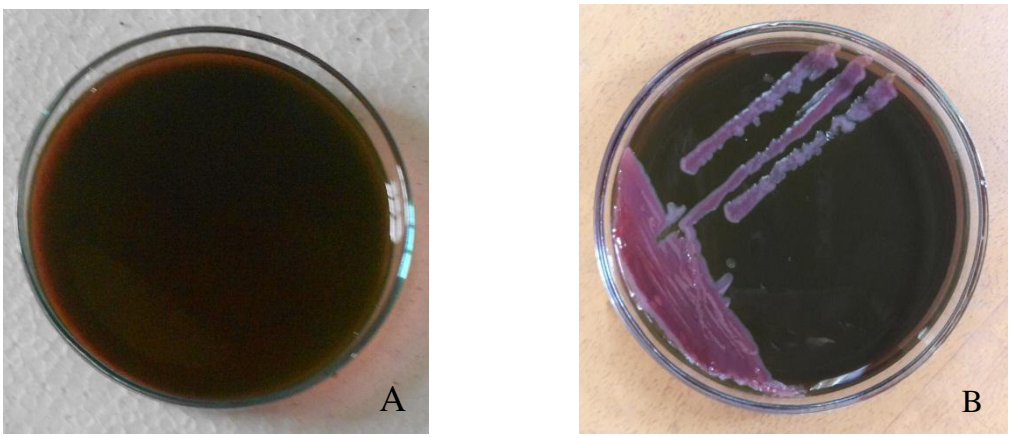


Plate 6: A: EMB agar (control media); B: Colony morphology of *Klebsiella spp.* on EMB agar

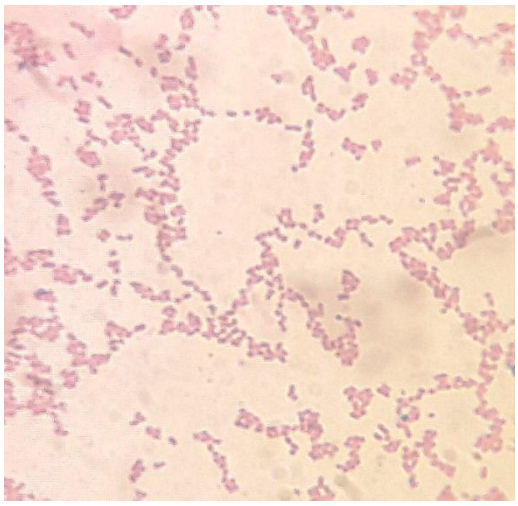


Fig. 21: Microscopic morphology of *Klebsiella spp.*

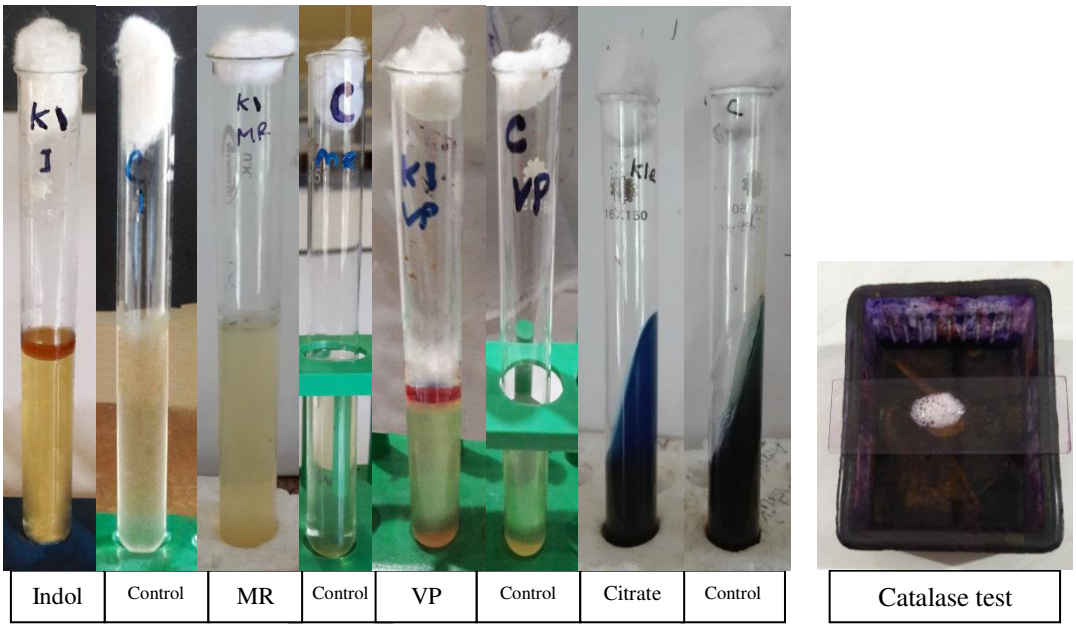


Fig. 22: Biochemical characteristics of *Klebsiella spp.*

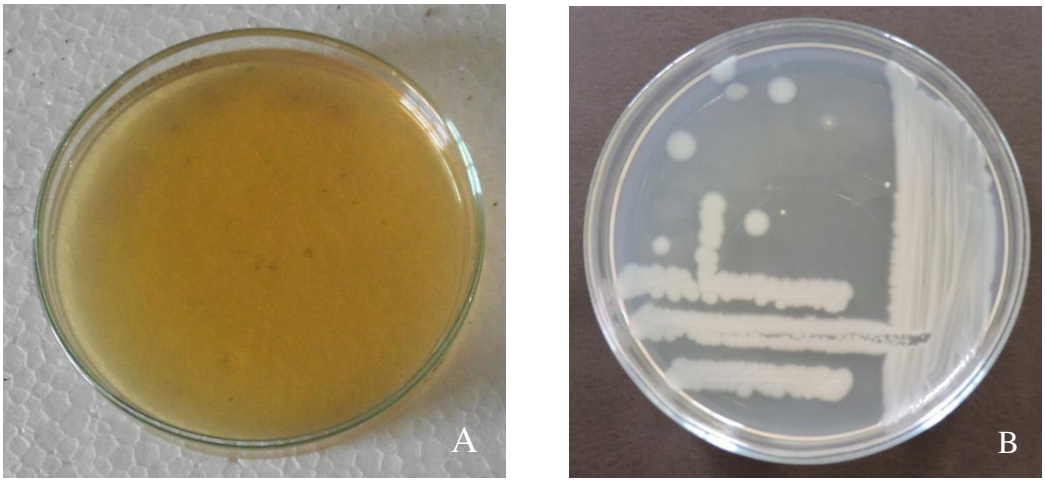


Plate 7: A: Nutrient agar (control media); B: Colony morphology of *Bacillus spp.* on Nutrient agar

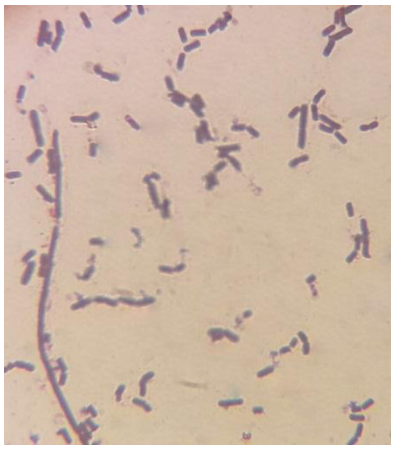


Fig. 23: Microscopic morphology of *Bacillus spp.*

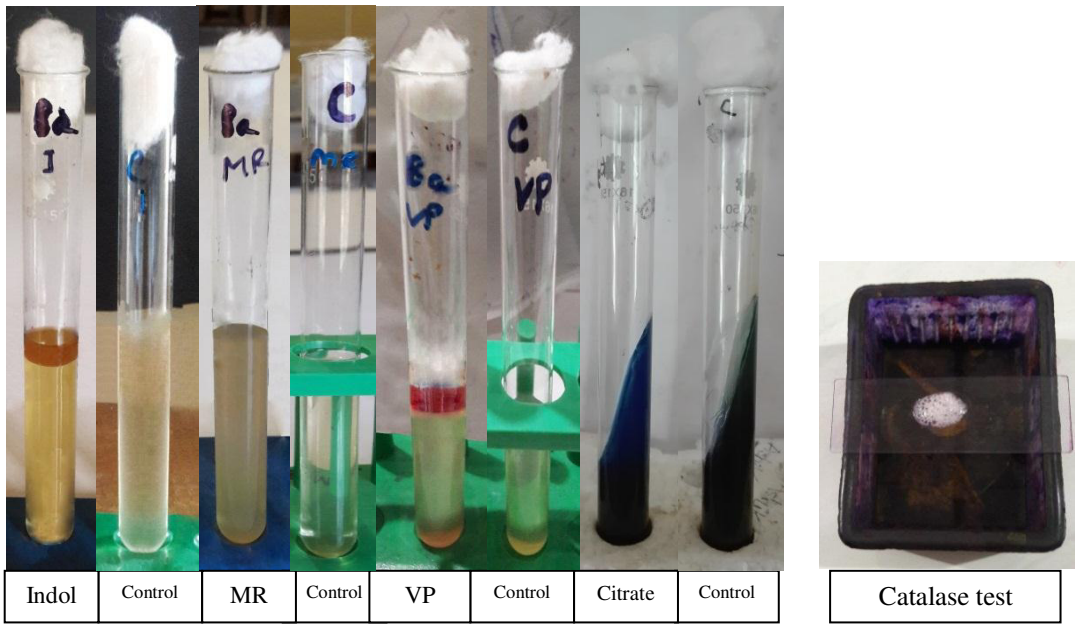


Fig. 24: Biochemical characteristics of *Bacillus spp.*

Table 10. Prevalence of bacterial isolated from milk of mastitic goat

Bacterial isolates	**No. of positive cases**	**Prevalence (%)**
Staphylococcus spp.	13	32.5
Escherichia coli	09	22.5
Pseudomonas spp.	05	12.5
Klebsiella spp.	05	12.5
Bacillus spp.	02	5
Staphylococcus spp + E. coli	04	10
Klebsiella spp + E. coli	01	2.5
Staphylococcus spp + Pseudomonas spp	01	2.5

4.5 Results of antibiogram study of the isolated bacteria

All of the bacterial isolates (40) were tested for the antibiotic sensitivity and resistance pattern against commonly used antibiotic. The result of sensitivity against antibiotic discs (zone of inhibition) were categorized as resistance, less sensitive, moderately sensitive, highly sensitive. The results of antibiotic sensitivity are given in the Table 11-15.

4.5.1 Antibiotic sensitivity pattern of *Staphylococcus spp.*

From the antibiotic sensitivity test of *Staphylococcus spp,* it was found that 23% of the *Staphylococcus spp* were moderately sensitive and 77% were highly sensitive to Azithromycin. 100% of the isolates were resistant to Amoxicillin. The organism showed 23% of the *Staphylococcus spp* were moderately sensitive and 77% were highly sensitive to Ciprofloxacin. 62% were less sensitive and 38% were moderately sensitive to Chloramphenicol. The isolates showed 85% of the *Staphylococcus spp* were resistant and 15% were less sensitive to Cloxacillin. 100% of the isolates were highly sensitive to Levofloxacin. 23% were moderately sensitive and 77% were highly sensitive to Gentamicin. Among the isolates, 54% of *Staphylococcus spp* were less sensitive and 46% were moderately sensitive to Erythromycin. 69% of the isolates were resistant and 31% were less sensitive to Tetracycline. Finally100% of the isolates were resistant to Penicillin.

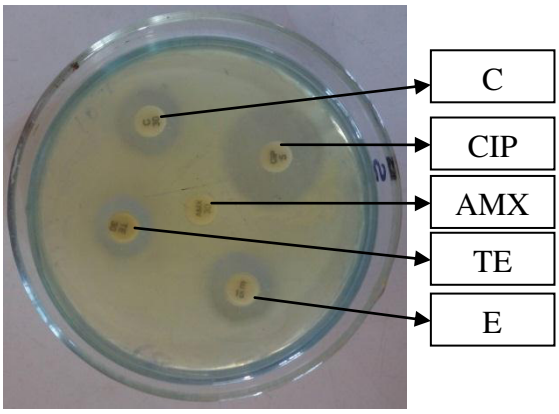


Plate 8: Antibiotic sensitivity test for *Staphylococcus spp.* on nutrient agar.
(C = Chloramphenicol, CIP = Ciprofloxacin, AMX Amoxicillin, TE = Tetracycline, E = Erythromycin)

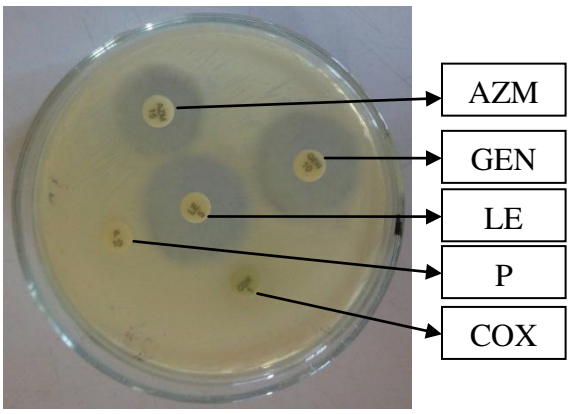


Plate 9: Antibiotic sensitivity test for *Staphylococcus spp.* on nutrient agar.
(AZM = Azithromycin, GEN = Gentamicin, LE = Levofloxacin, P = Penicillin, COX = Cloxacillin)

4.5.2 Antibiotic sensitivity pattern of *Escherichia coli*

From the antibiotic sensitivity test of *Escherichia coli,* it was found that 100% of the *E. coli* were moderately sensitive to Azithromycin. 89% of the isolates were resistant and 11% were less sensitive to Amoxicillin. 22% of the *E. coli* were moderately sensitive and 78% were highly sensitive to Ciprofloxacin. 100% were moderately sensitive to Chloramphenicol. The isolates showed 78% of the *E. coli* were resistant and 22% were less sensitive to Cloxacillin. Among the isolates, 100% of the *E. coli* were highly sensitive to Levofloxacin and 100% were highly sensitive to Gentamicin. 67% of the isolates of *E. coli* were less sensitive and 33% were moderately sensitive to Erythromycin. 89% of the isolates were resistant and 11% were less sensitive to Tetracycline. Finally, 100% of the isolates were resistant to Penicillin.

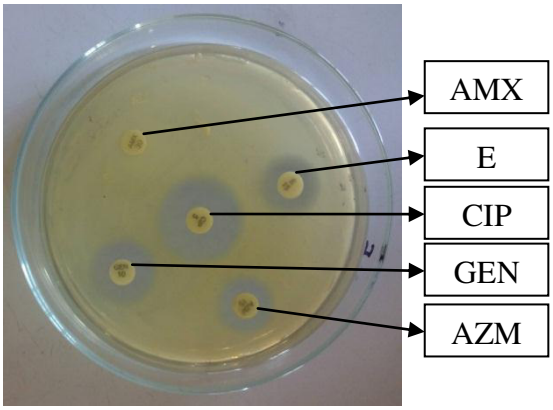


Plate 10: Antibiotic sensitivity test for *Escherichia coli* on nutrient agar.
(AMX Amoxicillin, E = Erythromycin, CIP = Ciprofloxacin, GEN = Gentamicin, AZM = Azithromycin)

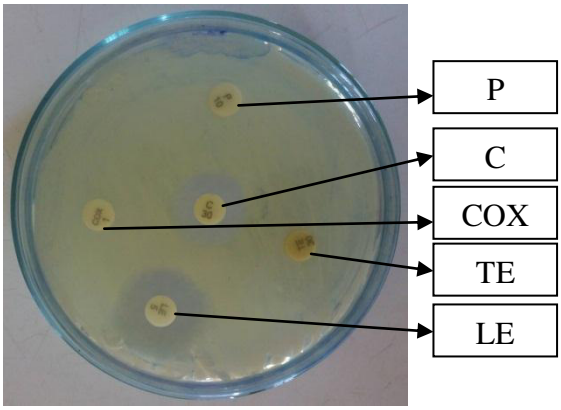


Plate 11: Antibiotic sensitivity test for *Escherichia coli* on nutrient agar.
(P = Penicillin, C = Chloramphenicol, COX = Cloxacillin, TE = Tetracycline, LE = Levofloxacin)

4.5.3 Antibiotic sensitivity pattern of *Pseudomonas spp.*

From the antibiotic sensitivity test of *Pseudomonas spp,* it was found that 100% of the *Pseudomonas spp* were resistant to Azithromycin and Amoxicillin. The organism appeared highly sensitive (100%) sensitive to Ciprofloxacin. Among the isolates, 40% were resistant and 60% were less sensitive to Chloramphenicol (Now a day chloromphenicol is not used in livestock industry, the result should not be like that). The 80% isolates were resistant and 20% isolates were less sensitive to Cloxacillin. The isolates were highly sensitive to Levofloxacin and Gentamicin. 60% of the isolates of *Pseudomonas spp* were resistant and 40% were less sensitive to Erythromycin. 80% of the isolates were resistant and 20% were less sensitive to Tetracycline. Finally, 100% of the isolates were resistant to Penicillin.

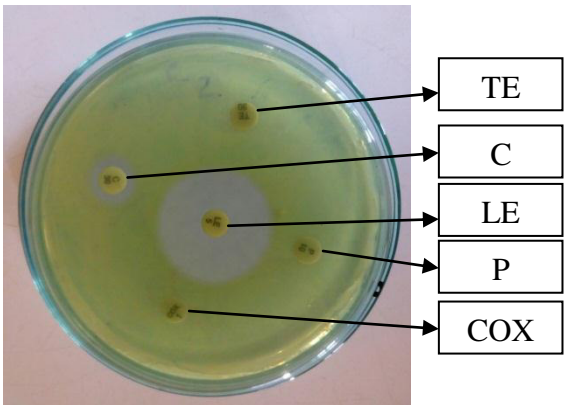


Plate 12: Antibiotic sensitivity test for *Pseudomonas spp.* on nutrient agar.
(TE = Tetracycline, C = Chloramphenicol, LE = Levofloxacin, P = Penicillin, COX = Cloxacillin)

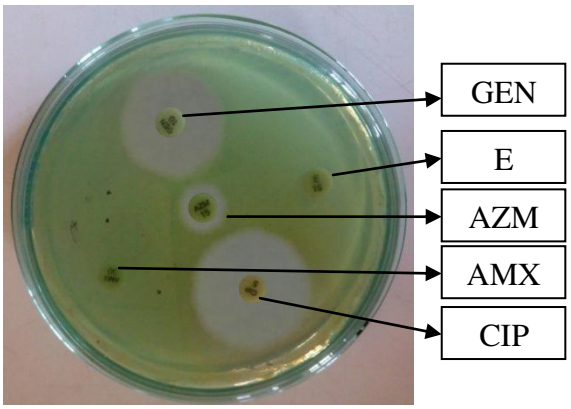


Plate 13: Antibiotic sensitivity test for *Pseudomonas spp.* on nutrient agar.
(GEN = Gentamicin, E = Erythromycin, AZM = Azithromycin, AMX Amoxicillin, CIP = Ciprofloxacin)

4.5.4 Antibiotic sensitivity pattern of *Klebsiella spp.*

From the antibiotic sensitivity test 80% of the *Klebsiella spp* were resistant and 20% were less sensitive to Cefixime. 100% of the isolates were resistant to Amoxicillin. The organism showed 100% sensitive to Ciprofloxacin. 40% of the *Klebsiella spp* were moderately sensitive and 60% were highly sensitive to Chloramphenicol. 100% of the isolates were resistant to Cloxacillin and 100% of the isolates were sensitive to Levofloxacin. Among the isolates, 60% were less sensitive and 40% were moderately sensitive to Gentamicin. 100% of the isolates of *Klebsiella spp* were resistant to Erythromycin. The organism showed 100% of the *Klebsiella spp* were resistant to Vancomycin. Finally, 100% of the isolates were resistant to Amikacin.

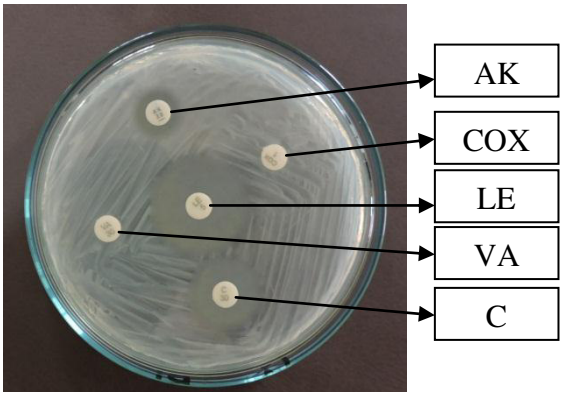


Plate 14: Antibiotic sensitivity test for *Klebsiella spp.* on nutrient agar.
(AK = Amikacin, COX = Cloxacillin, LE = Levofloxacin, VA = Vancomycin, C = Chloramphenicol)

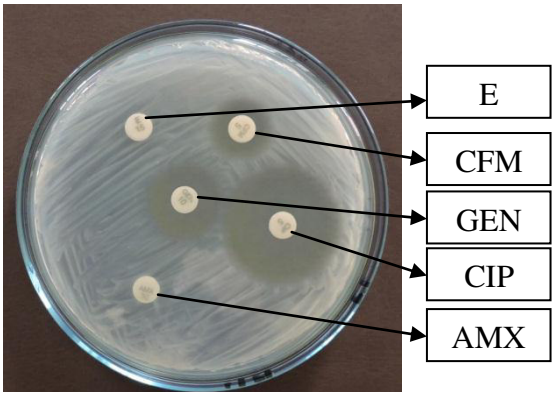


Plate 15: Antibiotic sensitivity test for *Klebsiella spp.* on nutrient agar.
(E = Erythromycin, CFM = Cefixime, GEN = Gentamicin, CIP = Ciprofloxacin, AMX = Amoxicillin)

4.5.5 Antibiotic sensitivity pattern of *Bacillus spp.*

From the antibiotic sensitivity test of *Bacillus spp,* 100% were resistant to Cefixime and Amoxicillin. 50% of the isolates were resistant and 50% were highly sensitive to Cephalexin. The organism showed 100% sensitivity to Chloramphenicol. The isolates were resistant to Cloxacillin and Cephradine whereas, but were highly sensitive to Gentamicin. Among the isolates, 100% were moderately sensitive to Erythromycin. 100% of the isolates were highly sensitive to Vancomycin. Finally, 50% of the isolates were resistant and 50% were less sensitive to Amikacin.

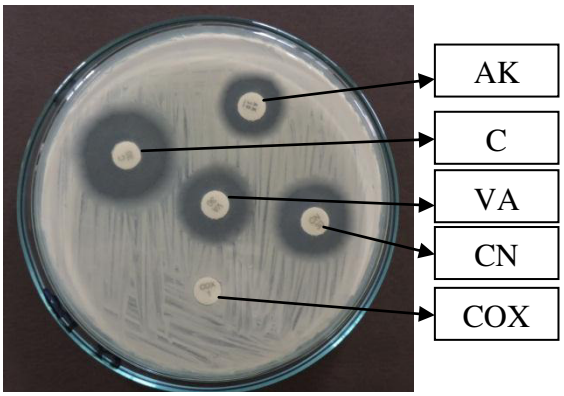


Plate 16: Antibiotic sensitivity test for *Bacillus spp.* on nutrient agar.
(AK = Amikacin, C = Chloramphenicol, VA = Vancomycin, CN = Cephalexin, COX = Cloxacillin)

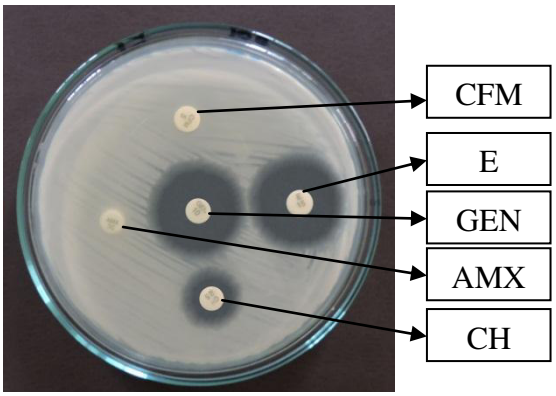


Plate 17: Antibiotic sensitivity test for *Bacillus spp.* on nutrient agar.
(CFM = Cefixime, E = Erythromycin, GEN = Gentamicin, AMX Amoxicillin, CH = Cephradine)

Table 11. Results of antibiotic sensitivity tests of the *Staphylococcus spp.*

Sensitivity	**% of isolated organism sensitive to various antibiotics**									
	AZM	**AMX**	**CIP**	**C**	**COX**	**LE**	**GN**	**E**	**TE**	**P**
Resistance	00	100	00	00	85	00	00	00	69	100
Less sensitive	00	00	00	62	15	00	00	54	31	00
Moderate sensitive	23	00	23	38	00	00	23	46	00	00
Highly sensitive	77	00	77	00	00	100	77	00	00	00

Table 12. Results of antibiotic sensitivity tests of the *Escherichia coli*

Sensitivity	**% of isolated organism sensitive to various antibiotics**									
	AZM	**AMX**	**CIP**	**C**	**COX**	**LE**	**GN**	**E**	**TE**	**P**
Resistance	00	89	00	00	78	00	00	00	89	100
Less sensitive	00	11	00	00	22	00	00	67	11	00
Moderate sensitive	100	00	78	100	00	00	00	33	00	00
Highly sensitive	00	00	22	00	00	100	100	00	00	00

Table 13. Results of antibiotic sensitivity tests of the *Pseudomonas spp.*

Sensitivity	**% of isolated organism sensitive to various antibiotics**									
	AZM	**AMX**	**CIP**	**C**	**COX**	**LE**	**GN**	**E**	**TE**	**P**
Resistance	100	100	00	40	80	00	00	60	80	100
Less sensitive	00	00	00	60	20	00	00	40	20	00
Moderate sensitive	00	00	00	00	00	00	00	00	00	00
Highly sensitive	00	00	100	00	00	100	100	00	00	00

Table 14. Results of antibiotic sensitivity tests of the *Klebsiella spp.*

Sensitivity	**% of isolated organism sensitive to various antibiotics**									
	CFM	**AMX**	**CIP**	**C**	**COX**	**LE**	**GN**	**E**	**Va**	**AK**
Resistance	80	100	00	00	100	00	00	100	100	100
Less sensitive	20	00	00	00	00	00	60	00	00	00
Moderate sensitive	00	00	00	40	00	00	40	00	00	00
Highly sensitive	00	00	100	60	00	100	00	00	00	00

Table 15. Results of antibiotic sensitivity tests of the *Bacillus spp.*

Sensitivity	**% of isolated organism sensitive to various antibiotics**									
	CFM	**AMX**	**CN**	**C**	**COX**	**CH**	**GN**	**E**	**Va**	**AK**
Resistance	100	100	50	00	100	100	00	00	00	50
Less sensitive	00	00	00	00	00	00	00	00	00	50
Moderate sensitive	00	00	00	00	00	00	00	100	00	00
Highly sensitive	00	00	50	100	00	00	100	00	100	00

CHAPTER 5

DISCUSSION

CHAPTER 5

DISCUSSION

The current study was designed to investigate the prevalence of Caprine mastitis, identify its etiological agents and antimicrobial sensitivity pattern of different types of bacteria isolated from the milk of apparently healthy and mastitic goats. For this study 240 milk samples from 120 lactating goats of different places of Dinajpur town and adjacent villages of HSTU campus were screened by clinical examination and California Mastitis Test (CMT). Then the milk samples were subjected to bacteriological culture for isolation and identification of major mastitic pathogen which were subjected to antimicrobial sensitivity test to know the *in vitro* sensitivity spectrum of these bacterial isolates against the commonly used antibiotic.

The study showed the overall prevalence of clinical and subclinical mastitis to be 11.67% and 38.75% respectively, which is in agreement with the reports of Mugabe *et al.* (2017), who found the highest prevalence of sub-clinical mastitis (13.5%) than clinical mastitis (4.29%). A 10% prevalence of clinical mastitis had been reported in dairy goats in Nigeria (Ameh *et al.,* 1993) which is similar to my study. The prevalence of mastitis was not same in various geographical regions. Previously the prevalence of subclinical mastitis reported was 18.29% (Rizwan *et al.,* 2016), 18.64% (Razi *et al*., 2012), 24.6% (Roukbi *et al.,* 2015)**,** 29.92% (Al-Ramahi and Al-Nassrawi*,* 2007) and 36% (Islam *et al.,* 2012). The prevalence of clinical mastitis reported in Pakistan was 3.4% (Rizwan *et al.,* 2016). The prevalence of mastitis in goats reported was 2.4% in Ethiopia (Assefa *et al.,* 2006) and 6% in Mymensingh, Bangladesh (Islam *et al.,* 2012), the prevalence were much lower in contrast to present study. The difference in the prevalence of caprine mastitis could be assigned to the difference in rearing system, milking technique, breed consideration, environmental effect etc.

In this study, animal wise and quarter wise subclinical mastitis was detected at 43.33% and 38.75% respectively which agreed the observation of Sharma *et al.* (2005). They reported the prevalence of subclinical mastitis at 56% on animal basis and 35% on quarter basis by examining 100 udder halves of 50 healthy goats in Bikaner, India. The result of present study is also similar with the study of Swai *et al.* (2008) who reported the prevalence of subclinical mastitis based on CMT at doe and udder half level as 38.92% and 28.9% respectively.

Side wise prevalence was detected as 10.83% in left quarter and 12.5% in right quarter in case of clinical mastitis whereas, 38.33% in left quarter and 39.17% in right quarter in case of subclinical mastitis which is comparable with those of previous report of Al-Ramahi and Al-Nassrawi (2007) as they found the percentage of affection of left half was 48.2% ,while the right halves give the percentage of 51.8%, with no significant difference by screening 274 milk samples from 137 clinically healthy local breed does. In contrast to this, Swai *et al.* (2008) reported a higher prevalence of mastitis in right halves (32.2%) than the corresponding left halves (25.5%). It may be due to, in goats which were fed to its full capacity the rumen gets engorged and the animal tends to lie on it's right side resulting in direct contact of right sided teats with ground which harbours microbes (Shittu *et al.*, 2008).

Among 17 clinically affected mastitic goat, 11 had bilateral and 6 had unilateral mastitis and among 52 goats with subclinical mastitis, 41 had bilateral and 11 had unilateral mastitis which is supported by Kumar *et al.* (2016). They reported that 32 udder halves were unilaterally affected and 152 udder halves had bilateral affection by screening 397 quarter foremilk samples from 200 apparently healthy randomly selected lactating beetal crossbred goats. As opposed to this, Sarker and Samad (2011) reported that 49 had unilateral and 5 goats had bilateral clinical mastitis among 54 Black Bengal lactating goats. In view of several variabilities, i.e., the disposition of the quarters, milking, various managemental practices, this observation cannot be generalized. On the other hand it may vary from animal to animal (Priya *et al.*, 2016).

In this study, California Mastitis Test kit was used for early detection of subclinical mastitis. The test is based on the reaction between the CMT reagent and the DNA genetic material of the somatic cells. A higher concentration in somatic cells leads to a higher CMT score. CMT scores are directly related to average somatic cell counts (Leite-Browning, 2008). Mammary gland irritation may lead to higher level of neutrophils and hence higher somatic cells (Smith and Roguinsky, 1997). Therefore CMT was shown to be useful way for screening goats for the presence of increased somatic cell count and presence of an intramammary infection (Razi *et al*., 2012). However, at population level, false negative CMT results may be observed (McDougall *et al.,* 2010).

In current study, bacterial pathogen of mastitic goats were isolated by conventional methods including colony morphology, Gram's staining and different biochemical tests

that (Ibrahim *et al.*, 2009; Assefa *et al.*, 2006) are the traditional methods to isolate and identify bacteria from milk samples.

Many infectious agent have been implicated as causes of mastitis but in this study *Staphylococcus spp.* (32.5%), *Escherichia coli* (22.5%), *Pseudomonas spp.* (12.5%) *Klebsiella spp*. (7.5%) and *Bacillus spp.* (5%) were isolated from the mastitic milk. Result of the present study is in agreement with the previous reports made by Najeeb *et al.* (2013), Mbindyo *et al.* (2014), Mugabe *et al.* (2017), Marogna *et al.* (2012), Gebrewahid *et al.* (2012), Zhao *et al.* (2015), Haftay *et al.* (2016). The major mastitic pathogen identified in this study was *Staphylococcus spp.* Similar findings were also reported by Kumar *et al.* (2016), Rashid *et al.* (2017), Marogna *et al.* (2012) and Hristov *et al.* (2016). The highest prevalence of Coagulase Negative Staphylococci (CNS) was reported by Gelasakis *et al.* (2016), Nickerson *et al.* (2015), Jakee *et al.* (2013), Silanikove *et al.* (2014). Other than single infection (85%), mixed infections (15%) were also found in this study which was supported by the observation of Sarker and Samad (2011). They reported that both the clinical and sub-clinical mastitis affected udder-halves had single (clinical 76.27% and sub-clinical 71.43%) and mixed (clinical 16.95% and sub-clinical 24.49%) bacterial infections. Ayodhya *et al.* (2013) also revealed mixed infection of *Staphylococcus spp., E. coli* and *Klebsiella spp.*

In this study there was a significant association of age (p=0.02), parity (p=0.00), litter size (p=0.00), teat lesion (p=0.00) with mastitis (CM and SCM) which is similar with previous study that age showed statistically significant association with the prevalence of subclinical mastitis (p = 0.009) (Haftay *et al.*, 2016) and caprine mastitis was significantly (P>0.05) associated to risk factors; parity class, breed, housing floors, flock size and suckling litter number (Mugabe *et al.*, 2017). Gebrewahid *et al.* (2012) revealed insignificant association between risk factors and mastitis like Age (p = 0.779), parity (p = 0.201) and stage of lactation (p = 0.952).

There was insignificant association of lactation stage (p= 0.669) with mastitis which is supported by Haftay *et al.* (2016). They reported that parity number and length of lactation did not show statistically significant relation with the prevalence of subclinical mastitis in goats. The possible reason for this could be some of the lactating goats may be aged without increasing their parity and this could also be associated with the inefficiency in the reproductive potential of these goats.

Highest prevalence of clinical (23.08%) and subclinical (57.69%) mastitis was found at the age 4-5 years and lowest prevalence of clinical (5.41%) and subclinical (35.14%) mastitis was found at the age 2-3 years. In this study, a trend in increase in the rate of prevalence of clinical and subclinical mastitis was observed with the advancement of age of the goats. Higher age (3 years or above) was found epidemiologically associated with increased caprine subclinical mastitis. Our present findings support the earlier observations of Sharma *et al.* (2007); Ali *et al.* (2010) and Razi *et al.* (2012). This increased prevalence of subclinical mastitis in older animal might be due to increased length of exposure of older animal to pathogens compared to younger animal.

An increased prevalence of CM (24.24%) and SCM (60.61%) was found at 5^{th} parity and lowest prevalence of CM (9.09%) and SCM (22.73%) was found at 2^{nd} parity. The prevalence of subclinical mastitis was higher in animals that were at later stage of their parity e.g., at the 6th and 5th parity, as reported earlier by Boscos *et al.* (1996), Sanchez *et al.* (1996) and Razi *et al.* (2012). A conclusion can be easily drawn from this and the previous studies that old age, the added burden of high milk production for longer period and multiple numbers of parturitions produce stress on their body. As a result such animals are easily become the host of infectious agents due to low immunity level (Ali *et al.*, 2010).

Highest prevalence of CM (25.81%) and SCM (64.52%) was found in does having 3 kids and lowest prevalence of CM (12.16%) recorded in does having 2 kids and SCM (13.33%) in does having single kid. Bergonier *et al.* (2003) reported that incidence of sub clinical mastitis is more in multiparous than primiparious French goats.

The highest prevalence of CM (16.90%) and SCM (45.07%) was detected in animals with an early lactation period which was gradually decreased as the length of lactation period increased. The similar result was declared by Las Heras *et al.* (1999), Leitner *et al.* (2001) that the High incidence may be observed in dairy ewes during suckling, suckling-milking periods or during first third of lactation in goats (Bergonier *et al.,* 2003). The highest distribution during the early period of lactation may be due to the residual infections in udder which flare up during the early lactation period (Saxena *et al.,* 1993). Quarter having different lesions was shown to be CM (44.74%) and SCM (47.39%) whereas quarter having no lesion was shown to be 0% and 41.46% clinically and subclinically mastitis positive which is in agreement with the report of Ali *et al.*

(2010). They found that the prevalence of subclinical mastitis was 57.7% in the presence of teat lesion and 42.2% in the absence of teat lesion. Injury in teats and udder gives access variety of pathogenic microorganisms to enter into it and as a result occurrence of mastitis (Gebrewahid *et al.,* 2012).

The *in-vitro* antibiotic sensitivity test of five different types of bacterial isolates to 15 different antibiotic such as Azithromycin (AZM) 15 µg, Cloxacillin (COX) 1 µg, Gentamicin (GN) 10 µg, Erythomycin (ERY) 15 µg, Tetracycline (OT) 30 µg, Levofloxacin (LE) 5 µg, Amoxycillin (AMX) 30 µg, Ciprofloxacin (CIP) 05 µg, Chloramphenicol (C) 30 µg, Penicillin (P) 10 µg, Cefixime (CFM), Cephalexin (CN), Cephradine (CH), Vankomycin (Va), Amikacin (AK) were studied. A major variation was noticed in the results of sensitivity test of bacterial isolates.

The antibiograms of various isolates of *Staphylococcus spp.* was found moderately sensitive to highly sensitive against Azithromycin, Ciprofloxacin and Gentamicin. The organism showed resistant to Amoxicillin and Penicillin. All isolates of *Staphylococcus spp.* were less sensitive to moderately sensitive against Chloramphenicol and Erythromycin. The isolates showed resistant to less sensitive against Cloxacillin and Tetracycline. All the isolates were highly sensitive to Levofloxacin. The similar results were declared by Sarker and Samad (2011) where they reported that moderately to highly sensitivity (2+ to 3+) was recorded in all the tested isolates of *Staphylococcus spp.* to gentamicin, ciprofloxacin, chloramphenicol, enrofloxacin and oxytetracycline, whereas less to moderate sensitivity was obtained with erythromycin (1+ to 2+). Ampicillin, amoxycillin, doxycycline and streptomycin were found resistant to less sensitive (- to 1+). The results were also supported by the report given by Najeeb *et al.* (2013), Razi *et al.* (2012) and Nathawat *et al.* (2013).

From the antibiotic sensitivity test of *Escherichia coli,* it was found that all of the *Escherichia coli* were moderately sensitive to Azithromycin and Chloramphenicol. The isolates were resistant to less sensitive against Amoxicillin, Cloxacillin and Tetracycline. The organism showed moderately sensitive to highly sensitive against Ciprofloxacin. All the isolates of *Escherichia coli* were highly sensitive to Levofloxacin and Gentamicin. *Escherichia coli* were less sensitive to moderately sensitive against Erythromycin. Finally, all of the isolates were resistant to Penicillin. The results were in agreement with the previous report of Sarker and Samad (2011). They reported that all the tested *E. coli*

isolates were highly sensitive (3+) to gentamicin and ciprofloxacillin, moderately to highly sensitive (2+ to 3+) to erythromycin and oxytetracycline, moderately sensitive (2+) to chloramphenicol and less sensitive (1+) to doxycycline, whereas resistant to less sensitive (- to 1+) results were obtained with ampicillin and streptomycin. The results were also supported by the report given by Najeeb *et al.* (2013) and Mbindyo *et al.* (2014).

From the antibiotic sensitivity test of *Pseudomonas spp,* it was found that all of the *Pseudomonas spp* were resistant to Azithromycin, Amoxicillin and Penicillin. The organism showed highly sensitive to Ciprofloxacin, Levofloxacin and Gentamicin. The isolates were resistant to less sensitive against Chloramphenicol, Cloxacillin, Erythromycin and Tetracycline. The results of antibiogram studies of *Pseudomonas spp.* could not be compared due to lack of similar inland reports on caprine mastitis.

From the antibiotic sensitivity test of *Klebsiella spp,* it was found that *Klebsiella spp* were resistant to less sensitive to Cefixime (write either trade or genric name). The isolates were resistant to Amoxicillin, Cloxacillin, Erythromycin, Vancomycin and Amikacin. The organism showed highly sensitive to Ciprofloxacin and Levofloxacin. All the *Klebsiella spp* were moderately sensitive to highly sensitive against Chloramphenicol. All of them were less sensitive to moderately sensitive against Gentamicin. The results of present study can be compared with the report of Ali *et al.* (2010). They observed that the given bacteria (*Staphylococcus aureus*, *Streptococcus spp*., *E. coli Klebsiella spp.*) were highly sensitive to gentamicin (96.15%) while least sensitive to penicillin G (42.13%).

From the antibiotic sensitivity test of *Bacillus spp,* it was found the organisms were resistant to Cefixime and Amoxicillin, Cloxacillin and Cephradine. *Bacillus spp* were resistant to highly sensitive against Cephalexin. The organism were highly sensitive to Chloramphenicol, Gentamicin and vancomycin. All the isolates were moderately sensitive to Erythromycin. Finally, the isolates were resistant to less sensitive against Amikacin. These results were supported by Sarker and Samad (2011) who observed that isolates of *Baccilus spp.* were moderately to highly sensitive (2+ to 3+) to gentamicin, ciprofloxacin and enrofloxacin, less to moderate sensitive (1+ to 2+) to oxytetracycline and doxycycline, less sensitive (1+) to chloramphenicol, resistant to less sensitive (- to 1+) to erythromycin and streptomycin, whereas ampicillin and amoxycillin were fully

resistant (-) to these isolates. Rizwan *et al.* (2016) also declared Chloramphenical, Norfloxacin, Gentamicin, Penicillin and Ciprofloxacin as most sensitive antibiotic for all isolates (*Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysagalactiae, Bacillus cereus*).

Overall effective drugs against isolated organisms in order by Levofloxacin, Gentamicin, Ciprofloxacin, Chloramphenicol, Erythromycin, Azithromycin, Vancomycin, Cloxacillin, Cephalexin, Cefixime, Tetracycline, Amoxicillin, Amikacin but resistant to Penicillin, Amoxicillin, Azithromycin, Cloxacillin, Erythromycin, Vancomycin, Amikacin, Cefixime, Cephradine were also observed. Indiscriminate and frequent use of these antibiotics in goats could be the reason for their ineffectiveness against these bacterial pathogens. Milk consumed from these goats suffering from clinical or sub-clinical mastitis may transmit antibiotic resistance genes to micro flora of human beings. This situation may be a biggest human health hazard in near future. Levofloxacin, Gentamicin, Ciprofloxacin, Chloramphenicol proved to be the drug of choice for the treatment of caprine mastitis in Bangladesh because of higher efficacy of these drugs.

This study indicates high percentage of clinical and sub-clinical mastitis due to presence of teat lesion. The measure should include elimination of objects that can cause injury to the udder. Good management practices with proper sanitation are required to prevent the incidence of intra-mammary infection in dairy goats. Strict hygienic measures should be adopted during the production and handling of milk to lessen the public health hazards. Awareness of the small ruminant's handlers about the significance of hygienic milking practices would minimize the adverse effects of mastitis on the yield and quality of goat milk. It would be beneficial if the lactating goats in the herd are screened regularly to diagnose sub-clinical mastitis, apply antibiogram assay and treat the goats accordingly to achieve healthy and optimum milk production.

Present study was carried out on a small population in Dinajpur district. Further and intensive epidemiological studies requires to be carried at regional and national level to determine the prevalence of caprine subclinical mastitis, identification of the risk factors and characterization of the associated bacterial agents for the treatment and control of the disease.

CHAPTER 6

CONCLUSION

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A cross sectional study was carried out to determine the prevalence of clinical and subclinical caprine mastitis according to different variables (age, parity, litter size, lactation stage and teat lesion), etiology of caprine mastitis and antimicrobial sensitivity pattern of mastitic bacteria. A total of 240 milk samples from 120 lactating goats were taken from different places of Dinajpur town and adjacent rural area of HSTU campus. Immediately after collection, milk samples were subjected to physical examination, performed indirect test e.g. CMT for rapid diagnosis of mastitis. The CMT positive milk samples were subjected to bacteriological culture to identify major mastitis causing pathogens and the isolated pathogens were subjected to *in vitro* antibiotic sensitivity test to know the sensitivity pattern of these pathogens against commonly used antibiotics.

The prevalence of clinical and subclinical mastitis in Black Bengal goats found 11.67% and 38.75% respectively in this study. The major pathogens isolated from mastitic milk were *Staphylococcus spp.* (32.5%) followed by *Escherichia coli* (22.5%), *Pseudomonas spp.* (12.5%), *Klebsiella spp.* (12.5%), *Bacillus spp.* (5%). Mixed infections were found as *Staphylococcus spp.+E. Coli* (10%), *Klebsiella spp.+E. Coli* (2.5%) and *Staphylococcus spp. + Pseudomonas spp.* (2.5%).

A significant relationship was observed between age, parity, litter size and teat lesion with enhance clinical and subclinical mastitis whereas significant association of lactation stage with mastitis was not seen. The major prevalent mastitis pathogens were highly sensitive against Levofloxacin, Gentamicin and Ciprofloxacin but all the isolates were complete resistance to Penicillin.

From the findings of this study it may be concluded that,

i. The prevalence of clinical and subclinical mastitis in goats were 11.67% and 38.75% respectively.

ii. Age of the animal, parity, litter size and teat lesion influenced the prevalence of clinical and subclinical mastitis.

iii. The major pathogens isolated from mastitic milk were *Staphylococcus spp., Escherichia coli, Pseudomonas spp., Klebsiella spp.* and *Bacillus spp.*

iv. Antibiogram results indicated that Levofloxacin, Gentamicin and Ciprofloxacin are the drug of choice to treat most cases of mastitis.

v. Antibiotic sensitivity result suggested not to use penicillin, Amoxicillin, Azithromycin, Cefixime and Amikacin in clinical and subclinical cases of mastitis in goats.

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APPENDICES

APPENDICES

APPENDIX I

Questionnaire for data collection

Research title: Prevalence of mastitis in goats of Dinajpur district, isolation of etiological bacteria and identification of their antibiotic resistance properties.

I. Tag no./ Case no..... Date: .....

II. Name and address of owner.....

III. Description of the goat

1. Breed..... 2. Age.....

3. Sex..... 4. Type.....

5. Color..... 6. Source of doe.....

7. Parity No..... 8. Gestation length.....

9. Service with..... 10. No. of kid borne.....

11. No. of kid died..... 12. Stage of lactation.....

13. Milk production...../d 14. Age of 1st service.....

15. Kidding interval..... 16. Others.....

IV. Feeding and management practices

1. Status of owner: small/ marginal/ landless farmer

2. No. of goats in the flock .....

3. Source and composition of feed: Grazing/ Stall feeding/ Concentrate/ Road side grass/ Tree leaves

4. Breeding practice: Natural service/ AI

5. Floor: Raised/ Ground

V. Structure and status of udder and teat

1. Size of udder (cm)..... 2. Teat length.....

3. Teat end to floor distance..... 4. Teat lesions/ injuries.....

5. Teat diameter..... 6. Supernumerary teats.....

7. Shape of tips: rounded/ plate/ funnel/pocket/ pointed

VI. Milking system and management

1. Hand milking/ machine..... 2. No. of milking..... /d

3. Use of antibiotic..... 4. Owners complaint.....

VII. Disease history

1. Name of disease..... 2. Duration.....

3. Treatment given..... 4. Result.....

VIII. Treatment history.....

IX. Examination of udder and teat

1. Sign of inflammation..... 2. Palpation findings.....

3. Systemic reactions Rectal temperature..... °F

4. Others.....

X. Examination of milk

1. Physical examination a) Color b) Clots/ Flakes.....

2. CMT.....

3. Bacteriological test results.....

XI. Recommendations.....

Signature of the interviewer

APPENDIX II

Composition of Different Media

1. Nutrient agar (Hi Media)

Ingredients:	g/L
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Final pH (at 25^{0}C)	7.4 ± 0.2

2. Nutrient broth

Ingredients:	g/L
Peptone	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Final p^H	7.2

Staphylococcus Agar no. 110

Ingredients:	g/L
Agar	15
Dipotassium phosphate	5
Sodium chloride	75
D- Mannitol	10
Lactose	2
Gelatin	30
Yeast extract	2.50
Casein enzyme hydryosate	10.00
pH	7.0 + 0.2 at 25 °c
Distilled water	1 ltr

Cetrimide Agar (Hi Media)

Ingredients:	g/L
Gelatin peptone	20
Magnesium chloride	1.40
Potassium sulphate	10
Cetrimide	.30
Agar	15

3. Eosine methylene blue Agar (Hi Media)

Ingredients:	**g/L**
Peptic digest of animal tissue	10
Lactose	5.0
Sucrose	5.0
Dipotassium phosphate	2.0
Eosin - Y	0.40
Methylene blue	0.065
Agar	20.0
Final pH (at 25^{0}C)	7.2 ± 0.2

4. MacConkey agar (Mreck)

Ingredients:	**g/L**
Peptic digest of animal tissue	17.0
Protease peptone	3.0
Lactose monohydrate	10
Bile salt	1.5
Sodium chloride	5.0
Agar-agar	15.0
Neutral red	0.03
Final pH (at 25^{0}C)	7.1 ± 0.2

6. Simmons Citrate Agar

Ingredients:	**Gms / Litre**
Magnesium sulphate	0.200
Ammonium dihydrogen phosphate	1.000
Dipotassium phosphate	1.000
Sodium citrate	2.000
Sodium chloride	5.000
Bromothymol blue	0.080
Agar	15.000
Final pH (at 25°C)	6.8±0.2

8. MR-VP broth (Hi Media)

Ingredients:	**g/L**
Bacto MR – VP	1.7
Distilled water	1000
Final pH (at 25°C)	6.9 ± 0.2

Preparation of reagents

Kovacs reagent

P-dimethyl aminobenzal dehyde	5 gm
Amyl alcoho	l75gm
Conc.HCL	25 ml

1. V-P reagent 1

5% alpha –naptholin absolute ethyl alcohol

2. V-P reagent 2

40% potassium hydroxide containing 0.3 creatine. The ingredients were dissolved by heating gently over steam bath. When in solution add 0.052 gm of cotton blue dye.

3. Methyl red solution

Methyl red	0.05 gm
Ethanol (absolute)	28 ml
Distilled water	22 ml

4. Phenol red solution

0.2% aqueous solution of phenol red

5. Potassium hydroxide solution

40% aqueous solution of KOH

6. Gram stain solution

 Stock crystal violet

Crystal violet	10 gm
Ethyl alcohol (95%)	1000 ml

 Stock oxalate solution

Ammonium oxalate	1 gm
Distilled water	1000 ml

 Lugols iodine solution

Iodine crystal	1 gm
Potassium iodide	2 gm

 Ethyl alcohol 250 ml

 Acetone 250 ml

 Counterstain

Safranine	2.5 ml
Ethyl alcohol (95%)	100 ml

Safranine working solution

The stock safranine is diluted 1:4 with distilled water.