COLIFORM AND STAPHYLOCOCCAL MASTITIS IN COWS RISK FACTORS AND TRENDS IN ANTIMICROBIAL RESISTANCE

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DHAKA-1207

DECEMBER, 2020

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REGISTRATION NO. 19-10019

A Thesis

Submitted to Department of Medicine & Public Health

Sher-e-Bangla Agricultural University, Dhaka-1207

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE (MS) IN MEDICINE

SEMESTER: JULY-DECEMBER, 2020

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CERTIFICATE

This is to certify that the thesis entitled ***"COLIFORM AND STAPHYLOCOCCAL MASTITIS IN COWS RISK FACTORS AND TRENDS IN ANTIMICROBIAL RESISTANCE "*** *submitted to the Department of Medicine and Public Health, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE in MEDICINE****, embodies the result of a piece of bona fide research work carried out by* **SYIDUL ISLAM,** *Registration No.* ***19-10019*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

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My Beloved Parents and Respected Research Supervisor

DECLARATION

I declare that the thesis hereby submitted by me for the MS degree at the Sher-e-Bangla Agricultural University is my own independent work and has not previously been submitted by me at another university/faculty for any degree.

Date: 21.10.2021 **SYIDUL ISLAM**

ACKNOWLEDGEMENTS

*All praises and compliments are due to the Supreme Regulator and Ruler of the Universe, "**Almighty Allah**" for the blessing upon the successful accomplishment of education, to complete the research work and thesis leading to Master of Science (MS) in Medicine.*

*The author likes to express his heartfelt respect, deep sense of gratitude and profound indebtedness to his reverend Supervisor **Prof. Dr. K. B. M. Saiful Islam,** Department of Medicine and Public Health, Sher-e-Bangla Agricultural University, Dhaka for his scholastic guidance, valuable advice, important suggestions, affection feelings, endless encouragement, and supervision throughout this research work and in preparing this thesis.*

*The author also extends his sincere appreciation, profound regards and cordial thanks to his Co-supervisor, **Prof. Dr. Uday Kumar Mohanta,** Department of Microbiology and Parasitology, Sher-e- Bangla Agricultural University, Dhaka for his kind help, constructive advice, fruitful criticism, creative suggestion, necessary correction and encouragement during the compilation of this thesis.*

The author also grateful to Minister of Science and Technology, The People's Republic of Bangladesh for providing him a National Science and Technology (NST) fellowship to conduct his research properly.

The author wishes to express his wholehearted thanks to his well-wishers, classmates and all other friends for their keen help as well as heartiest co-operation and encouragement.

DECEMBER, 2020 ***The Author***

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LIST OF ABBREVIATIONS AND ACRONYMS

ABBREVIATION	FULL WORD
ABR	Antibiotic Resistance
AFP	Agence-France-Presse
AMR	Antimicrobial Resistance
AMX	Amoxicillin
AMP	Ampicillin
BM	Bovine Mastitis
BA	Blood Agar
CM	Clinical Mastitis
ChM	Chronic Mastitis
^{o}C	Degree Celsius
CNS	Coagulase Negative Staphylococcus
CLSI	Clinical & Laboratory Standard
CMT	California Mastitis Test
CXM	Cefuroxime
CFT	Ceftriaxone
CFM	Cefixime
COT	Co-trimethaxole
DLS	Department of Livestock Services
et.al	and others (at elli)
EMB	Eosine Methylene Blue
E. coli	*Escherchia coli*
FAO	Food And Agricultural Organization
FDA	Food And Drug Administration
GDP	Gross Domestic Product
GEN	Gentamicin
H_2O_2	Hydrogen Per Oxide
LMICS	Low and Middle Income Countries

LIST OF ABBREVIATIONS AND ACRONYMS

ABBREVIATION	FULL WORD
l/day	Liter per day
MOHFW	Ministry of Health & Family Welfare
MR	Methylene Red
NO.	Number
NB	Nutrient Broth
OIE	Office International des Epizooties
MSA	Mannitol Salt Agar
mm	milimeter
MC Agar	MacConkey Agar
ml	mililiter
mg	miligram
PBS	Phosphate Buffer Solution
RPl	Resistance Plasmid
S	Streptomycine
S. aureus	*Staphylococcus aureus*
SCM	Sub-Clinical Mastitis
SCC	Somatic Cell Count
TE	Tetracycline
U.S.	United States
UTI	*Escherchia coli*
µl	Micro liter
Vol	Volume
WHo	World Health Organization

LIST OF SYYMBOLS

Symbols	Full Meaning
@	At the rate of
%	percentage
&	and
<	Less than
>	Greater than
≥	Greater than equal
≤	Less than equal

ABSTRACT

This study was undertaken to know the prevalence of *E coli & Staphylococcus aureus causing mastitis*. Total Prevalence of mastitis was determined from different farms of Amtali, Barguna. Identification of different bacteria from positive samples were performed by cultural characteristics, and biochemical tests to some extent. The overall prevalence of mastitis was 5%. The prevalence of mastitis in cross breed cow was 6.42% and in local cow 3.75%. Prevalence of mastitis was higher in older animal of 7-8 years old. The prevalence of mastitis in 3-4, 5-6, 7-8 and 9-10 year old cows were 3.08%, 4.29%, 5.88% and 5%, respectively. The higher number of mastitis incidence was 8.75% during 3^{rd}-4^{th} parity than 3.33% during 1^{st}-2^{nd} parity and 3.84% when $\geq 5^{th}$ parity. Among 15 mastitis infected cattle, the prevalence of mastitis in cow had peri-parturient disease were 86.67% and cows without histories of peri-parturient disease were 13.33%. Among 15 mastitis infected cattle, the prevalence of mastitis were 26.67% at 1st-2^{nd} month of lactation 53.33%, at 3^{rd}-4^{th} month of lactation and 20% and at 5^{th} -6^{th} month of lactation respectively. The prevalence of mastitis in dry and wet season was 33.33% and 66.67% respectively. The occurrence of mastitis was 26.67% in cows in farms with brick-block floor and 20% in cows in farms with soil floor. Only 53.33% cows were affected with mastitis when the floor was wet and soiled. The occurrence of mastitis had relation with the cleanliness of farm. Among 15 mastitis infected cattle, 73.33% infected cow were reared in dirty farm and 26.67% infected cows are reared in clean farm. The prevalence of *E. coli* and *S. aureus* in mastitis milk samples were 73.33% and 66.67% % respectively. Nine antibiotics were used to know the susceptibility, intermediate resistance and resistance percentage against the isolated bacteria. Amoxicillin showed 36.36% sensitivity, Ampicillin showed 36.36%, Tetracycline shows 54.54% sensitivity, Streptomycine and Co-trimethaxole/Trimethoprim showed 81.81% sensitivity, Gentamycin, Cefttriaxone shown 90.91% sensitivity, Cefuroxime and Cefixime shown 100% sensitivity against *E. coli*. Cefuroxime and Cefixime were highly sensitive and Ceftriaxone and Gentamycin shown 9.09% resistance. Amoxicilin showed higly resistance among these 9 antibiotics. Amoxicilin showed 45.45% resistance. Amoxicillin, Ampicillin and Tetracycline showed 18.18%, 27.27% and 9.09% intermediate resistance respectively against *E. coli*. Against *S. aureus;* Cotrimethaxole/Trimethoprim showed 60% sensitivity, Tetracycline showed 70% sensitivity, Amoxicillin, Ampicillin, Streptomycine showed 80% sensitivity, Gentamycin, Cefttriaxone shown 90% sensitivity, Cefuroxime and Cefixime showed 100% sensitivity. It was observed that Cefuroxime and Cefixime were highly sensitive and ceftriaxone showed 10% resistance Cotrimethaxole showed higly resistance among these 9 antibiotics. Cotrimethaxole/ Trimethoprim showed 30% resistance. Amoxicillin, Tetracycline, Streptomycine and Cotrimethaxole/Trimethoprim showed 10% intermediate resistance against *S. aureus.* This study showed that antibiotic resistance against *E. coli* and *S. aureus* was increased. So, prevention and conrol of the outbreak of this disease is very necessary through good therapeutic management and hygienic care of the farm and dairy cow.

Keywords: Mastitis, prevalence, *E. coli, S. aureus*, Antibiotic Resistance

CHAPTER 1
INTRODUCTION

Milk production in Bangladesh is characterized by small marginal rural milk producers scattered all over the country accounting for about 70% of the production. Dairy farming is one of the major enterprises for livelihood of rural farmers in Bangladesh. One of the main obstacles on the economy of dairy farming is mastitis as these rural farmers often face a great setback due to high prevalence and incidence of mastitis in dairy cow.

Mastitis is the inflammation of the udder accompanied by physical, chemical and bacteriological changes in milk. Bovine mastitis is one of the devastating diseases causing huge loss to the dairy industry worldwide. The costs associated with mastitis are innumerous and include antibiotic treatment, reduced milk quality, reduced milk yield, increased culling rate and hazards to public health (Kurijogi and Kaliwal, 2011). It has a high incidence rate and prevalence in dairy cows, affecting the net earnings of milk producers' worldwide (Frola *et al*., 2011). Generally, clinical mastitis is easily diagnosed by visible clinical manifestations, such as red, hot, and swollen mammary glands (Sharma *et al.,*2007). Mastitis in cow is the major constraints of Bangladeshi dairy industry. Mastitis is widespread disease among dairy animals in our country. It has been estimated that the mastitis alone can cause approximately 70% of all avoidable losses incurred during milk production in Bangladesh (Sumathi *et.*al., 2008). The disease also results in partial or complete damage to udder tissues and decreases productive lifespan of the animal. Mastitis is one of the major causes of antibiotic use in dairy cows (Mitchell *et al.,* 1998). Treatment failure in mastitis is due to indiscriminate use of antibiotics without testing in vitro sensitivity. Also the mastitis bacteria are rapidly acquiring resistance due to frequent and indiscriminate use of antimicrobials in treatment, which has been growing concern worldwide (WHO, 2000). The monitoring of antibiotic resistance is needed not only for effective treatment and control of mastitis but is an increasing threat in human and veterinary medicine also.

Hence, its monitoring is recommended by (OIE, 2001). The milk from an infected animal is the main source of pathogenic bacteria (Gilmour & Harvey. 1990) and some bacterial

toxins produced in the milk cannot be destroyed by heating or drying (National Mastitis council, 1996). Milk is considered as the excellent medium for growing of many microorganisms. Milk can be contaminated with several bacteria during milking process from the milking personnel, utensils used for milking (Rehman *et al.*, 2014). Besides, microorganisms may enter the udder through teat canal and the bacteria may come out through milk (Smith *et al.*, 2007). *Staphylococcus aureus* and *Escherichia coli* are the two major contaminants of milk. The presence of the pathogen in milk largely depends on fecal contamination and the presence of pathogen in feces mainly originates from feed contamination (Aycicek *et al*., 2005). Food borne diseases are of great concern around the world. However, this is an important issue in developing countries where poor sanitation is maintained during collection and processing of milk from cattle and buffaloes (Le *et al.*, 2003).

S. aureus is an important pathogen for dairy cows causing inflammatory reactions. The organism is believed to cause 30-40% inflammatory reaction in udder in mastitis (Akineden *et al*., 2001; Asperger and Zangeri, 2003; Cabral *et al.*, 2004; Katsuda *et al*., 2005). The organism can be excreted directly from udder through milk (Rehman *et al.*, 2014). Presence of *S. aureus* in milk indicates the hygienic standard followed during the milking process. Information on antibiotic resistance against *S. aureus* can be useful in treating the disease caused by the organism (Jahan *et al*., 2015). *E. coli* is one of the important bacteria of gut flora (Eckburg *et al.*, 2005). Among the pathogenic *E. coli*, Shiga toxigenic *E. coli* (STEC) strains have been reported mostly in Latin America, India, Bangladesh and many other developing countries (Kaddu-Mulindw *et al.*, 2001; Rehman *et al.*, 2014). Pathogenic *E. coli* have been isolated by several researchers in Bangladesh (Nazir *et al.*, 2005; Khatun *et al.*, 2015; Himi *et al.*, 2015) from fecal samples of healthy cattle (Hassan *et al.*, 2014), raw milk of cattle and buffaloes (Alam, 2006; Islam *et al.*, 2008; Hossain *et al.*, 2011; Jahan *et al.*, 2015). In Bangladesh, about 20% of all diarrheal cases is associated with enterotoxigenic *E. coli* (Qadri *et al.*, 2005). Moreover, very few works have been reported in Bangladesh on molecular detection of pathogenic organisms from raw cow milk and buffalo milk. Besides, selection of appropriate antibiotic against the *S. aureus* and *E. coli* is crucial for proper treatment of mastitis in cattle and buffaloes. Mastitis is a major economic burden on the dairy industry, affecting milk production and

milk quality (Abebe *et al.,* 2016; Hogeveen and Van Der Voort, 2017). Numerous microorganisms associated with cases of mastitis have been isolated (Kromker and Leimbach, 2017; Vakkamaki *et al.,* 2017); the most frequently isolated pathogens associated with clinical mastitis (CM) in China are *Escherichia coli*, *Klebsiella* spp., NAS, *Streptococcus dysgalactiae*, and *Staphylococcus aureus* (Gao *et al.,* 2017).

Identification of pathogens causing CM enables appropriate choices for antimicrobial treatment (Pinzon-Sanchez *et al.,* 2011) and preventive mastitis management. Antimicrobials are used in the dairy industry for prevention and control of mastitis and other bacterial diseases affecting dairy cows (Oliver and Murinda, 2012). Therefore, dependence on antimicrobials has become a widespread phenomenon on dairy farms.

Antimicrobial resistance (AMR) occurs when microorganisms are able to overcome effects of antimicrobials that were effective in the past. Based on EARS-Net data in 2016, AMR remains a serious threat to public health in Europe (ECDC, 2017). Additionally, AMR is one of the biggest threats to global health, food security, and development (WHO, 2015).

In 2010, China had the largest share of global antimicrobial use in food animal production (23%), with projections for use of 30% by 2030 (Van Boeckel *et al.,* 2015). Furthermore, AMR is becoming a serious healthcare problem, with high resistance rates of most common bacteria to clinically important antimicrobial agents (Shi *et al.,* 2010; Xiao *et al.,* 2011; Wang *et al.,* 2015). Prevalence of AMR in clinical bovine mastitis pathogens has been investigated numerous times recently in China, including regional results for *Staph. aureus* (Gao *et al.,* 2012; Liu *et al.,* 2017), *E. coli* (Liu *et al.,* 2014), and *Strep. dysgalactiae* (Zhang *et al.,* 2018).

Therefore The present investigation was undertaken to monitor antimicrobial resistance trends in cow mastitis and to generate the data for therapeutic decisions.

Objectives of the Investigation:

- To know the prevalence of mastitis in Amtali, Barguna
- To Isolate and identify bacteria harbors in mastitis infected cow's milk .
- To investigate the antibiotic resistance pattern of the isolated bacteria and efficacy of some antimicrobial drug for proper treatment.

CHAPTER 2
REVIEW OF LITERATURE

Bangladesh is a densely populated country where livestock has been an important component of farming system practiced for centuries. Livestock sector has been playing a vital role in the socio-economic development of Bangladesh. This sector also has high potential for the perspective of economic development of the country. This labor intensive and fast income generating sector contributes significantly to poverty reduction and foreign currency earnings as well as employment generation for the poor and marginal people. Nearly 85% of the populations of the country are engaged in agriculture and livestock sector (Raha 2000). Farmers get more than 50% of their annual income through dairy farming irrespective of their gender or land ownership, and on average milk of 0.85 L/day are available for a family that keeps dairy stock (FAO, 2013).The livestock sector generates 20% of full-time employment and 50% of part-time employment in Bangladesh (DLS , 2020). Contribution of Livestock in Gross Domestic Product (GDP) (Constant Prices) is 1.47% (DLS, 2020). About 44 percent of the protein comes from livestock sources. The government has set strategic targets for meeting protein demands, employment generation, up-scaling export earnings and women‘s empowerment through the livestock sector. Cattle farming play an important role for the development of Bangladesh. About 90% of cattle used for draught purposes, a substantial amount of cattle for transportation of goods, meat and milk production. Raw materials like hides, bones, horns for industry purpose, manure for crop fields and fuel for domestic uses derived from the livestock of the country. Dairy farming is an important and potential sector in Bangladesh. In Bangladesh the No. of cattle production is 243.91 lakh (DLS, 2020). Milk is an ideal food for human being irrespective of ages and undoubtedly the most important one among the foods of animal origin. In Bangladesh, cows are the main source of milk. In Bangladesh, cattle, goat and buffalo are considered as dairy animals. Out of total milk production, about 90% share is from cattle, 8% from goat and the remaining 2% from buffalo (DLS, 2015). According to DLS, In Bangladesh the milk production is 106.80 lakh metric tons (DLS, 2020). Smallholder producers dominate the dairy sector in Bangladesh. More than 70% of the dairy farmers are smallholders and produce around 70–80% of the country‘s total milk (Uddin *et al.,* 2012). It is estimated that there are about 1.4 million

dairy farms with an average herd size of 1–3 cows (Hemme *et al*., 2008). Dairy cow rearing has been increasingly viewed as a source of alleviating poverty in Bangladesh. It is also turned as a means of improving the livelihood of landless and small households and acts as a critical cash reserve and steady cash income for many landless and marginal farmers (Saadullah, 2001). This sector meets the demand for animal protein partially in the form of meat, milk, and milk products (Miazi *et al.*, 2007). The dairy sector offers good opportunities for on-farm and off-farm employment, especially at the rural level. Dairying in Bangladesh is growing faster, but it also faces lots of problem including high input cost and low output prices. Disease, along with non-availability of feed resources and nutrition are the most important constraints to milk production. Mastitis is the disease of the mammary gland caused by bacterial infection and the most common and costly health disorder of dairy cows (Ruegg,2003).

2.1 Mastitis

Mastitis is defined as the inflammation of the mammary gland that can be caused by physical or chemical agents but the majority of the causes are infectious, and usually caused by bacteria that invade the udder, multiply, and produce toxins that are harmful to the mammary gland (Radostits *et al.,* 2007).

Bovine mastitis, an inflammation of the mammary gland, causes physical, chemical, and usually, bacteriological changes in milk and pathological changes in the glandular tissues of the udder that affect the quality and quantity of milk (Sharma and jeong, 2013). It has a high incidence and prevalence in dairy cows, affecting the net earnings of milk producers' worldwide (Frola *et al.,* 2011).

Mastitis is an inflammation of the udder accompanied by physical, chemical and bacteriological changes in milk and glandular tissue.

Mastitis is universally recognized as one of the costliest diseases in the dairy industry (Rahman *et al.,* 2009). It is of particular concern in developing countries like Bangladesh, where milk and milk products are scarce.

Mastitis is the inflammation of mammary glands with physical, chemical and microbiological changes characterized by an increase in somatic cells, especially leukocytes, in the milk and by the pathological changes in the mammary tissue.

Moreover, mastitis poses a threat to human health since it may be responsible for zoonoses and for food toxin infections (Blum *et al.,* 2008; Fernandes e*t al.,* 2011).

Mastitis is an infectious disease condition resulting in an inflammatory reaction in the mammary gland of the cow. It is the most common disease in dairy cattle characterized by various degrees of severity - ranging from a mild disease with no gross changes in the secretion (milk) but an increase in inflammatory cells (somatic cells) in the milk, to a moderate disease with an increase in inflammatory cells and gross changes in the milk. It may be accompanied by signs of inflammation in the mammary gland including swelling, redness, and painfulness. Mastitis may progress to a severe disease with all of the above changes in the milk and systemic signs including fever, depression, and ―off-feed‖ and occasionally even death in the most severe cases. Mastitis reduces milk production and milk quality.

2.2 Types of mastitis

Literally bovine mastitis means an inflammatory response of the bovine mammary gland tissue to noxious agents; the agents can be either infectious or non-infectious by nature. Most frequently the aetiology is infectious by nature, organisms as diverse as bacteria, mycoplasma, yeasts and algae have been implicated as causes of the disease (Watts, 1988; Quinn *et al.,* 2000; Radostits *et al.,* 2000).

Mastitis can be classified into three major types: clinical mastitis (CM), sub-clinical mastitis (SCM) and chronic mastitis (ChM) (Anonymous, 2003).

Mastitis can be further subdivided into two categories based on the source of infections: 1) Contagious mastitis infections acquired by transmission of contagious bacteria from cow to cow during the milking process; and, 2) Environmental infections acquired from bacteria in the environment of the cow. .

The main contagious microorganisms are S. aureus and Streptococcus species, being their main source the mammary gland of infected cows. On the other hand, the primary source of environmental mastitis pathogens is the habitat of the cow and Streptococcus species and Gram negative bacteria (Escherichia coli and Klebsiella) are examples of microorganisms included in this group (Bogni *et al.,* 2011)

Mastitis, which is the inflammation of the udder and teats, exists in two primary forms: clinical mastitis and subclinical mastitis (Ruegg.2017, Taponen *et.al.,* 2017). Clinical mastitis, which is less prevalent, is characterized by systemic signs in the cow and visible abnormalities in the udder and milk (Radostits *et.al.,* 1973, Jamali *et.al.,* 2018). In contrast, subclinical mastitis is more common and results to reduced milk production without observable clinical signs or abnormalities in the udder or milk (Zeryehun and Abera2017, Ndahetuye *et. al.,* 2019). For this reason, subclinical mastitis is challenging to diagnose, persists longer in the herd, and is associated with higher losses compared to clinical mastitis (Abrahmsén *et. al.*, 2014).

2.3 Etiology

Milk can be contaminated with several bacteria during milking process from the milking personnel, utensils used for milking (Rehman *et al.,* 2014). Besides, microorganisms may enter the udder through teat canal, and the bacteria may come out through milk (Smith *et al.,* 2007).

Mastitis causing pathogens include bacteria (mostly *Staphylococcus aureus*, coagulase-negative *staphylococcus*, *Streptococcus uberis, Streptococcus dyslactiae, Streptococcus agalactiae,* enterococci and coliform bacteria including *Escherichia coli)* and Mycoplasmas.

Bacteria are the primary causes of mastitis, and more than 140 different pathogenic species have been reported (Motaung *et. al.,* 2017). Previously, studies had documented major pathogens of mastitis such as *Staphylococcus aureus, Streptococcus agalactiae,* and Coliforms (Bradley *et.al*.,2007; Zadoks and Fitzpatrick 2009).

Major mastitis causing organisms are *Staphylococci spp., Streptococci spp.* and other gram-negative bacteria (Mubarack *et al.,* 2012). Bacteria are the most important microorganisms that generate mastitis in dairy herds, and can act like an opportunistic pathogen or contagious pathogen.

According to its infectious etiology, mastitis can also be divided into contagious and environmental forms. Contagious mastitis is caused by microorganisms such as *Staphylococcus aureus, Streptococcus agalactiae*, *Arcanobacterium pyogenes* and

Mycoplasma spp.; and its reservoirs are the mammary gland and the milk of infected cows. Its transmission can occur at the time of milking by poor practices such as the sharing of towels to wash and dry teats; by the contaminated hands of farm workers or by the sharing of non-disinfected teat liners between cows in the milking (Blowey and Edmondson 2010; Andrade-Becerra *et al,* 2014). *Streptococcus agalactiae* is a highly contagious pathogen in cows with mastitis, having a common transmission in herds that allow the microorganism to colonize, invade and it replicates in the udder (Carvalho-Castro *et al.* 2017.) Environmental mastitis is caused by Gram-negative germs, normally found in the environment such as *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Pseudomonas* spp., and *Proteus* spp. and some Gram-positive bacteria such as *Streptococcus uberis* and *Streptococcus dysgalactiae,* which cause mild and moderate forms of mastitis and within the rare germs are yeasts and molds (Haubert *et al.* 2017). Also, mastitis can be due yeast and molds, but this presentation in dairy herds is low (Williamson and Di Menna 2007). *Escherichia coli* is another of the common infectious agents of BM being considered one of the major agents worldwide (Kempf *et al.,* 2015, Blum *et al.,* 2015). *E. coli* is a pathogen able to infect the mammary gland by entering the udder via the teat canal (Lipman *et al.,* 1995). Mastitis due *E. coli* is reported worldwide and is clinically important due to the possibility of endotoxic shock in the cow if there is a large bacterial presence. Its pathogenicity factors (endotoxins) are responsible for this problem (Yangliang *et al.,* 2016; Zhang *et al.,* 2017). Generally, mastitis due *E. coli* is clinical, but this can vary due the number of microorganisms in the mammary gland, also the number of endotoxins presents (Andrade-Becerra *et al.* 2012). Its presentation is related with poor cleaning practices at the milking time. Lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, is considered to be the primary virulence factor in coliform bacteria (Hogan & Smith, 2003), being responsible for most pathophysiological reactions in *E. coli* mastitis (Burvenich *et al.,* 2003; Gonen *et al.,*2007). It is released from the bacteria following cell death and during multiplication (Lohuis *et al.,* 1988; Burvenich *et al.,* 2003). Clinical signs in acute coliform mastitis are induced by LPS and the subsequent release of inflammatory mediators (Lohuis *et al.,* 1988; Burvenich *et al.,* 2003; Kornalijnslijper *et al.,* 2004). No specific virulence determinants have been detected in *E. coli* isolated in mastitis (Lehtolainen *et al.,*

2003; Wenz *et al.,* 2006a; Dyer *et al.,* 2007; Suojala *et al.,* 2011). Clinical signs of *E. coli* mastitis can vary from only mild inflammation in the quarter, with minor changes in the appearance of the milk and no systemic signs, to severe clinical signs and strongly decreased milk production (Jones & Ward, 1990; Pyor€ al € a€ *et al.,* 1994; Shpigel *et al.,* 1997; Burvenich *et al.,* 2007). Severity of acute E. coli mastitis differs greatly between individual cows and is associated with the age of the cow and the lactation stage (Vandeputte-Van Messom *et al.,* 1993; Shuster *et al.,* 1996). Cows infected during the puerperal period more often die and only 30–50% of them return to full lactation (Jones & Ward, 1990; Burvenich *et al.,* 2007) regardless of antimicrobial and supportive therapy (Erskine *et al.,* 1991). A major determinant of the severity of *E. coli* mastitis is growth of bacteria during the acute phase of infection, before the efficient influx of neutrophils (Katholm & Andersen, 1992; Rantala *et al.,* 2002; Kornalijnslijper *et al.,* 2004). Bacteremia may develop in *E. coli* mastitis cases, and it has been found significantly more often in cows with severe clinical signs (Cebra *et al.,* 1996; Wenz *et al.,* 2001). *Staphylococcus aureus* is one of the most commonly reported pathogens worldwide as a cause of mastitis, due to its pathogenic characteristics (exotoxins) and it is easily transmitted to the teat and is especially important due to the generation of resistance to antibiotics (Haubert *et al.,* 2017). *Staphylococci* are the bacteria most commonly isolated from BM (Leitner *et al.,* 2011). *Staphylococcus aureus* is a common cause of this disease (Oliveira *et al.,* 2007). However, *coagulase-negative staphylococci* (CNS) have become the most common BM isolate in many countries and are now predominant over S. aureus in most countries and could therefore be described as emerging mastitis pathogens (Tremblay *et al.,* 2013). Apart from *staphylococci*, Coliforms, *Enterococci* and *Streptococci* are also frequently isolated from cows with mastitis (Smulski *et al.,* 2011). S. aureus is part of the commensal flora of several mammalian species. However, when subjected to a combination of endogenous and exogenous factors they can become pathogenic and a source of mastitis (Melchior *et.al.,* 2006a; Melchior *et.al.,* 2006b).This pathogen is responsible for between 5 and 70% of cows and 90% of herds affected worldwide with BM (Zecconi and Scali 2013). *Staphylococcus aureus* and *Escherichia coli* are the two major contaminants of milk. The presence of the pathogen in milk largely depends on fecal

contamination, and the presence of pathogen in feces mainly originates from feed contamination (Aycicek *et al.,* 2005).

2.4 Epidemiology

A wide range of microbes have been documented as causative agents of mastitis globally (*Motaung et.al. 2017,* Jamali *et al.,* 2018). These include both contagious and environmental bacteria, in addition to fungi, algae, and viruses. Evidence-based studies have shown significant variation in the distribution of mastitis and mastitis-causing pathogens among countries, regions, and farms (Verbeke *et al.,* 2014, Gao *et al.,* 2017). These variations are influenced by farm management practices and regional environmental factors (Taponen *et al.,* 2017, Amer *et al.,* 2018). It is the outcome of the interaction of various factors associated with the host, pathogens and the environment, accounting for 38% of all morbidity (Smith and Hagsted, 1996).

Prevalence of infection increases in multiparous cows, within 2-3 months of lactation, abnormally large udder, unhygienic environment, means of milking, unclean milker‘s hand, udder wound and mismanagement of milking machine (Alom, 2001).

The prevalence of mastitis was higher in wet season in compared to dry season. The overall prevalence of Bovine subclinical mastitis was 19.9% and 44.8% in dry and wet seasons, respectively in Sylhet District (Rahman *et.al,* 2010).It appeared that the floor was a potential source for mastitis organisms to enter the udder through the teat orifice (Kivaria *et al.,* 2004).

2.5 Host factor

All breeds of dairy cows are susceptible to mastitis. Exotic and crossbred cows are more prone to mastitis than the zebu cows (Roy *et al.,* 1989). High yielding dairy cattle are more prone to mastitis than low milk yielding cattle. In the high-yielding cows the glandular tissues are more susceptible to infection (Slettbakk *et al.,* 1995; Radostits *et al.,* 2000).

The defence mechanism in aged cows is poorer than in younger cows (Dulin *et al.,* 1988). So, older cows are more susceptible to mastitis than younger cow.

The lower immunity level of peri-parturient cows makes the cow more prone to infection in the udder (Rainard and Riollet, 2006). Once a cow gets infected or diseased during the peri-parturient period, it becomes more susceptible to udder infection due to lowered immunity (Nickerson, 1994; Peeler *et al.,* 1994). Cows having infected uterine discharge and retained placenta infection has more risk that the udder and teats being contaminated (Peeler *et al.,* 1994). Mastitis can occur at any stage of lactation, including the dry period, but is most likely in the first month after calving and in late lactation. The prevalence of mastitis increased with age in dry and wet seasons. The prevalence of mastitis was the lowest in first lactation (12.3 to 31.6%) in both seasons and the highest in 6th to 13th lactation (41.3%) in dry season and 5th lactation (65%) in wet season (Rahman *et al.,* 2010).

2.6 Sources of infection

Sources of infection may include:

- Contaminated milking hands
- Dirty milking unit liners
- Improperly cleaned milking units
- Soiled bedding
- Polluted teat dip
- Dirty water used to clean udders prior to milking
- Pond water
- Mud holes
- Teat trauma
- Flies

2.7 Transmission

Epidemiological study revealed that infectious agents of mastitis may be transmitted from infected animals to another animals by milker‘s hand (Philpot .1975; Oliver,1975). These pathogens infect the udder via the teat canal (Eberhart. 1984). According to (Nemeth *et al.,*

1994) open for one to two hours after milking (Jone, 2006), and between milking, the teat end is constantly exposed to environmental pathogens; this is in contrast to contagious pathogens, which usually affect the teat only during milking (Smith.*et.al.*, 1985). *E. coli* may be due to poor hygienic conditions as *E.coli* originate from the cows environment and infect the udder via the teat canal (Sudhakar *et al.,* 2009). Mastitis is a result of interaction between three elements like bacteria, cow and environment. In the present study the prevalence of *Staphylococcus species* may be due to the incomplete milking and especially when it is associated with the painful lesions or any wounds on the outer surface of the udder. *Staphylococcus* is an opportunistic pathogenic bacteria which survive on the skin of the udder and can infect the udder via teat canal or any wound (Kaliwal *et al.,* 2011).

2.8 Economic impact

Bovine mastitis (BM) is responsible for major economic losses on dairy farms worldwide, caused by the decrease in milk production, increase in health care costs and increase in culling and death rates (Melchior *et al.*, 2006b).

An average clinical case of mastitis costs the dairy producer approximately $200. While the incidence of clinical mastitis varies greatly from herd to herd, Mastitis is one of the top three reasons producers cull dairy cows. In addition, Mastitis adversely affects reproductive performance of dairy cows and on average, it takes 40 days longer to get cows pregnant that have had a case of mastitis compared to herd mates that have not had a case of mastitis. Treatment of clinical cases of mastitis not only cost the producer in increased labor and treatment costs, and in milk discard, it also increases the risk of antibiotic residue in the bulk tank.

In both clinical and subclinical mastitis there is a substantial loss in milk production. Production losses due to clinical mastitis have been estimated (Grohn *et al.,* 2004, Hortet *et al.,* 1998, Houbenet. *et al.,* 1993).

The economic consequences of mastitis (clinical or subclinical) are due to treatment, production losses, culling, changes in product quality and the risk of other diseases. The associated costs can be divided among the following factors: Milk production losses,

Drugs, Discarded milk. Veterinary services, Labour, Product, quality Materials and investment, Diagnostics, Other diseases & Culling (Halasa *et al.,* 2007).

2.9 Symptoms

When signs of mastitis become apparent, the infection can either be subclinical or clinical. The difference is dependent upon:

- Duration of infection
- Host immune status
- Pathogen virulence

Subclinical Mastitis (SCM)

In SCM, there are no visible abnormalities in the udder tissues and milk except an elevated somatic cell count (SCC) (MacDougall *et al.,* 2001)
Most commonly associated with *S. aureus, Strep. Spp*

Milk appears normal and there is no visible sign of inflammation of the mammary gland. Diagnosis will be made on the basis on an increase in somatic cell counts in the milk.

Clinical Mastitis (CM)

The signs of CM are inflammation of the udder and changes in milk (Kader *et al.,* 2003). Depending on the type of pathogens involved, fever and depression could be associated with the disease. Evidence of mammary gland inflammation (redness, heat, swelling, pain) Physical changes in the milk from a few milk clots to appearing like serum with clumps of fibrin.

Acute mastitis (organisms most commonly associated: Coliform organisms including *E. coli* and *Klebsiella,* and *Strep. Spp.* and Enterococci).

Clinical signs (fever, depression and loss of appetite) are severe. The udder is swollen, hard and painful. The milk may contain clots or flakes and can be watery, serous or purulent.

Acute gangrenous mastitis

Most commonly associated with *S. aureus, Cl. Perfringens,* and *E. coli*.

- Anorexia, dehydration, depression, fevers.
- Toxemia sometimes leads to death.
- Early in the disease, the gland is red, swollen and warm
- Within a few hours the teat becomes cold
- The secretions become watery and bloody
- The mammary gland becomes necrotic

Chronic mastitis

Chronic mastitis most commonly associated with coagulase-negative staphylococci, *S. aureus, and S. uberis.* Clinical signs of an acute infection from time to time with no clinical signs for prolonged intervals. Milk periodically contains clots, flakes or shreds of fibrin. The Somatic Cell Count is elevated.

2.10 Diagnosis

Among the diseases mastitis is a significant one and most of the farmers are under the threat of mastitis (Rahman *et al.,* 2010). Diagnosis is made on clinical signs of abnormal milk, swelling of the udder (tender to the touch) and general signs of illness (fever, depression, loss of appetite) and in many cases a reduction in milk production. Development of reliable tests for detection of mastitis was a priority for early researchers who wanted to ensure public safety, produce high-quality dairy products, and have a practical means of managing affected cows (Halversen *et al.,* 1934; Shaw *et al.,* 1937). Detection methods that were evaluated included direct microscopic examination of milk for bacteria, enumeration of milk leukocytes, microbial culture, and detection of various abnormal milk constituents (such as chloride content; Halversen *et al.,* 1934). The evaluation of various diagnostic methods with regard to non-specific mastitis (Switzer & Gates, 1931; Klein and Learmouth, 1935; Starr *et al.,* 1936; Johns and Hastings, 1938a, b), however, indicated that other methods (i.e., chemical, biological and microscopic cytological) were more suitable than bacteriological examinations (Rosell, 1933b). Mastitis

caused by Staphylococcus aureus (S. aureus) is often subclinical and is typically manifest as an elevation of the somatic cell count (SCC) of the milk from the affected quarter (Radostits *et al.,* 2000; Bradley 2002). For subclinical mastitis, the diagnosis will be made on the basis of an increase in somatic cells in the milk.. Somatic cell count has been accepted as the best index to use to both evaluate milk quality and predict udder infection in the cow (Poutrel and Rainard, 1982). Under field conditions, determination of SCC in cow‘s milk is usually performed by the California Mastitis Test (CMT). Microbiological culturing and identification of the microorganisms to species level was performed according to standard procedures described by Hogan *et al.,* 1999. Bacteriology provides useful information about the likely source of the bacteria and aids in developing a mastitis control plan at reliably identify cows with *S. aureus* IMI are needed.

2.11 Treatment

There are two aims of mastitis treatment:

1) Returning milk to normal with an acceptable cell count so that it can be sold again

2) Getting rid of the bacteria

Antimicrobial

Bacterial infections are the predominant cause of bovine mastitis; therefore, antimicrobial therapy is commonly implemented for mastitis prevention and control (Oliver *et al.,* 2011) Mastitis is the most common disease of dairy cows and the most common reason that cows are treated with antibiotics (Pol and Ruegg, 2007; Saini *et al.,* 2012). There are two options: intramammary antibiotics, the classic mastitis tube and systemic antibiotics given by the intramuscular or subcutaneous route.

Intramammary antibiotics should be the first-line treatment for cows with mild uncomplicated mastitis in a single quarter. Systemic antibiotics should be used when more than one quarter is affected, when udder changes are marked or when the cow is obviously ill. Combination therapy, with both systemic and intramammary antibiotics, may increase bacteriological cure rates but should only be used based on advice from your veterinarian.

Anti-inflammatory drugs

These are aspirin-like drug, which reduce the inflammation and pain associated with mastitis. They have proven very useful in severe cases of mastitis, but there is now increasing evidence of their usefulness in mild to moderate cases. Cows treated with intramammary antibiotics and NSAIDs had lower cell counts, better cure rates and better fertility than cows treated with antibiotics alone.

2.12 Prevention

- Proper use of a functional milking machine with appropriate milking machine maintenance.
- Teat dipping, pre- and post-milking with an effective, approved teat dips.
- Dry cow therapy at the end of lactation with one of a dry cow mastitis product.
- Use of an internal teat sealant.
- Culling of cows with chronic mastitis.
- Appropriate vaccination with a Gram-negative core antigen vaccine to prevent coliform infections.
- Regular cleaning or changing of bedding
- Reducing heat stress
- Removing udder hair
- Preventing teat trauma
- Reducing udder edema in peri-parturient cows by nutritional management of potassium and sodium intake.
- Avoidance of areas that accumulate water
- Maintenance of stalls for proper lying behavior
- Preventing frostbite and fly exposure

2.13 Control

Dairy farmers in Bangladesh are not always aware of the best practices to control mastitis (Rahman *et al.,* 1997). Besides bacterial infection, there are many risk factors associated with mastitis. The disease cannot be eradicated but can be reduced to low levels by good management. Practices such as good nutrition, proper milking hygiene, and the culling of chronically infected cows can help. Ensuring that cows have clean, dry bedding decreases the risk of infection and transmission. Dairy workers should wear rubber gloves while milking, and machines should be cleaned regularly to decrease the incidence of transmission.

2.14 Commonly used antibiotic in treatment of mastitis

The main treatment of mastitis is commonly administered by intramammary infusion of an ointment or intramuscular or intravenous injection of antibiotics, such as streptomycin, ampicillin, cloxacillin, penicillin, and tetracycline (Bhosale *et al.,* 2014).

Only two antimicrobial classes are represented among commercially available products that are approved by the U.S. Food and Drug Administration (FDA). Those classes are β-lactams (amoxicillin, ceftiofur, cephapirin, cloxicillin, hetacillin, and penicillin) and a lincosamide (pirlimycin). The drugs considered for treatment of mastitis include the more common penicillins, aminoglycosides and macrolides; oxytetracyline, chloramphenicol, trimethoprim, and several sulphonamides (MacDiarmid, 1978).

2.15 Antimicrobial resistance

The word antimicrobial was derived from the Greek words anti (against), mikros (little) and bios (life) and refers to all agents that act against microbial organisms. Antimicrobials include all compounds that act against all types of microorganisms, such as bacteria (antibacterial), viruses (antiviral), fungi (antifungal) and protozoa (antiprotozoal). An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For

example, antibiotics are used against bacteria, and anti-fungal are used against fungi. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis.

Antibiotics are group of antimicrobial agents synthesized by microorganisms like bacteria or fungi and have the property of inhibiting the growth of other microorganism (bacteria). Antibiotic is a common antimicrobial agent. Antibiotics are widely used for preventing and treating various infections in humans and animals. Antibiotics are also used as growth promoters in animal food production sectors, where its addition in feed enhances animal growth and improves the quality of products (Cheng *et al*., 2014). But their indiscriminate and irrational use in different fields like agriculture, fisheries, livestock industry, etc., has given rise to development of resistant bacteria (Aarestrup, 2005) and this results in the spread of resistance by transfer of its resistant determinants to other bacteria (Stanton, 2013).

Antibiotic resistance happens when germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them. That means the germs are not killed and continue to grow.

Importance of antimicrobial resistance.Antibiotic resistance presents a significant challenge to clinical, veterinary and plant health and is recognized by the world helath organization (WHO) as emerging problem of global significance.

Antimicrobial resistance (AMR) is an ongoing problem with multidrug-resistant strains of bacteria and fungi impacting medical progress in many regions of the world. Collecting AMR surveillance data is an essential approach to (1) define the scope of the resistance problem, (2) develop interventions that improve the appropriate application of antimicrobial agents, and (3) decrease resistance selection pressure (Jones 1996, Núñez *et.al.,*2018*).* Other important efforts are underway to understand the mechanisms of resistance whereby microorganisms avoid the effects of antimicrobials and to use that information to discover/develop new compounds, or modify older agents, that retain potent activity against key target pathogens (Boucher *et al.*, 2017).

One important aspect of any antimicrobial surveillance program is longitudinality. By conducting surveillance of specific pathogens over time, one can assess the emergence of

specific strains or species and discover changes in the antimicrobial susceptibility profile of the organisms. Furthermore, when longitudinal surveillance encompasses a broad geographic distribution, one may eventually develop a useful understanding of regional, national, or even global trends of species distributions and AMR (Jones 1996).

Antibacterial agents do however leave survivors resistant to that particular agent or antibiotic. Suddenly freed from so many competitors, these survivors reproduce quickly, spread, and colonize. Places associated with scrupulous hygiene, such as hospital wards and operating theatres, are particularly vulnerable. A survey of over 2000 US hospitals in 2009–10 found that some 20 per cent of such hospital-acquired infections involved multidrug-resistant organisms (the so called superbugs) (Sievert *et al.*, 2013).

By the production of biofilms, bacteria adopt a multicellular behavior that can facilitate and/or prolong their survival in diverse environmental niches. In hospital settings, the formation of biofilms on vents and medical equipment enables bacteria such as *Pseudomonas aeruginosa* to persist as reservoirs that can readily spread to patients (Kostakioti *et al*., 2011).

Alert to this crisis, the May, 2015, world health organization assembly adopted a global action plan on antimicrobial resistance, which outlines five objectives.

- To improve awareness and understanding of antimicrobial resistance through effective communication, education and training.
- To strengthen the knowledge and evidence base through surveillance and research.
- To reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures.
- To optimize the use of antimicrobial medicines in human and animal health.
- To develop the economic case for sustainable investment that takes account of the needs of all countries and to increase investment in new medicines, diagnostic tools, vaccines and other interventions.

Antibiotic resistance is one of the biggest threats to global health, food security, and development today. Antibiotic resistance occurs naturally, but misuse of antibiotics in humans and animals is accelerating the process.

Antibiotic resistance is rising to dangerously high levels in all parts of the world. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases (WHO).

Antibiotic resistance has the potential to affect people at any stage of life, as well as the healthcare, veterinary, and agriculture industries, making it one of the world‘s most urgent public health problems.

2.16 Causes of Antimicrobial resistance

A significant milestone in our understanding of microbes and their role in infection was the discovery that microbial antibiotic resistance genes themselves are transmissible and promiscuous, so spreading from organism to organism. Bacterial resistance can come about for a number of reasons, classically due to a mutation of the antibiotic-target gene in the bacterial chromosome, or elsewhere following the addition of extra-chromosomal DNA (Ventola, 2015a and 2015b). Blanco *et al., (*2016) reported that bacterial multidrug efflux pumps are antibiotic resistance determinants present in all microorganisms: efflux pumps are ancient, highly conserved determinants, which have been selected long before the recent use of antibiotics for the therapy of human infections. The role of efflux pumps as relevant antibiotic resistance determinants in bacterial pathogens is likely secondary to other functional roles with reference to bacterial physiology. Shaikh *et al*., (2015) state that transferase enzymes are the most diverse family of resistant enzymes which inactivate antibiotics, including beta-lactams, aminoglycosides, chloramphenicol, and streptogramin A. The mechanism involves chemical substitution, i.e. the addition of chemical groups to the periphery of the antibiotic molecule, which impairs their binding to a target. It can be noticed that bacteria acquire antibiotic resistance genes most commonly by conjugation, whereby a resistant ‗donor‘ strain can transfer a plasmid to an antibiotic-susceptible recipient in what is termed a horizontal exchange. Plasmids are extra-chromosomal loops of DNA that act as vectors which can carry and transfer antibiotics resistance genes. Such transfer can occur both within members of the same species and also between genera or species. One plasmid with a broad host range is the resistance plasmid RP1, first identified in a clinical strain of *P. aeruginosa*. This plasmid can transfer to most, if not all Gram-negative bacteria (Bennett, 2009) and carries resistance to ampicillin, tetracycline, and kanamycin. Newly resistant cells can then transmit resistance vertically to daughter generations.

Other causes of antibiotic resistance are-

- Over- prescription of antibiotics.
- Patients were not finishing the entire antibiotic course.
- Over use of antibiotics in livestock and fish farming.
- Poor infection control in health care settings.
- Poor hygiene and sanitation.

2.17 Mechanism and Origin of antibiotic resistance

Antibiotic resistance is a global crisis driven by appropriate and inappropriate antibiotic use to treat human illness and promote animal growth. The antimicrobial resistance epidemic continues to spread due to the triple threat of unfettered access, minimal product regulation and oversight of antibiotic prescription, and lack of clinical diagnostic tools to support antibiotic de-escalation in low-resource settings. Though the antibiotics were more successful as therapeutics against many bacterial infections in the history of medicine, their irrational and indiscriminate use has created enormous pressure resulting in the development of antibiotic resistance in bacteria (Witte, 1998). Antibiotic resistance can be an intrinsic property of bacteria themselves or it can be acquired later. Apparently most pathogenic microorganisms have the capability of developing resistance to at least some antimicrobial agents. The main mechanisms of resistance are: limiting uptake of a drug, modification of a drug target, inactivation of a drug, and active efflux of a drug. In natural or intrinsic resistance to a drug occurs without any additional changes in their genetic elements, whereas acquired resistance results through random mutations or acquisition of foreign genetic material carrying resistance determinants (Hollenbeck and Rice, 2012), The antimicrobial agent becomes effective against a target bacterial species only when a susceptible antibiotic target site exists in the cell, the antibiotic reaches the target in sufficient quantity and the antibiotic is not inactivated or modified by the bacterial cell wall (Sutcliffe *et al*., 1999).

Factors that have contributed to the growing resistance problem include: increased consumption of antimicrobial drugs, both by humans and animals; and improper prescribing of antimicrobial therapy. Overuse of many common antimicrobials agents by physicians may occur because the choice of drug is based on a combination of low cost

and low toxicity (Griffith *et al.,* 2012). There may also be improper prescribing of antimicrobials drugs, such as the initial prescription of a broad-spectrum drug that is unnecessary, or ultimately found to be ineffective for the organism(s) causing the infection (Yu. 2011). The danger is that excessive use of antibiotics in humans leads to emergence of resistant organisms (Goossens 2009),(Pakyz *et al.,* 2008). In addition, prior use of antimicrobial drugs puts a patient at risk for infection with a drug resistant organism, and those patients with the highest exposure to antimicrobials are most often those who are infected with resistant bacteria(Griffith *et al.,* 2012),(Tacconelli,2009). There is evidence to support the idea that feeding antibiotics to animals may result in development of antimicrobial resistant organisms, and that those resistant organisms may be transferred to the humans who consume those animals (Landers *et al.,* 2012),(Wegener, 2012). The antimicrobial resistance patterns seen in the animals who reflect the types and amounts of antibiotics given to the animals. The transmission of antimicrobial resistance from the animals to humans may occur in various ways, with the direct oral route being the most common (includes eating meat plus ingestion of feces in contaminated food or water). Another common route is from direct contact with the animals by humans (Wegener2012). Antibiotic resistance in bacterial pathogens (*E. coli, Staphyllococcus aureus, Streptococcus sp.)* are common in all over the world.

Mastitis is one of the most frequent infectious diseases in dairy cattle and is a reason for antimicrobial drug usage in dairy cows. The bacteria involved in bovine mastitis are mainly *Streptococcus* spp., *Staphylococcus* spp., and coliforms *Escherichia coli*, a member of the normal gut flora of humans and animals, possess many beneficial functions. Nonetheless, their pathogenic role is also well recognized as they cause many bacterial infections including urinary tract infection (UTI), diarrhea, meningitis and pneumonia. Bovine mastitis caused by *Escherichia coli* can range from being a subclinical infection of the mammary gland to a severe systemic disease. Cow-dependent factors such as lactation stage and age affect the severity of coliform mastitis. Evidence for the efficacy of antimicrobial treatment for *E. coli* mastitis is very limited (Suojala *et al.,* 2013). Coliform bacteria are a frequent cause of bovine clinical mastitis. By far the most common species, isolated in more than 80% of cases of coliform mastitis, is *Escherichia coli* (Bradley *et al.,*2007; Botrel *et al.,* 2010). This species is the most common cause of clinical mastitis in

well-managed dairy herds with low milk somatic cell counts (SCC) (Barkema *et al.,* 1998; Bradley *et al.,* 2007). Broad-spectrum antimicrobials are commonly used for the treatment of *E. coli* mastitis (Erskine *et al.,* 2003; Ruegg, 2010). The dairy industry suffers from considerable economic losses due to *staphylococcal* mastitis in cattle (Wells et al., 1998), with the prevalence of udder infections being closely linked to milking hygiene, as well as udder and leg hygiene (Neave *et al.,* 1969; Schreiner and Ruegg, 2003). Intramammary infections caused by *Staphylococcus aureus* are difficult to cure and are particularly challenging, as they are prone to chronicity and resurgence (Peton and Le Loir, 2014).Though antibiotic treatment is widely used to fight bovine mastitis, its merits are controversial. Use of antimicrobial agents is not only economically questionable and favors the development of antibiotic resistance, but it is also unsuitable to address the issue of intracellular persistence of the organism (Steeneveld *et al.,* 2011; Fluit, 2012; Saini *et al.,* 2012). Bovine intramammary *Staph. aureus* infections are also of relevance in the context of food-borne intoxications in humans. Ingestion of food containing *staphylococcal* enterotoxins leads to staphylococcal food poisoning characterized by violent vomiting, diarrhea, and prostration (Fetsch and Johler, 2018). Although food handlers contaminating food with *Staph. aureus* are considered the most common source of *staphylococcal* food poisoning, outbreaks have also been linked to consumption of raw milk or raw milk cheese originating from dairy animals suffering of mastitis (Giezendanner *et al.,* 2009; Johler *et al.,* 2015).

Streptococcal species are major mastitis pathogens, along with *Staphylococcus aureus* and coliforms. *Streptococcus agalactiae* is cow-associated and well adapted to the mammary gland, whereas *Streptococcus dysgalactiae* and *Streptococcus uberis* are environmental pathogens; *Strep. uberis* is one of the most common pathogens isolated from clinical mastitis (Botrel *et al.,* 2010).

2.18 Present Scenario of Antimicrobial Resistance in Bangladesh

The overuse of antibiotics in animals in low- and middle-income countries (LMICs) is a serious issue that is contributing to the increasing global burden of antimicrobial resistance (AMR) (VanBTP *et.al.*,2019, LaxminarayanR *et al.*,2013). Although improving awareness and understanding of AMR is a key strategic objective of the World Health Organisation's (WHO) global AMR action plan (WHO;2015) South East Asia has been identified as the global area posing the greatest risk to AMR dissemination (ChereauF et.al. 2017). The WHO warned on 20th November, 2020 that growing antimicrobial resistance is every bit as dangerous as the coreonavirus pandemic and threatens to reverse a century of medical progress, reports AFP. WHO chief Tedros Adhanom Ghebreyesus called the issue – one of the greatest health threats of our time. Rsistance is when bugs become immune to existing drugs-antibiotic, antiviral or antifungal treatments rendering minor injuries and common infections potentially deadly resistance has grown in recent years due to over use of such drugs in humans and also in farm animals. –Antimicrobial resistance may not seem as urgent as a pandemic but it is just as dangerous, Tedros told a virtual press conference. Bangladesh, a developing country of Southeast Asia with a high degree of ABR, poses a regional and global threat. According to the 2014 WHO report on global surveillance of antimicrobial resistance, significant gaps in surveillance prevail, along with a lack of standards for methodology, data sharing, and coordination. However, the Southeast Asia, African, and Eastern Mediterranean regions have been identified as having major gaps (WHO, 2014). Multiple studies have demonstrated irrational antibiotic prescribing by physicians, a habit of self-medication among patients, and the indiscriminate use of antibiotics in agriculture and farming in different parts of the country (Biswas *et al.*, 2014a,b; Mostafa Shamsuzzaman and Kumar Biswas, 2012; Sutradhar *et al.*, 2014). The international AMR community now acknowledges that, for maximal effect, large-scale awareness campaigns should be integrated within industry-wide AMR behavioural policy strategies (Huttner *et al.*, 2019, Haenssgen *et al.*, 2019). Prime Minister Sheikh Hasina has been made co-chair of One Health Global Leaders Group on Antimicrobial Resistance (AMR) along with Prime Minister of Barbados Mia Amor Mottley.Prime Minister Sheikh Hasina said,–We need worldwide coordinated

actions to monitor the nature of infections, to implement required control measures and raise global awareness against the widespread use of antibiotics."Prime minister Sheikh Hasina on 25th January, 2021 urged global leaders to promote and ensure rational antimicrobial agents in human health, livestock, fisheries and agricultural sectors as the silently emerging antimicrobial resistance (AMR) could endanger all the significant advances of modern medicine.

The regulatory regime in Bangladesh is weak concerning human, technical and logistic capacity to oversee this vast market (Ahmed *et al.,* 2017).Policies and regulations that support appropriate and rational use of antimicrobials are essential for effective interventions to contain the development and spread of AMR. Bangladesh has recently approved a National Action Plan (NAP) for containing AMR, in alignment with the WHO GAP guidelines (MoHFW. 2017).

CHAPTER 3
MATERIALS & METHODS

3.1 Brief description of experimental design:

The entire study was divided into two major steps: The first step included selection of sources, collection of samples, isolation, identification and characterization of microorganisms (E. *coli*, *Staphylococcus aureus*, on the basis of their colony morphology and biochemical characteristics.

In the second step, the current status of drug sensitivity and resistance pattern of microorganisms isolated from mastitis infected milk was determined

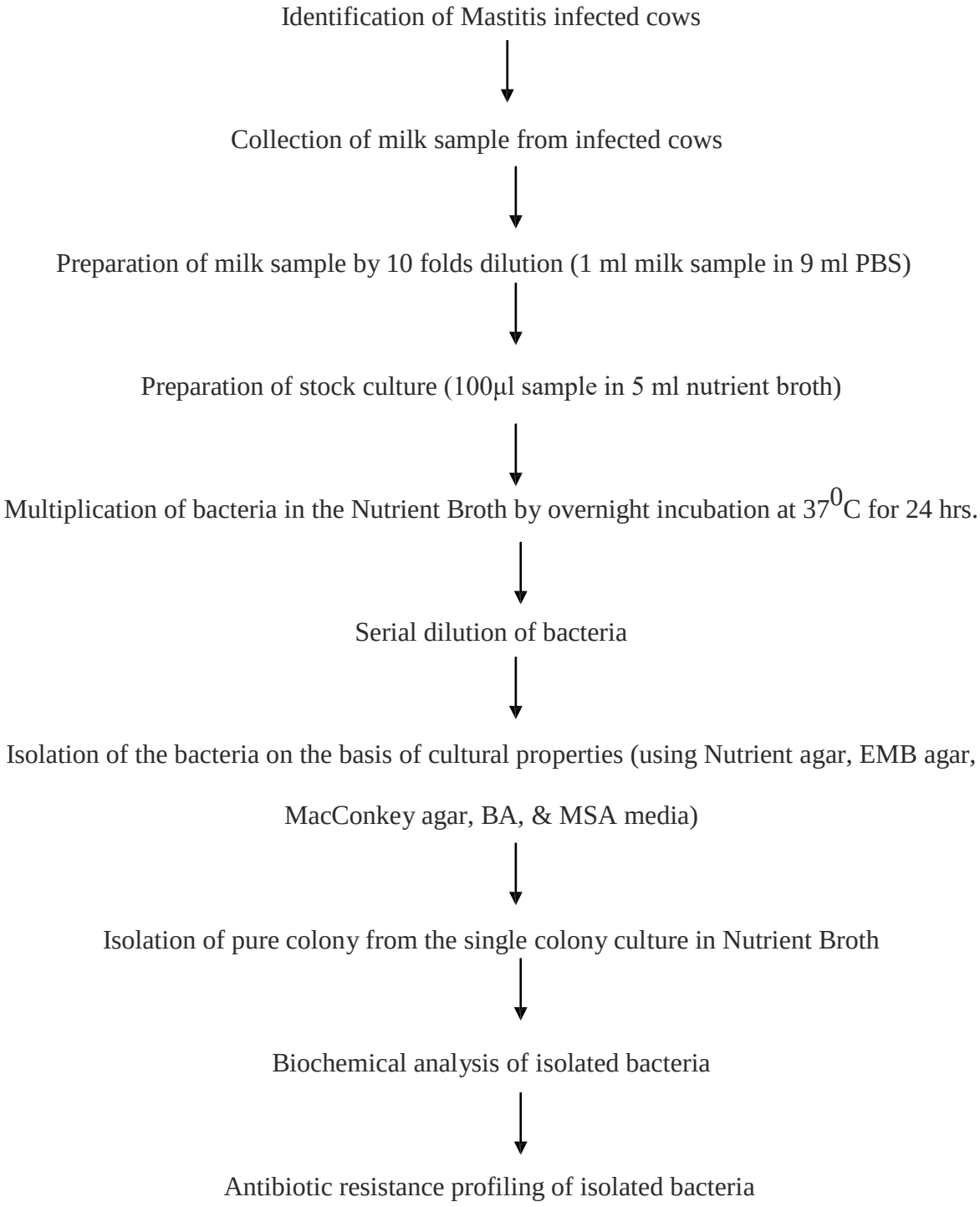


Figure 1: Layout of the experiment design

3.2 Study area

The study was conducted at Amtali, Barguna; Bangladesh.

3.3 Study period

The study was performed from 1st January 2020 to 31st December, 2020.

3.4 Study size

A total number of 15 cases were recorded during the study period. The study was conducted on Antimicrobial resistance in mastitis infected cows at various age, seasons and breeds.

3.5 Data collection

The data were collected directly from the farmer by interview and observe the cow. A pre-test questionnaire had been prepared before the data collection. The complain of affected animal was recorded carefully asking questions to the animal owner or farmers. Month, age, sex and breed also recorded with the date and time. The diseases were diagnosed on basis of clinical signs, owner‘s statement and Physical examination of udder and teats of infected animal. The risk factor of mastitis like peri -parturient disease, floor condition and hygienic management of farm considered during data collection.

The cows were categorized in the following groups-

Breed

According to the breed of animals, the cows were classified into

a) Local: Native non descriptive animals with well developed hump.

b) Crossbred: Breed between local and others foreign breed.

Parity

According to the parity (number of calving) of cows, Cows were divided into following groups-

Parity-1: one-two times calved

Parity-2: Three-four times calved

Parity 3: five times or more calved

Age

According to the age of cows, the cow had been divided into 3-4 years, 5-6 years, 7-8 years and 9-10 years old cow.

Season:

According to the season, the cows were classified into two seasonal groups:

- Dry season (January-mid June and late October- December 2020)
- Wet season (late June- mid October)

Peri-parturient Disease

According to the presence or absence of disease cow had been classified into

- Cow with history of having peri-parturient disease infected by mastitis
- Cow without history of having peri-parturient disease infected by mastitis

Lactating time

Cow infected with mastitis during their lactation time were classified into 3 sub-categories, includes:

- 1st-2nd month of lactation
- 3rd -4th month of lactation
- ≥5th month of lactation

Floor condition of the farm

During study the floor condition of farm has also considered to know the whether it was responsible for mastitis infection. The floors of different farm are brick block, soiled floor and partly or completely soiled and wet floor. The mastitis infected cows were categorized on the basis of floor condition of farm.

Cleanliness of farm

Some farms were clean and some farms were dirty which was also considered as the risk factor of mastitis infection and mastitis infected cow were categorized on the basis of cleanliness of farm.

3.6 Diagnosis of Clinical Mastiis:

The diagnosis of clinical mastitis is based on clinical sign in infected cow. These clinical signs include-

- High rectal temperature. Udder swollen, hot & painful udder.
- Discoloration of milk from white to yellow. sometime pus cell & blood come out with milk
- Stop secretion of milk from one or two teat.
- Gangrene developed in chronic case.

3.7 Sample Collection

A total number of 300 milking cows were examined for mastitis and 15 mastitis infected milk samples were collected from Amtali, Barguna.

3.8 Sample preservation:

The collected sample were stored in deep freeze at -20^0c temperature. Then the sample were transferred to the SAU Medicine & Public Health Laboratory via cool-chain maintaining in cool-box and stored in freeze at -20^0c temperature untill working (Lab work).

3.9 Bacteriological media

3.9 .1 Agar media

Agar media used for bacteriological analysis were Nutrient agar, Eosin Methylene Blue (EMB) agar, MacConkey agar, Blood agar, Mannitol Salt agar (MSA), and Muller Hinton (MH) agar.

3.9 .2 Liquid media

The liquid media used for this study were Nutrient broth, Methyl-Red and Voges-Proskauer broth (MR-VP broth).

3.9.3 PBS (Phosphate Buffered Saline)

For preparation of phosphate buffered saline, 8 gm of sodium chloride (NaCl), 2.89 gm of disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$), 0.2 gm of potassium chloride (KCl) and 0.2 gm of potassium hydrogen phosphate (KH_2PO_4) were suspended in 1000 ml of distilled water. The solution was heated to dissolve completely and pH was adjusted to the level with the help of pH meter. The solution was then sterilized by autoclaving and stored at 4ºC for future use.

3.9.4 Chemical reagent

Reagent used during bacteriological study were Phosphate buffered saline (PBS), 3% Hydrogen peroxide, Phenol red, Methyl red, 10% Potassium hydroxide, Mineral oil, Normal saline and other common laboratory chemicals and reagents.

3.10 Glass ware and experimental appliances

Glass wares and appliances were used during the course of the experiment were Test tubes, Ependorf tube, test tube stand, petridishes, conical flask, pipette (1 ml, 2 ml, 5 ml, 10 ml) & micro-pipettes (1ml, 200µl, 100µl, 10 µl) slides and cover slips, hanging drop slides, bacteriological loop, sterilized cotton, cotton plug, test tube stand, water bath, bacteriological incubator, refrigerator, sterilizing instruments, thermometer, ice carrier,

hand gloves, spirit lamp, match lighter, laminar air flow, autoclave, hot air oven, centrifuge tubes and machine etc.

3.11 Antibiotic disc

Nine antibiotic disc like Amoxicillin, Ampicillin, Gentamicin, Streptomycin, Tetracycline, Ceftriaxone, Cefuroxime, Cefixime, Cotrimehaxole/Trimehoprim with their disc concentration were used to test the sensitivity and resistance pattern of the selected *E. coli* and *Staphylococcus aureus* isolated from mastitis infected milk sample.

Table-1: Drugs with their disc concentration for *E. coli*

SL. No.	Name of Antimicrobial/Antibiotic Drug	Disc cocentration (µg /disc)	Zone Diameter Interpretive Standard (mm)		
			R	IR	S
1	Amoxicillin	30µg	≤ 13	14–17	≥ 18
2	Ampicillin	25µg	≤ 13	14–16	≥ 17
3	Tetracycline	30 µg	≤ 11	12–14	≥ 15
4	Gentamycin	10µg	≤12	13-14	≥15
5	Ceftriaxone	30 µg	≤ 14	15–17	≥ 18
6	Cefuroxime	30 µg	≤ 14	15–17	≥ 18
7	Streptomycine	10µg	≤ 11	12–14	≥ 15
8	Trimethoprim/Co-trimethaxole	25µg	≤ 10	11–15	≥ 16
9	Cefixime	5µg	≤15	16-18	≥19

(Source: **Clinical & Laboratory Standards Institute: CLSI 2020**)

R= Resistance; IR= Intermediate Resistance; S= Sensitive; µg= Microgram; mm= Milimeter

Table 2: Drugs with their disc concentration for *Staphylococcus sp.*

SL. No.	Name of Antimicrobial/Antibiotic Drug	Disc Concentration (µg /disc)	Zone Diameter Interpretive Standard (mm)		
			Resistant	Intermediate	Susceptible
1	Amoxicillin	30µg	≤ 13	14–17	≥ 18
2	Ampicillin	25µgm	≤13	14–16	≥17
3	Tetracycline	30µgm	≤14	15–18	≥19
4	Gentamycin	10µgm	≤12	13-14	≥15
5	Ceftriaxone	30µgm	≤ 22	23-27	≥28
6	Cefuroxime	30µgm	14	15-22	23
7	streptomycine	10µgm	≤ 11	12–14	≥ 15
8	Co-trimethaxole	25µg	≤10	11–15	≥16
9	Cefixime	5µg	15	16-18	19

(Source: **Clinical & Laboratory Standards Institute: CLSI 2020**)

R= Resistance; IR= Intermediate Resistance; S= Sensitive; µg= Microgram; mm= Milimeter

3.12 Preparation of Bacteriological culture Media

The commercial media were prepared according to the direction of the manufacturers and the non-commercial media were prepared in the laboratory. The composition and the procedures for the preparation of media are presented in the appendix.

3.12.1 Nutrient Agar

Nutrient agar was prepared by dissolving 28 grams of dehydrated nutrient agar (HiMedia, India) in to 1000 ml of distilled water and was sterilized by autoclaving at 121ºC under 15 lb pressure per square inch for 15 minutes. Then the agar was dispensed into petridish (90 mm and 100 mm) and was incubated at 37ºC for overnight to check their sterility and stored at 4ºC in the refrigerator until used.

3.12.2 Nutrient Broth

Nutrient Broth was prepared by Suspended 25 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Media should be sterilized by autoclaving at 15 lbs pressure (121°C) for 30 minutes. The broth was filled in test tubes & incubated at 37ºC for overnight to check their sterility and stored at 4ºC in the refrigerator until used.

3.12.3 EMB agar media

Thirty-six grams powder of EMB agar base (HiMedia, India) was suspended in 1000 ml of distilled water. The suspension was heated to boil for few minutes to dissolve the powder completely with water. The medium was autoclaved for 30 minutes to make it sterile. After autoclaving the medium was put in to water bath at 45ºC to cool down its temperature at 40ºC. From water bath 10-20 ml of medium was poured in to small and medium sized sterile petridishes to make EMB agar plates. After solidification of the medium in the plates, the plates were incubated at 37ºC for overnight to check their sterility.

3.12.4 MacConkey agar Media

Suspend 49.53 grams of dehydrated medium in 1000 ml purified/distilled water. Heat was given to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well before pouring into sterile Petri plates.

3.12.5 Blood agar media

Suspend 28 g of nutrient agar powder in 1 liter of distilled water. Heat this mixture while stirring to fully dissolve all components. Autoclave the dissolved mixture at 121 degrees celsius temperature for 15 minutes. Once the nutrient agar has been autoclaved, allow it to cool but not solidify. When the agar has cooled to 45-50 °C, add 5% (vol/vol) sterile defibrinated blood that has been warmed to room temperature and mix gently but well. Avoid Air bubbles. Dispense into sterile plates while liquid.

3.12.6 Mannitol salt Agar Media

The medium is usually used at a concentration of 11.1 g in every 1000 ml distilled water (concentration may vary depending on the manufacturer). Prepare the medium as instructed by the manufacturer. Sterilize by autoclaving at 121^{O}C for 15 minutes**.** When the medium has cooled to 50-55^{O}C, mix well, and dispense it aseptically in sterile petri dishes. Date the medium and give it a batch number. Store the plates at 2-8^{O}C preferably in plastic bags to prevent loos of moisture.

3.12.7 Mueller Hinton agar media

Suspended 38.0 grams in 1000 ml distilled water & heated to boiling to dissolve the medium completely. After sterilization by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cooling was done to 45-50°C.Then it was mixed well and poured into sterile Petri-dishes. After solidification of the medium in the petridishes, the petridishes were allowed for incubation at 37ºC for overnight to check their sterility and then stored at 4ºC in a refrigerator for future use.

3.12.8 Methylene Blue (MR) broth

A quantity of 3.4 gm of MR medium (HiMedia, India) was dissolved in 250 ml of distilled water, distributed in 2 ml quantities in test tube and then autoclaved. After autoclaving, the tubes containing medium were incubated at 37ºC for overnight to check their sterility and then stored at 4ºC for future use.

3.13 Isolation of bacteria

3.13.1 Primary stock

Primary stock was made by mixing of milk in PBS.

3.13.2 Ten-fold dilution (Spread Plate Technique)

Principle

The spread plate technique involves using a sterilized spreader with a smooth surface made of metal or glass to apply a small amount of bacteria suspended in a solution over a plate. The plate needs to be dry and at room temperature so that the agar can absorb the bacteria more readily. A successful spread plate will have a countable number of isolated bacterial colonies evenly distributed on the plate.

Procedure

1. At first a series of epindorf tubes, each containing 450 µl of PBS were taken.
2. From the original sample, 50 µl was transferred in the epindorf tube no. 1 and mixed thoroughly.
3. Then 50 µl from first tube is transferred to second tube and this way dilution was made up to last tube and finally 50 µl is discarded from the last tube.
4. For each tube, 3 Petri dishes were taken containing EMB agar media, Blood agar media and Nutrient agar media.
5. 0.1 ml of mixture was transferred from each Eppendorf tube to the center of the corresponding Petri dishes separately (one pipette or tip was used for each tube).
6. The samples were spreaded over the surface of the media using glass spreader (Before using glass spreader on each Petri dish it is sterilized by dipping into 70% alcohol and burning it in bunsen burner)
7. Incubated each plate over night for 37^0C.

8. Bacterial growth on agar media examined and subculture again for detection of specific bacteria.

3.13.3 Method for obtaining pure culture

Enriched culture from EMB/Blood agar media and Nutrient agar media was taken into selective NB/ Mannitol salt agar media and EMB agar media and incubated at 37ºC for 24 hours. Single colony appeared on the selective media.

3.13.4 Colony characteristics

Colony characteristics such as shape, size, surface texture, edge and elevation, color and opacity developed on selective media after 24 hours of incubation at 37°C was recorded.

3.14 Identification of suspected bacteria by biochemical test

3.14.1 Catalase test

This test was used to differentiate those bacteria that produced the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci. To perform the test an amount of 2-3 ml of 3% hydrogen peroxide solution was poured into a test tube. Using a sterile wooden stick or a glass rod, a good growth of the test organism was immersed into the solution. If the organisms are catalase producer, bubbles of oxygen are released

3.14.2 Methyl red test

The test was conducted by inoculating a colony of the test organism in 0.5 ml sterile glucose phosphate broth. After overnight incubation at 37^0C, a drop of methyl red solution was added. A red coloration was positive and indicates an acid pH resulting from the fermentation of glucose. A yellow coloration indicated negative result (Cheesbrough, 2006).

3.15 Maintenance of stock culture

Stock culture was mixed with a medium prepared by adding 0.5ml of 80% sterilized glycerol in 0.5ml of pure culture in nutrient broth and this was stored at -20°C for further use. Eighty percent Buffered Glycerol Saline is used to store it for a long time for further research or banking.

3.16 Antimicrobial resistance pattern of isolated bacteria

A total of 11 *E. coli* isolates , 10 *Staphylococcus aureus c*ollected from 15 Mastitis infected milk samples of Dairy cow were used for disc sensitivity testing. The antimicrobial sensitivity testing of each isolate was carried out by the Kirby-Bauer disc diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) procedures. Antibiotic sensitivity discs used were Gentamicin (GEN), Amoxicillin (AMX), Cefuroxime (CXM), Tetracycline (TE), Ampicillin (AMP), Ceftriaxone (CTR) and Cefixime (CFM), Colistin (CL), Streptomycine (S), Co-trimethaxole/ Trimethoprim (COT). This method allowed for the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that results from diffusion of the agent in the medium surrounding the disc. The suspension of the test organism was prepared in a test tube containing 5 ml nutrient broth by overnight incubation in shaking incubator. By micropipette 100µl of broth culture of the test organism was poured on Muller-Hinton agar plate. Sterile glass spreader was used to spread the culture homogenously on the medium. Inoculated plates were closed and allowed to dry for approximately 3-5 minutes. Then the antibiotic discs were applied aseptically to the surface of the inoculated agar plates at a special arrangement with the help of a sterile forceps. The plates were then inverted and incubated at 37ºC for 24 hours. After incubation the plates were examined and the diameter of the zone of complete inhibition was measured by mm scale. The zone diameters for individual antimicrobial agents were translated in to sensitive, intermediate and resistant categories by referring to an interpretation table.

CHAPTER 4
RESULT AND DISCUSSION

The results presented below demonstrated the prevalence of mastitis in dairy cows at Amtali, Barguna. In the present study, isolation and identification of bacteria through bacterial culture and biochemical test were demonstrated. The results also presented the sensitivity and resistance pattern of the isolates to different drugs.

4.1Overall prevalence of mastitis in dairy cattle

Three hundred dairy cows were investigated for identification of mastitis from different farms of Amtali, Barguna. Among these 15 cases were recorded in one year investigation. The prevalence of mastitis was 5%.

Table-3: Overall prevalence of mastitis in dairy cattle.

Total no. of cows	No. of mastitis cases	Prevalence%
300	15	5%

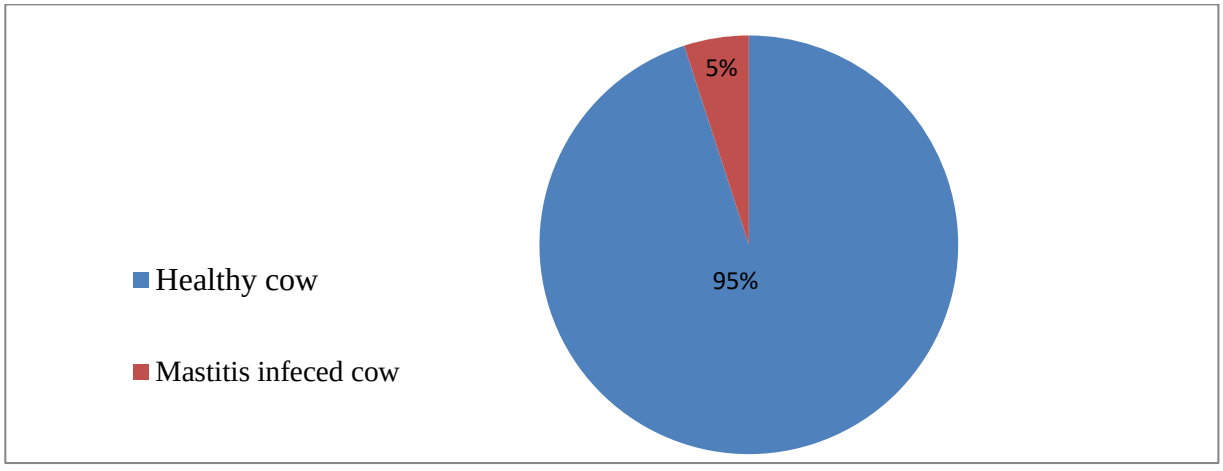


Fig.2: Pie chart showing prevalence of mastitis in cow.

4.2 Prevalence of mastitis in different breeds of cattle

Among 300 dairy cows 160 local cows are examined for mastitis case. 6 cows are diagnosed as mastitis positive and the prevalence rate is 3.75%. Among 140 crossbreed cows 9 cows are diagnosed as mastitis positive during the study period and the prevalence was 6.42% .

Table 4: Prevalence of mastitis in different breeds of cattle

Breed	Total No. of cow	No. of infected cow	Prevalence%
Local/ indigenous cattle	160	6	3.75%
Cross-breed cattle	140	9	6.42%

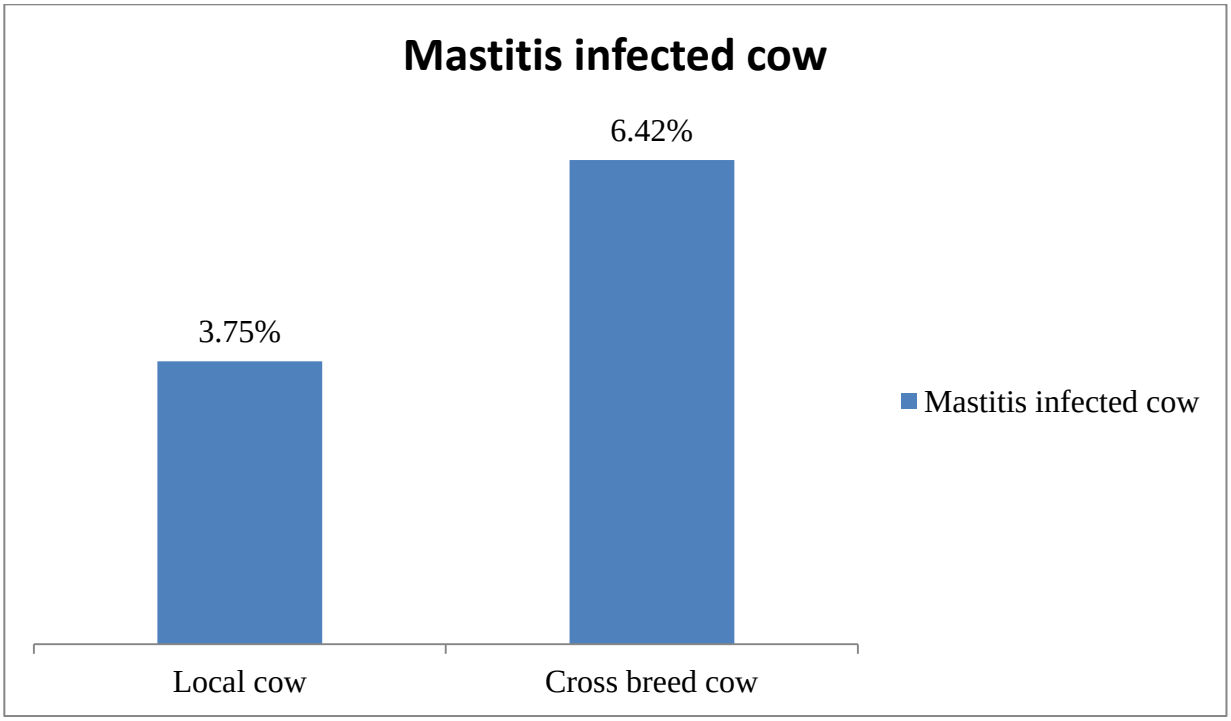


Fig. 3: Diagram showing the prevalence of mastitis in different breeds of cattle.

4.3 Prevalence of mastitis in different age group

The cows are subcategorized into different age group. The prevalence of mastitis in 3-4, 5-6, 7-8 and 9-10 years old cows were 3.08%, 4.29%, 5.88% and 6.25%, respectively (Table). The prevalence of mastitis is 3.08% in 3-4years aged cow which is lowest prevalence %. That means prevalence of mastitis in young cattle is lower. The prevalence of Mastitis is higher 6.25% in 9-10 years aged dairy cow. It is due to cow with 9-10 years age group have less immunity and loose sphincter of teat that helped the bacteria to entering into udder through teat canal in this period and had increased chance to get infected.

Table-5: Prevalence of mastitis in different age group.

Age	Total No. of cow	No. of infected cow	Prevalence%
3-4 years	65	2	3.08
5-6 years	70	3	4.29
7-8 years	85	5	5.88
9-10 years	80	5	6.25

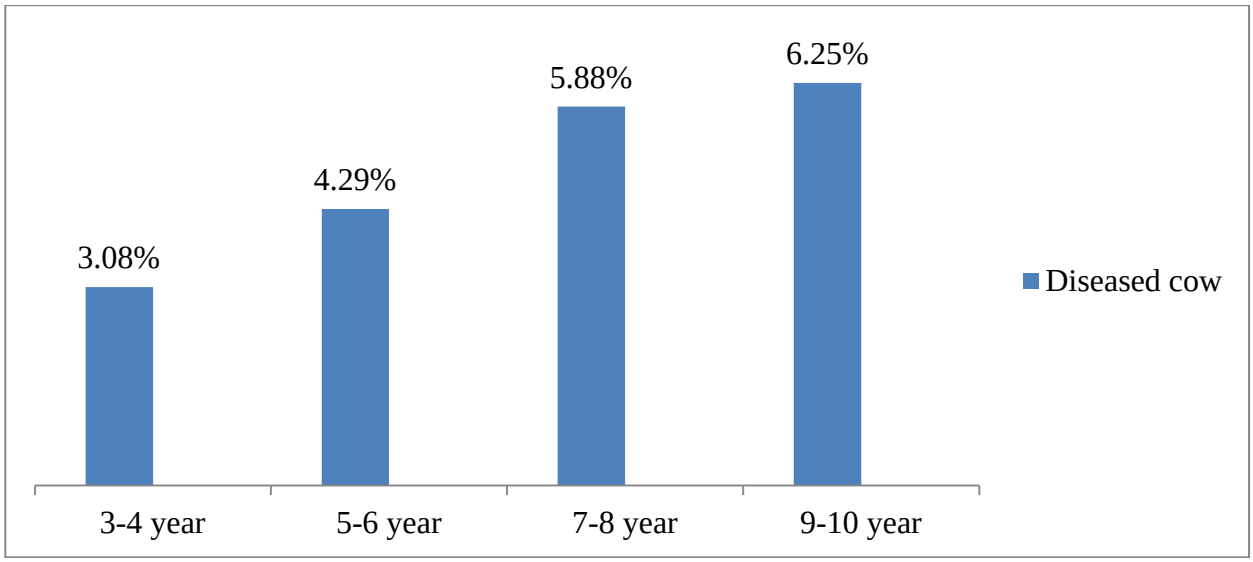


Fig.4: Diagram showing the prevalence of mastitis in different age group.

4.4 Prevalence of mastitis in different Pairity

Occurrence of mastitis during different pairity was represented in Table. From the table it has been shown that higher number of mastitis incidence 8.75% (7) during 3^{rd}-4^{th} Pairity than 1^{st}-2^{nd} pairity 3.33% (3) and $\geq 5^{th}$ Pairity Pairity 3.84% (5).

Table-6: prevalence of mastitis in different Pairity

Pairity	No. of cow	No. of infected cow	Prevalence%
1^{st}-2^{nd} Pairity	90	3	3.33
3^{rd}-4^{th} Pairity	80	7	8.75
$\geq 5^{th}$ Pairity	130	5	3.84

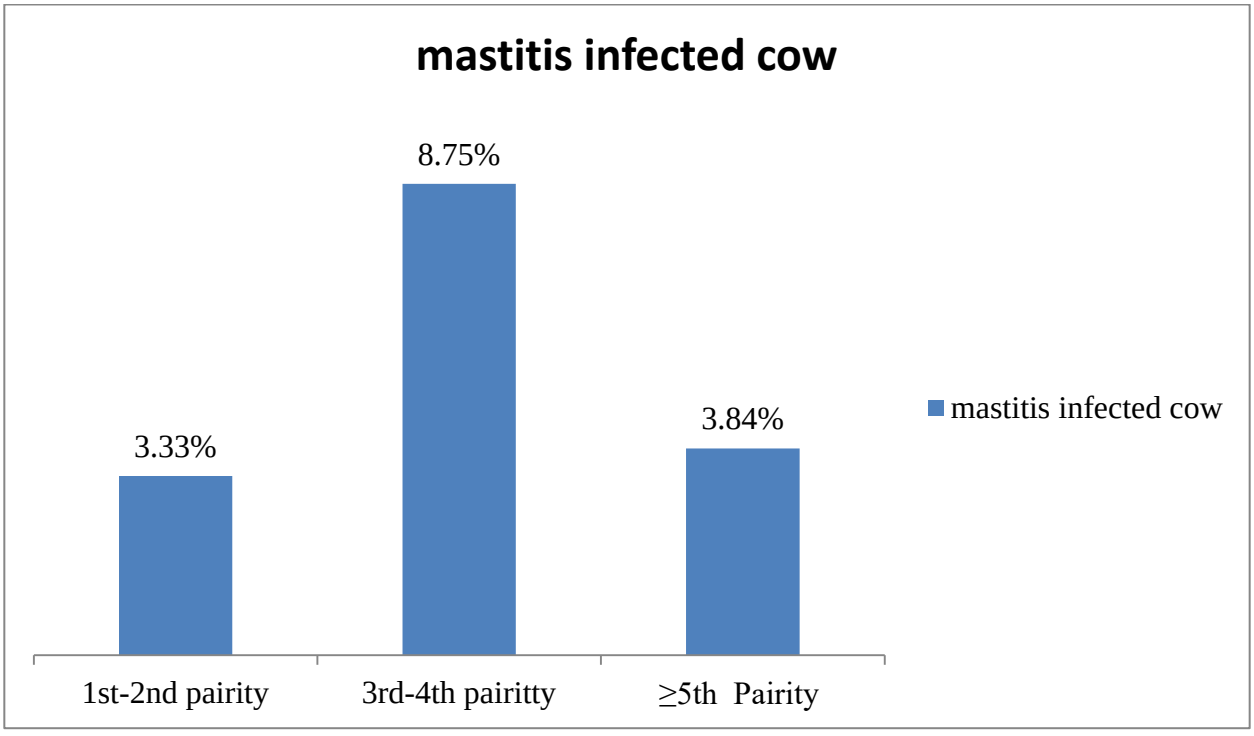


Fig. 5: Diagram showing the prevalence of mastitis in different Pairity.

4.4 Prevalence of Mastitis in different Season

The overall prevalence of mastitis in cow in dry and wet season was 3.70% and 6.06% respectively. The prevalence of mastitis is higher in wet season than dry season respectively.

Table-7: Prevalence of mastitis in different season.

Season	No. of infected cow	Prevalence%
Dry season	5	33.33%
Wet season	10	66.67%

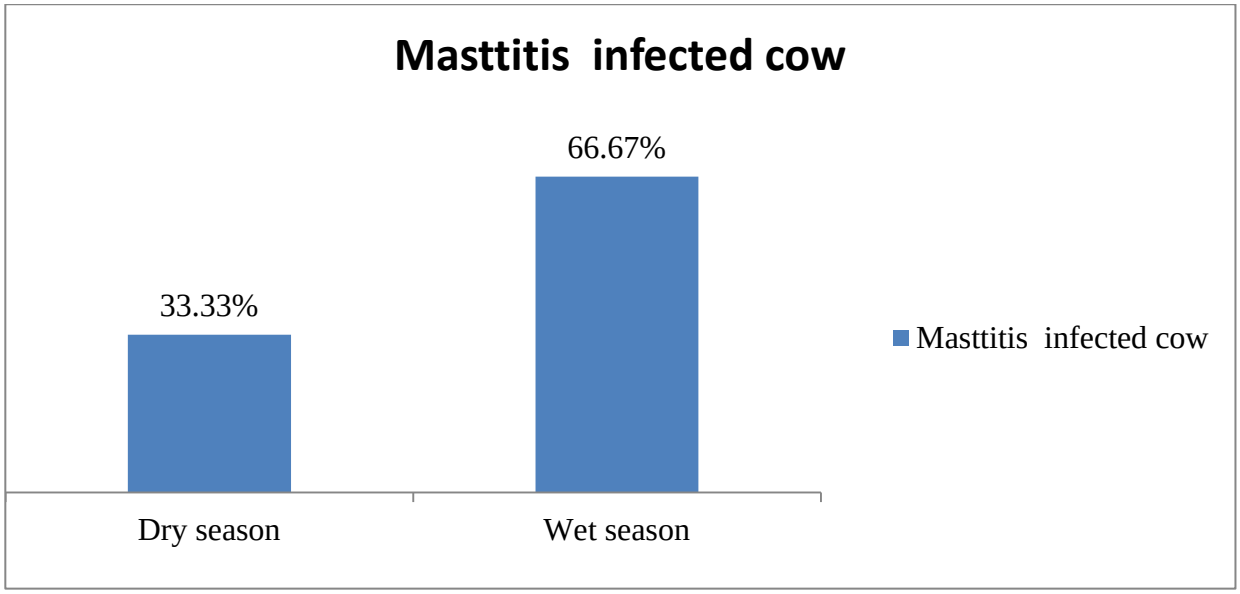


Fig.6: Diagram showing the prevalence of mastitis in different Season.

4.6 Occurence of mastitis in cow with history of periparturient disease.

The occurence of mastitis in cow having peri-parturient disease 86.67% and cows without a history of peri-parturient disease was 13.33% respectively. The cows infected with other disease were more prone to mastitis than healthy cows.

Table-8 Percentages of mastitis in cows with peri-parturient diseases

Condition	Affected animals (Cows)	Percentage (%)
Cows without history of peri-parturient disease	2	13.33
Cows with history of peri-parturient disease	13	86.67
Total	15	100

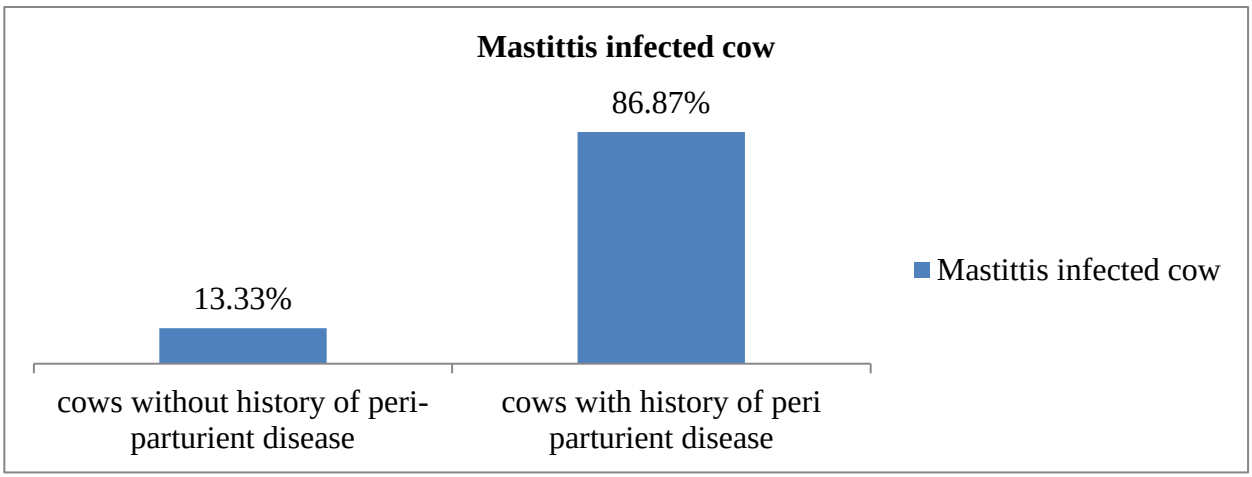


Fig7. Diagram showing the prevalence of mastitis in cows with/without history of peri-parturient disease.

4.7. Occurence of mastitis in different lactating time.

The occurrence of mastitis is 26.67% at 1st-2nd month of lactation ,53.33% at 3rd-4th month of lactation and 20% at 5th -6th month of lactation respectively. During third month of lactation milk production is higher in dairy cow and have more chance to get infected with

mastitis disease. So, mastitis prevalence is higher in dairy cow in third to fourth month of lactation.

Table -9: Occurence of mastitis during lactating time.

Months	No. of cows	Percentage (%)
1st to	4	26.67
3rd to	8	53.33
5th to	3	20
Total	15	100

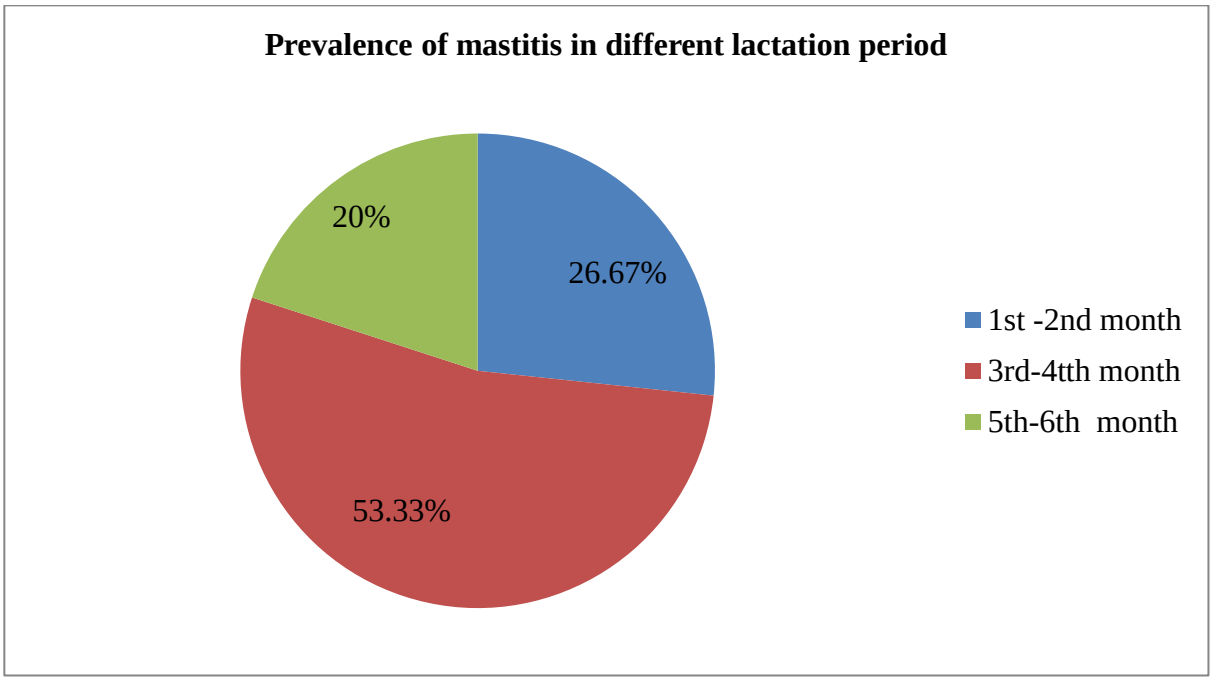


Fig.8: Diagram showing the prevalence of mastitis in different lactation period

4.8. Occurrence of mastitis depending on floor component

Occurrence of mastitis depending on floor condition is presented on the Table **.**The occurrence of mastitis was 26.67% (4) in cows in farms with brick-block floor and 20% (3) in cows in farms with soil floor. The occurrence of mastitis was also significantly affected by partly or completely wet and soiled floor. Only 08 (53.33%) cows were affected with mastitis when the floor was wet and soiled.

Table-10: Occurrence of mastitis depending on floor component

Floor condition	Total cases (Cows)	Percentage (%)
Brick block floor	4	26.67
Soiled floor	3	20
Partly or completely Wet & soiled	8	53.33
Total	15	100

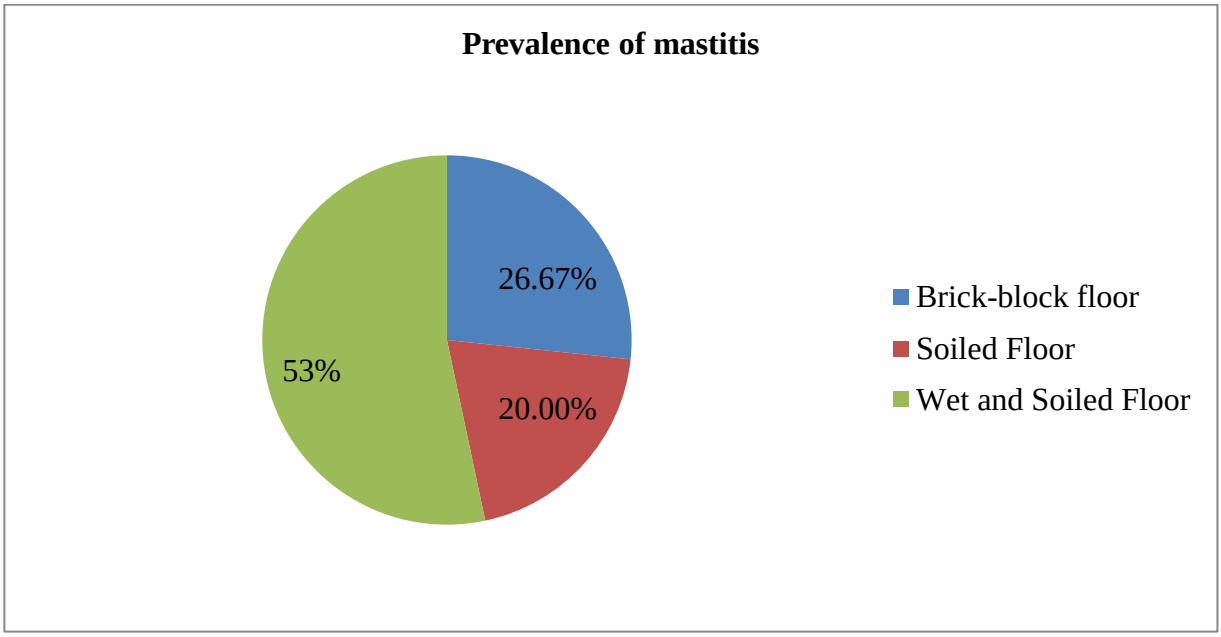


Fig 9. Diagram showing the prevalence of mastitis in different floor condition.

4.9 Occurrence of mastitis in relation to cleanliness of farm

The prevalence of mastitis is higher in dairy cows reared in dirty farm rather than clean farm. Among 15 mastitis infected cattle, (11) 73.33% infected cows reared in dirty farm and (04) 26.67 infected cows are reared in clean farm.

Table-11: Occurrence of mastitis in relation to cleanliness of farm

Category	Total cases (Cows)	Percentage (%)
Clean	04	26.67
Dirty	11	73.33
Total	15	100

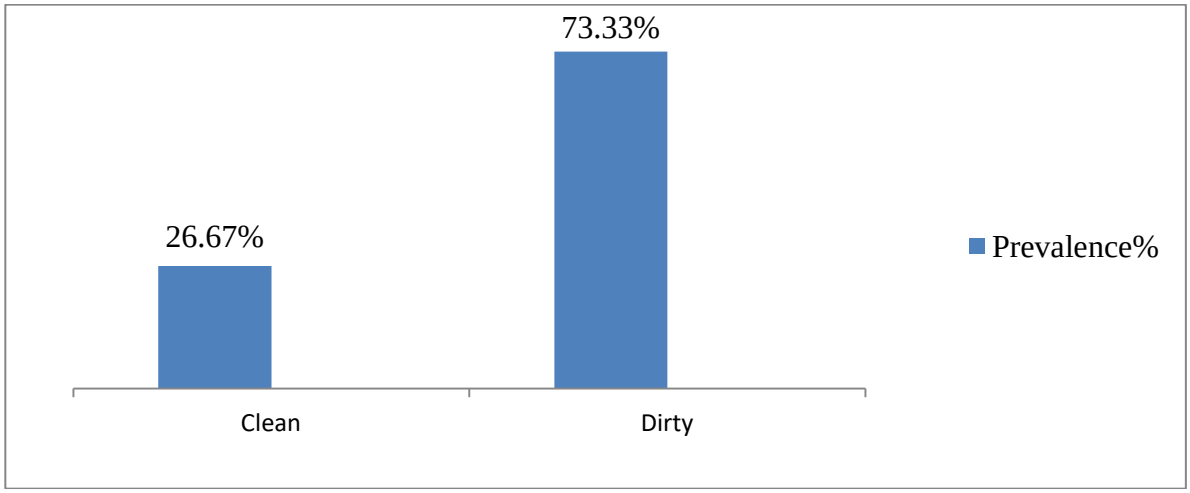


Fig.10: Prevalence of mastitis in relation to cleanness of farm

4.10 Overall prevalence of identified & isolatied causal agent

The overall prevalence of *E coli* is 73.33% and *Staphylococcus aureus* is 66.67% among the 15 sample which is identified by bacterial culture and biochemical test.

Table12: Prevalence of Isolatied & identified Causal agent

Causal Agent	Total Sample	No. of positive sample	Prevalence%
E. coli	15	11	73.33
Staphylococcus aureus	15	10	66.67

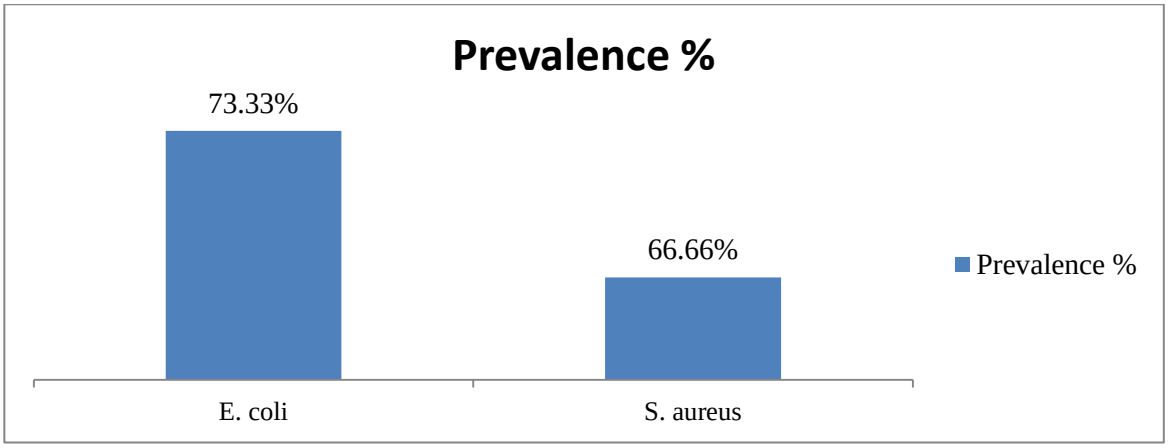


Fig11. Persistence of bacteria and their prevalence in mastitis case.

4. 11 Results of isolatied & identified *E.coli*

E. coli is isolated and identified through cultural examination and biochemical test performed in SAU Medicine and Public Health Laboratory.

4. 11.1 Culture in nutrient broth

All the *E. coli* isolates produced turbidity in nutrient broth.

4. 11.2 Culture on MacConkey agar

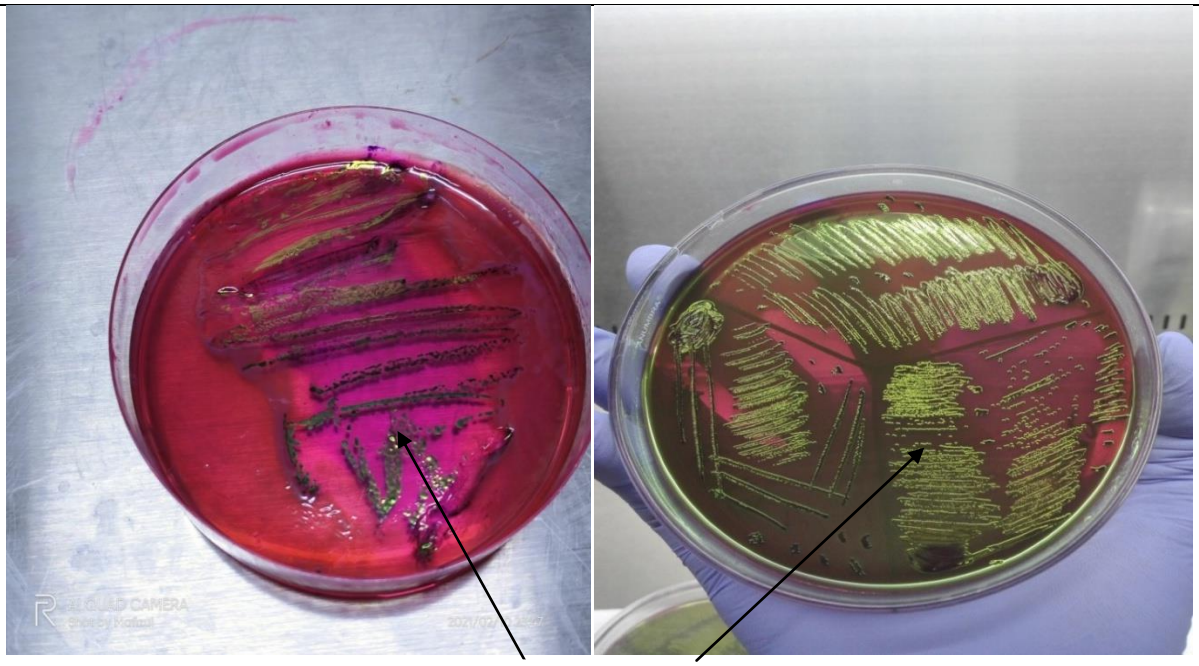
Bright pink colored colonies on MacConkey agar produced by the organisms after overnight incubation were presumptively selected as *E. coli.*

4. 11.3 Culture on Eosine Methylene Blue (EMB) agar

Greenish colonies with metallic sheen produced by the organisms on EMB agar after overnight incubation were tentatively confirmed as *E. coli.* Tentatively confirmed *E. coli* isolate from cattle produced Greenish red colonies with faint metallic sheen.

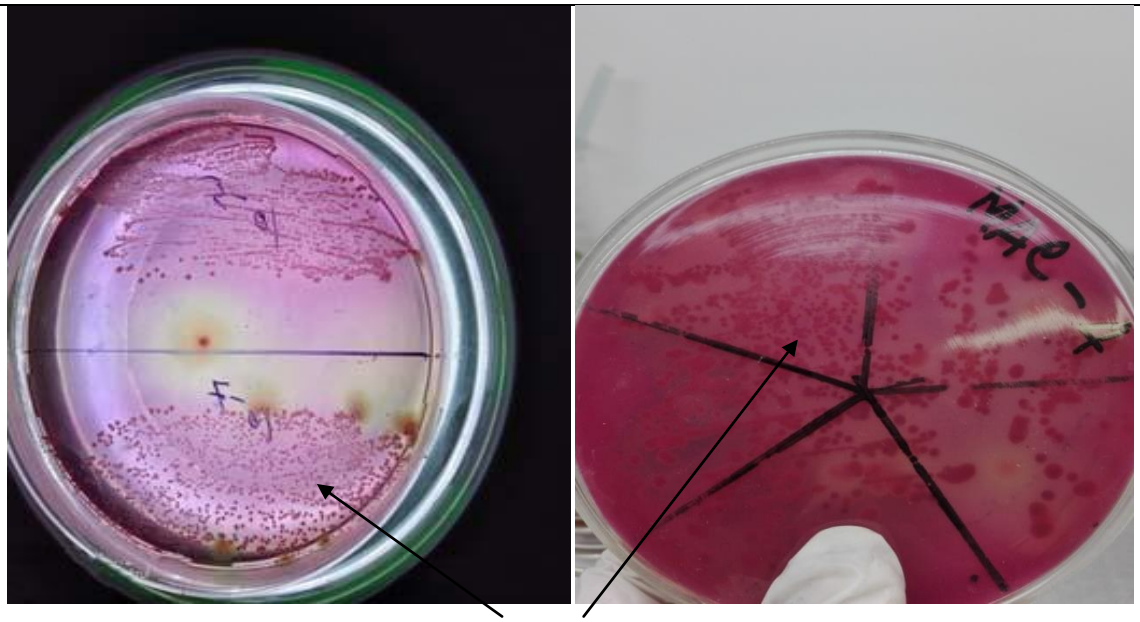
Table-13: Colony characteristics of *E. coli* in different agar media

Sources of *E coli*	**Colony Characteristics in different Agar media**	
	EMB Agar media	**MacConkey agar Media**
Sample No.- 1, 2, 3, 5, 7, 8, 9, 11, 13, 14, 15	Greenish colonies with metallic sheen	Bright pink colored colonies



Greenish colonies with metallic sheen

Plate 1: *E coli* in EMB Agar

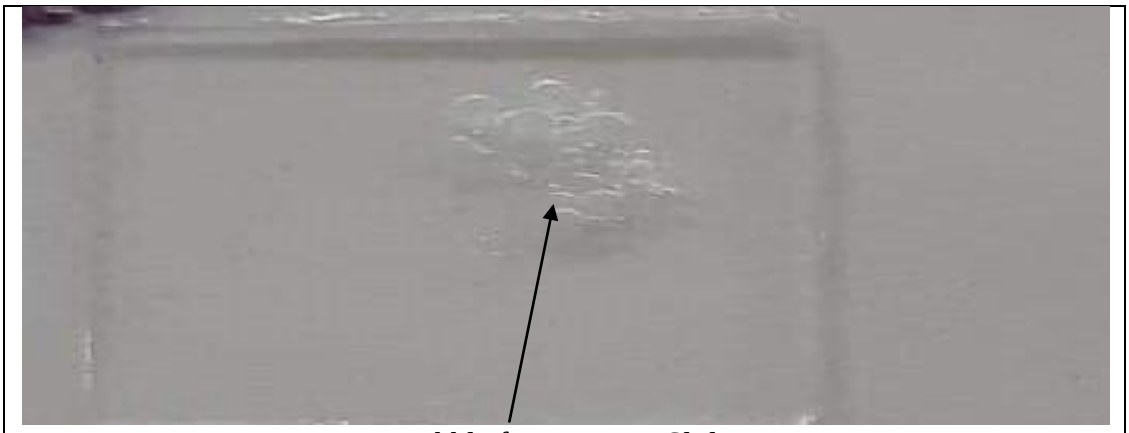


Bright pink colored colonies

Plate 2: *E coli* in MacConkey Agar

4. 11.4 Catalase test:

3% H_2O_2 when added with Bacterial colony form bubble formation in slide indicates *E. coli* positive.



Bubble formation in Slide

Plate 3. (E. coli Positive in Catalase test)

4.11.5 MR test:

In Methylene Red indicator red color indicate the test is positive for the presence of *E. coli.*

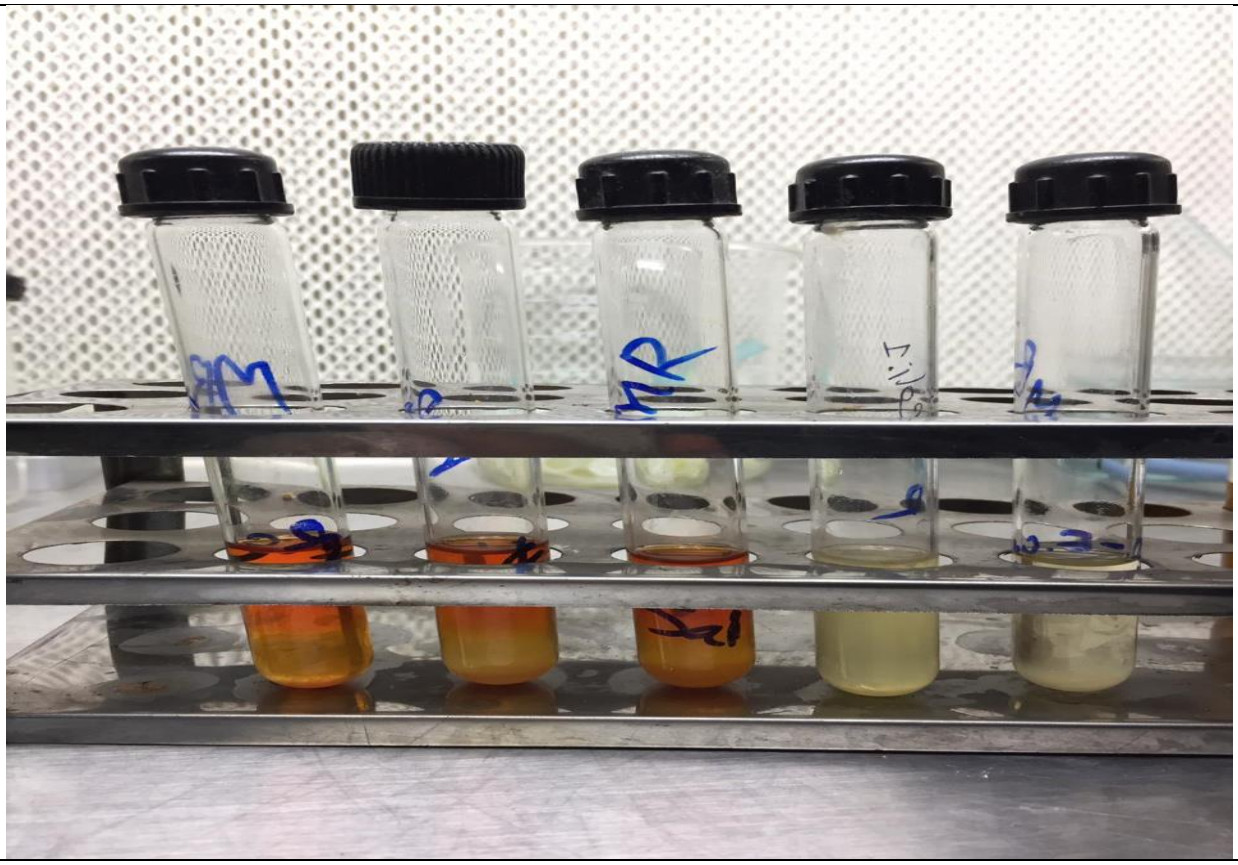


Plate 4: *E. coli* Positive in methylene red test

4.12 Results of isolation and identification of *Staphylococcus aureus*

For isolation of *S. aureus* bacteria was cultured in different selective media and different biochemical test had performed for their identification.

4.12.1 Culture in nutrient broth

All *Staphylococcus aureus* isolates produced turbidity in nutrient broth.

4.12.2 Culture on Blood Agar

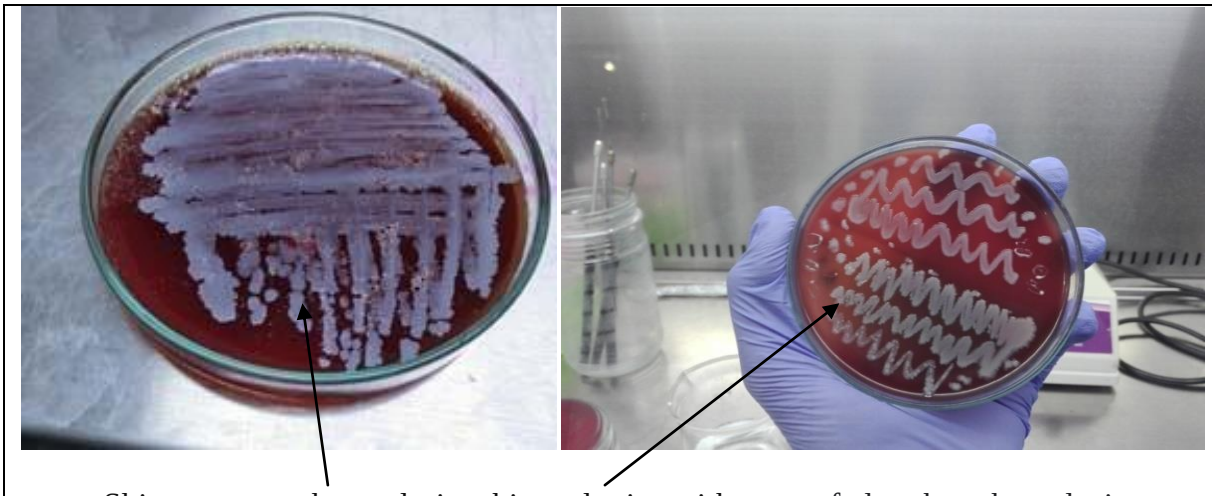
Golden colored colonies frequently surrounded by zones of clear beta-hemolysis on Blood agar produced by the organisms after overnight incubation were presumptively selected as *Staphylococcus aureus.*

4.12.3 Culture on Mannitol Salt Agar

Mannitol Salt Agar (MSA) is used as a selective and differential medium for the isolation and identification of *Staphylococcus aureus* . Yellow colonies surrounded by yellow zone on Mannitol Salt Agar media produced by the organisms after overnight incubation were presumptively selected as *Staphylococcus aureus*

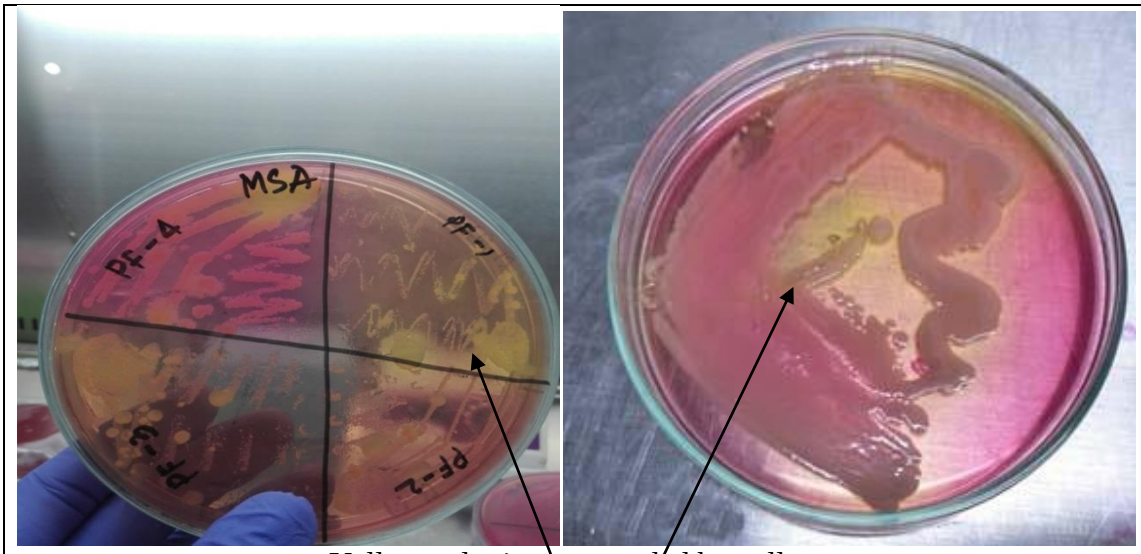
Table -14: Cultural characteristics of *Staphylococcus aureus* isolated from mastitis infected milk samples in different agar media

Sources of *Staphylococcus aureus*	**Colony Characteristics in different Agar media**	
	Blood Agar media	**Mannitol Salt agar Media**
Sample No.- 1, 2, 4, 5, 7, 8, 10, 11, 13, 15	*Staphylococcus aureus* are shiny, convex, haemolytic white colonies with zone of clear beta-hemolysis.	Yellow colonies surrounded by yellow zone.



Shiny, convex, haemolytic white colonies with zone of clear beta-hemolysis

Plate 5: *Staphylococcus aureus* in Blood Agar

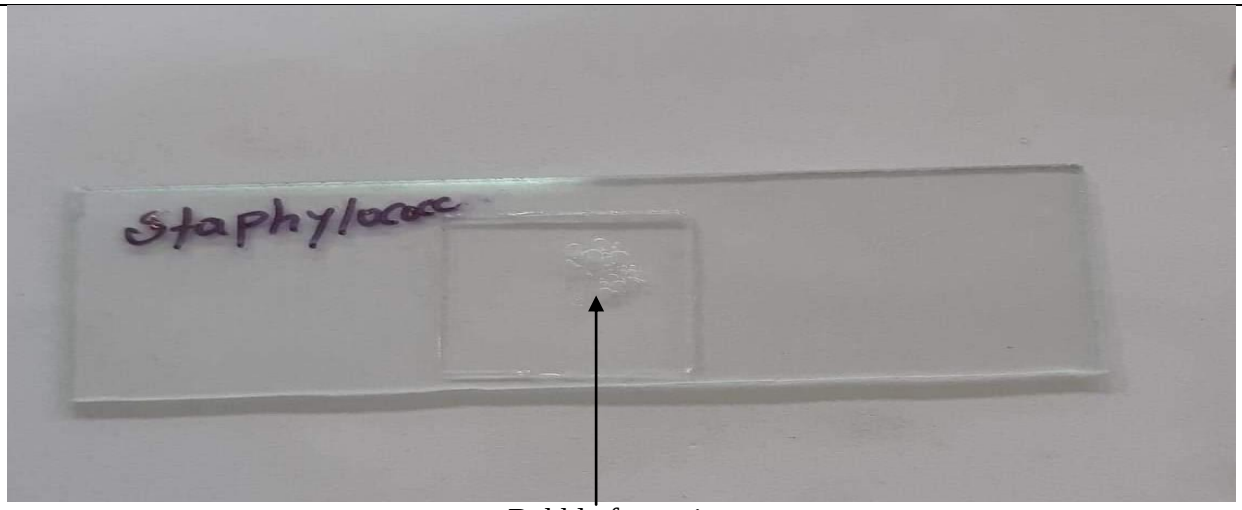


Yellow colonies surrounded by yellow zone.

Plate 6: *Staphylococcus aureus* in Mannitol Salt Agar

4.12.4 Catalase test:

Bubble formation on slide indicate the test is positive for *Staphylococcus aureus*



Bubble formation

Plate 7: *Staphylococcus aureus* Positive in Catalase test

4.12.5 MR test:

Red color develop in test tube indicates *Staphylococcus aureus* Positive in methylene red test.

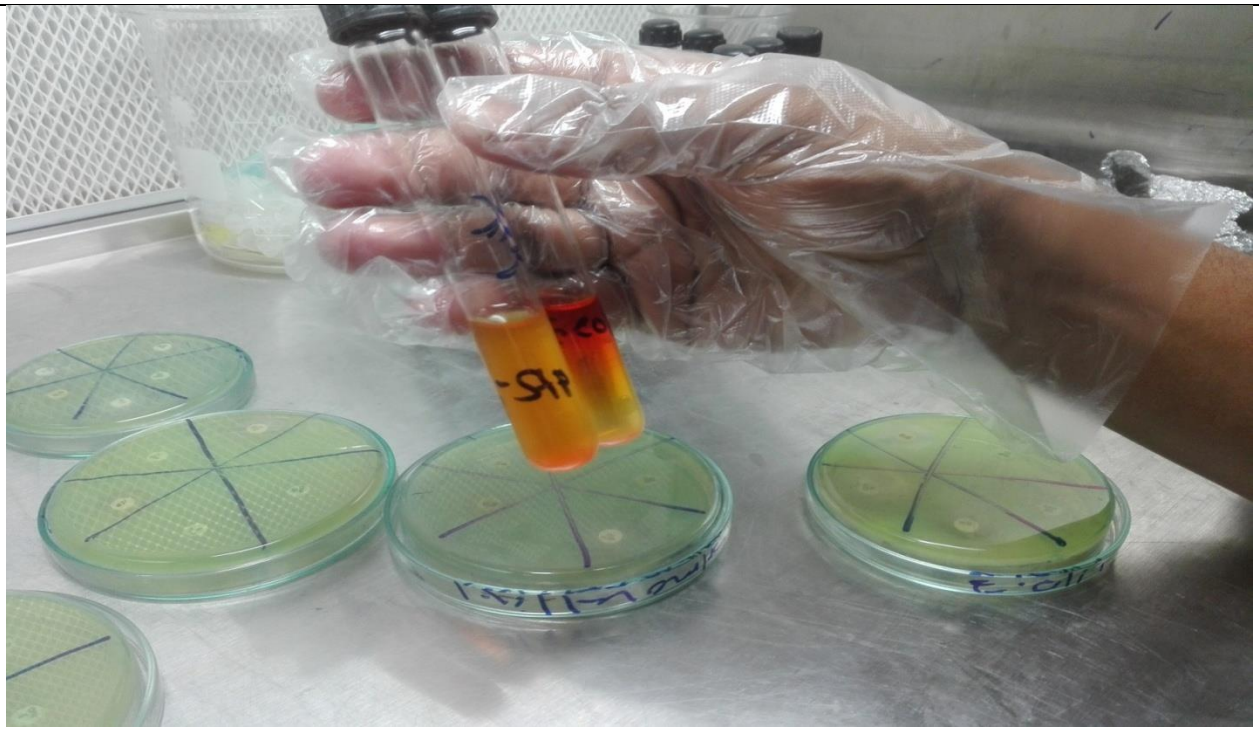


Plate 8: *Staphylococcus aureus* Positive in methylene red test

4.13 Antibiotic Resistance Profiling of *E. coli*

Antibiotic disc were used to know the sensitivity and resistance of *E. coli* organism. The disc were placed on bacterial growth containing muller hinton agar media in petridish and placed in incubator for 24 hour and count the zone of interpretive standard. On the basis of zone of interpretive standard the resistance percentage of bacteria was identified against antibiotic agents.

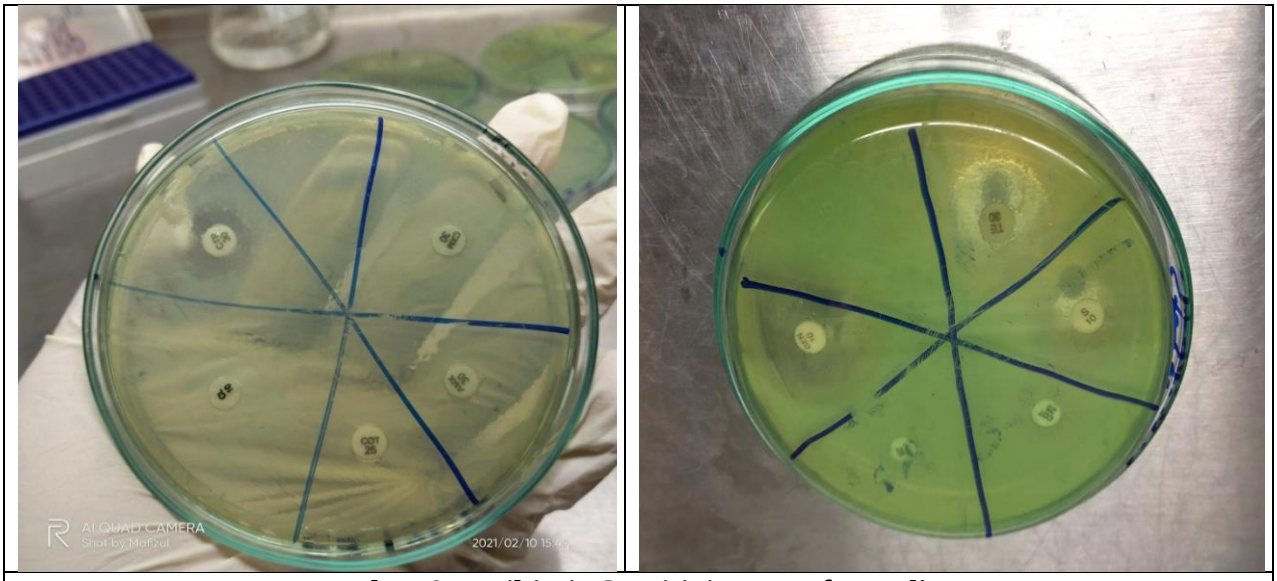


Plate 9. antibiotic Sensitivity test of *E. coli*

Table-15: AMR of *E. coli* to different antibiotics obtained from mastitis infected bovine milk.

AntiMicrobial drugs	R	IN	S	Percentage (%) of resistance to different drugs		
				R	IN	S
Amoxicilin (AMX_{30})	5	2	4	45.45	18.18	36.36
Ampicillin (AMP_{25})	4	2	5	36.36	27.27	45.45
Tetracycline (TE_{30})	4	1	6	36.36	9.09	54.54
Streptomycine (S_{10})	2	0	9	18.18	0	81.81
Gentamycin (GEN_{10})	1	0	10	9.09	0	90.91
Ceftriaxone (CTR_{30})	1	0	10	9.09	0	90.91
Cefuroxime (CXM_{30})	0	0	11	0	0	100
Cefixime (CFM_5)	0	0	11	0	0	100
Cotrimethaxole/ trimethoprim (COT_{25})	2	0	9	18.18	0	81.81

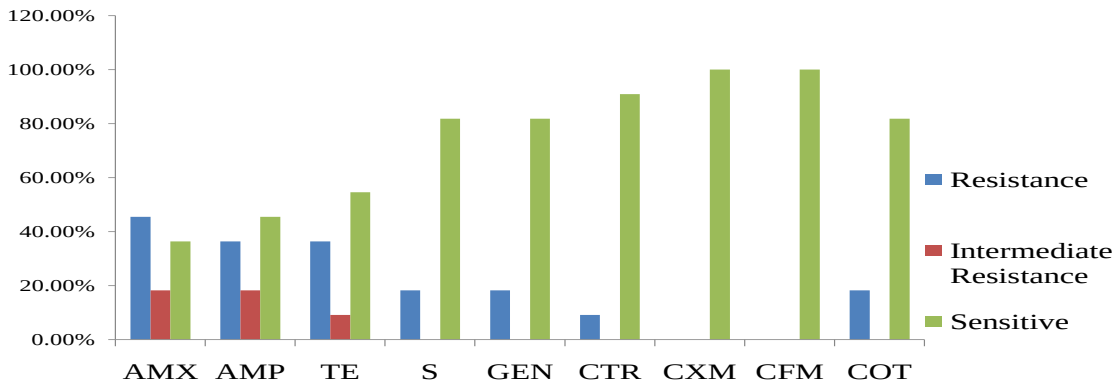


Fig 23 :Sensitivity and resistance patterns of isolated *E. coli*

4.14 Antibiotic Resistance Profiling of *Staphylococcus aureus*

Antibiotic disc were used to know the sensitivity and resistance of *E. coli* organism. The disc were placed on bacterial growth containing muller hinton agar media in petridish and placed in incubator for 24 hour and count the zone of interpretive standard. On the basis of zone of interpretive standard the resistance percentage of bacteria was identified against antibiotic agents.

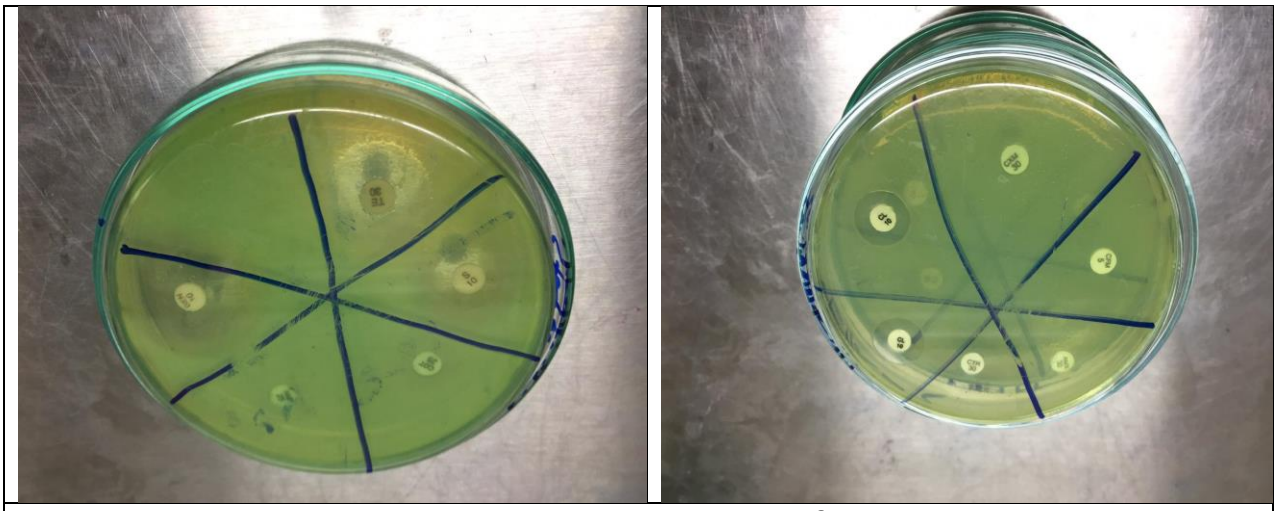


Plate 10. Antibiotic Sensitivity test of *S. aureus*

Table-16: AMR of *Staphylococcus aureus* to different antibiotics obtained from mastitis infected bovine milk.

AntiMicrobial drugs	*R*	*IN*	*S*	Percentage (%) of resistance to different drugs		
				R	IN	S
Amoxicilin (AMX_{30})	1	1	8	10	10	80
Ampicillin (AMP_{25})	2	0	8	20	0	80
Tetracycline (TE_{30})	2	1	7	20	10	70
Streptomycine (S_{10})	1	1	8	10	10	80
Gentamycin (GEN_{10})	1	0	9	10	0	90
Ceftriaxone (CTR_{30})	1	0	9	10	0	90
Cefuroxime (CXM_{30})	0	0	10	0	0	100
Cefixime (CFM_5)	0	0	10	0	0	100
Cotrimethaxole/ trimethoprim (COT_{25})	3	1	6	30	10	60

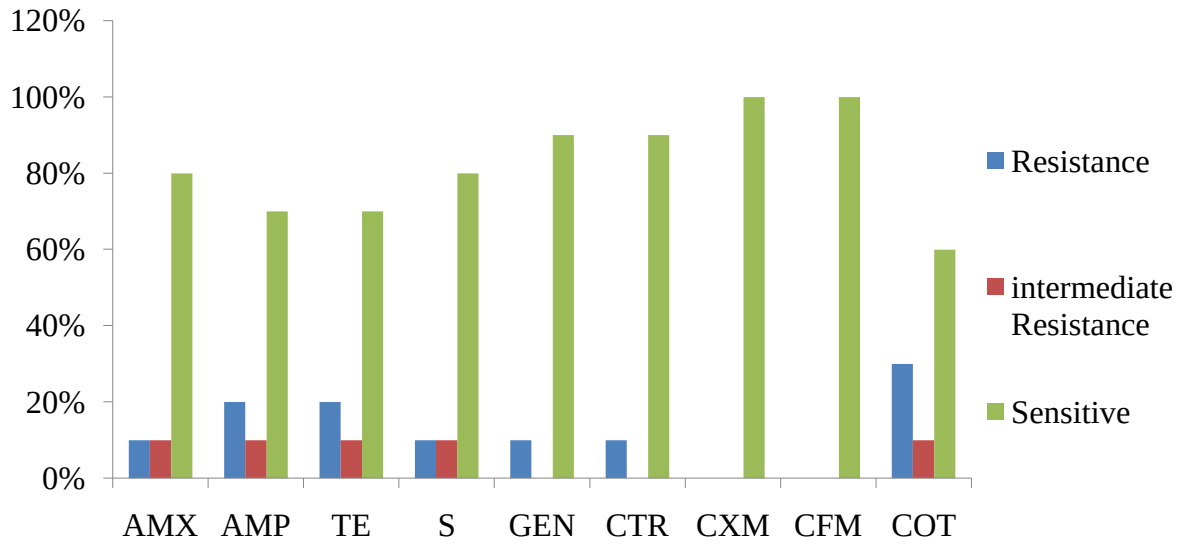


Fig 25 :Sensitivity and resistance patterns of isolated *Staphylococcus aureus*

4.15 Discusion

The oveall prevalence of mastitis were 5% at Amtali, Barguna(Table-3).These findings was in agreement with Raman *et al., (*1997) who reported 13.33% occurence of clinical mastitis in dairy cows. Rahman *et al.,* (2009) found 19.9% mastitis, Bari *et al.,* (2014) who reported that the overall prevalence of mastitis were 8.36% and Faruk *et al.,*(2018) shows that the overall prevalence of clinical mastitis in cows were 11.02%.. This finding is also support with the observation of Chisty *et al.,* (2007) who reported 16.72% prevalence of clinical mastitis in dairy cows at Pakistan. This result was very much lower than Sinha *et al.,*(2011) who found that the total percentage of mastitis cows was 42.7%. Prevalence of clinical mastitis in Bangladesh were about 13.3% (Prodhan *et al.,*1996). The differerence was due to smallholder farming system at Amtali, Barguna and good management of farm. Among 300 dairy cows 160 local cows were examined for mastitis case. 6 cows were diagnosed as mastitis positive and the prevalence rate were 3.75%. Among 140 crossbreed cows 9 cows were diagnosed as mastitis positive during the study period and the prevalence % were 6.42%(Table-4) . This finding was supported by Bari *et.al.,* (2014) where the prevalence of mastitis were found significantly higher ($P < 0.01$) in crossbred cows (10.09%) than in indigenous cows

(4.26%), Faruk et.al.,(2018) who reported that Mastitis was significantly (P<0.05) higher in cross breed cows (15.2 %) than the local breed (6.67%). This result is similar to the observations made by Rahman *et al*., (1997) & Nooruddin *et al*., (1997) who recorded higher frequency of mastitis in cross breeds. The findings of Hossain *et al*., (2004), Slettbark *et al.*, (1995) & Rodostits *et al*., (2000) also reported that high yielding cows were more prone to udder infection than low producing one. Higher prevalence of mastitis in crossbred cattle that revealed in current study was also supported Roy *et.al.*,(1989). It might be due to the larger udder size and genetic conformation that leads to susceptibility to various pathogens. The prevalence of mastitis in 3-4, 5-6, 7-8 and 9-10 years old cows were 3.08%, 4.29%, 5.88% and 6.25%, respectively (Table-5). The prevalence of mastitis is 3.08% in 3-4 years aged cow which is lowest prevalence %. That means prevalence of mastitis in young cattle were lower. The prevalence of Mastitis was higher 6.25% in 9-10 years aged dairy cows. It is due to cow with 9-10 years age group have less immunity in this period and have chance to get infected. Prevalence of mastitis in this study varied depending on the age and higher infection were observed with advancing of age. One of the findings of the current study was that the prevalence of mastitis increased with the advancing age which had an agreement with the statements of Rasool *et.al.,(*1985). Faruk *et.al.,(*2018) who found that, On age basis mastitis was higher in above 7 years cows (16.92%), moderate in 5-7 years cows (9.47%) and lowest in below 4 years cows (8.23%), Sinha *et al.*,(2011) who reported that the prevalence of mastitis in 3-4, 5-6, 7-8 and 9-10 years old cows were 33.3%, 42.5%, 45.3% and 52.8%, respectively. Many other studies were in agreement with the present findings of increased mastitis in advancing age (Slettbakk *et al*., 1995, Radostits *et al*., 2000, Quaderi, 2005). Husain, (2007) showed that older cows with about 14 years of age had 61% sub-clinical mastitis which correlates with the present findings. Increased age predisposed the cows to more susceptible to infection and decreasing potency of the teat sphincter (Pankey *et al.*, 1991). On the contrary, younger cows may have decreased susceptibility of mastitis because they possess more effective host defense mechanism (Dulin *et al*., 1988).

Seasonal influence on occurrence of mastitis has been shown on the Table-6. The data was recorded from different farm of Amtali Upazilla in last year (January- December, 2020). From the observation it had been shown that a great impact on seasonal influence on

mastitis. A total 5 cows in dry season, 10 cows in wet season were mastitis affected, and the overall prevalence of mastitis in dry and wet season were 33.33% and 66.67% respectively. The overall occurrences of mastitis were significantly higher in wet season than in dry season. The statement was supported by Bhuiyan *et.al.,* (2010) who reported that a total of 347 cows in dry season and 388 cows in wet season were studied, and the overall prevalence of mastitis were 19.9% and 44.8%. Rahman *et al.,* (2009) and explained that during the wet season, water was plenty, and in most cases, except the barn and household, land was submerged by flood-water. The cows remained on the floor all day and got dirty. The floor was muddy, and drainage was difficult to maintain. In addition, the warm temperature and high humidity favored the growth of organisms (Fox *et al*., 1995). The overall prevalence of mastitis in cow at different pairity was represented in Table-7. From the table it has been shown that higher number of mastitis incidence 8.75% during 3^{rd}-4^{th} Pairity than 1^{st}-2^{nd} pairity 3.33% and $\geq 5^{th}$ Pairity Pairity 3.84% . This result is in agreement with the observation made by Sinha *et al.,* 2011, who reported that the occurrence of mastitis in the cows at parity 1st - 2nd, 3rd - 4th and 5th - 7th was 32.9%, 50.6%, and 62.5%, respectively. Kapur & Sing 1978, who reported highest incidence rate of mastitis in 2^{nd} parity than 1^{st} one. The finding of present study also supported with the findings of Rasool *et al.,* (1985) who observed an increased prevalence of mastitis in old animals. Because the high parity cows have less defense apparatus as their teat sphincter get loosen and cellular defense lowered, resulting the organism to get easy entrance through the teat canal on the contrary younger cows are less susceptible to mastitis because of their more effective defense mechanism. Polymorphoneuclear leukocytes functions better in primiparous cows than multiparous ones (Dulin *et al.,* 1988 & Roy *et al.,* 1998).

The prevalence of mastitis in cow having peri-parturient disease 86.67% and Cows without a history of peri-parturient disease is 13.33% respectively(Table-8). This result was supported by the report of Bari *et al.,*(2014) where without a history of cows having periparturient disease had a prevalence of 3.73% mastitis, in contrast, 33.67% of cows with a history of periparturient disease had mastitis. The result is also supported by Rahman *et al.,* (2009). The lower immunity level of peri-parturient cows made the cow

more prone to infection in the udder (Rainard and Riollet, 2006). Once a cow gets infected or diseased during the peri-parturient period, it becomes more susceptible to udder infection due to lowered immunity (Nickerson, 1994; Peeler *et al.,* 1994). Calcium ions are necessary for muscle constriction. As a result, in milk fever, low level of calcium decreases the rigidity of the teat sphincter that perhaps allows the organism to pass into the udder (Paape and Guidry, 1993). In addition, cows having infected uterine discharge and retained placenta risk the udder and teats being contaminated (Peeler *et al.,* 1994).

The prevalence of mastitits was 26.67% at 1st-2nd month of lactation, 53.33% at 3rd-4th month of lactation and 20% at 5th -6th month of lactation respectively(Table-9). This result was supported to the observation made by Rahman *et al*., (1997) who recorded that higher frequency of mastitis at third months of lactation.

Occurrence of mastitis depending on floor condition was presented on the Table-10 .The occurrence of mastitis was 26.67% (4) in cows in farms with brick-block floor and 20%(3) in cows in farms with soil floor. The occurrence of mastitis was also significantly affected by partly or completely wet and soiled floor. Only 08 (53.33%) cows were affected with mastitis when the floor was wet and soiled. This can be explained by the fact that farms with soil floor would dry more quickly than the brick floor (Hogan *et al.,* 1990). As a result soiled floor animal were less affected than brick block floor. But wet soiled floor (less absorbable) are most harmful for dairy animal to cause mastitis. It appeared that the floor was a potential source for mastitis organisms to enter the udder through the teat orifice. Kivaria *et al.,* (2004) showed scarcity of water as one of the potential risk factors for the prevalence of mastitis. This is true for the area where the present investigator worked. But in the present study, during the wet season, water was plenty, and in most cases, except the barn and household, land was submerged by flood-water. The cows remained on the floor all day and got dirty. The floor was muddy, and drainage was difficult to maintain. In addition, the warm temperature and high humidity favored the growth of organisms (Fox *et al., 1995*). The occurrence of mastitis in relation to cleanliness of farm had shown in table-11. Factors such as barn cleanliness are one which a farm in frequently judged. Clean farm had shown lower occurrence of mastitis than the dirty farm. This result of present study was supported with the findings of Chishty

et al., (2007) who reported that the prevalence of mastitis were found to be highest in cows managed with lower drainage system.

The overall prevalence of *E coli* was 73.33% and *Staphylococcus aureus* was 66.67% among the 15 sample which was identified by bacterial culture and biochemical test. This result wass supported by Arthanari Eswaran M., (2018) who reported that The predominant mastitis causing pathogens were *Escherichia coli* (40%) followed by *Staphylococcus aureus*, (27%), *Klebsiella pneumoinae* (20%) and *Streptococcus dysgalactiae* (13%). Chandrasekaran *et.al.,(*2014) who reported that, Out of 401 clinical mastitis samples subjected to bacterial isolation, 184 (45.89%) were positive for, 162 (40.4%) were positive for S. aureus.

Among 15 samples, 10 (66.67%) showed ß-hemolysis on 5% sheep blood agar with circular, small, smooth raised whitish colony. Islam *et al.*,(2007b) reported that 89.3% *S. Aureus* from bovine origin were hemolytic.. After overnight incubation on MS agar media, some plates showed yellow colony and some plates showed whitish colony. All the suspected *S. aureus* which produced ß- hemolysis on 5% blood agar were able to ferment mannitol salt agar characterized by the formation of yellow colony and white/transparent colony indicated other *Staphylococcus spp*., as indicated by Cheesbrough (1985), Begum *et al.,* (2007) and Islam *et al.,* (2007a, b). Catalase test Was performed to differentiate Staphylococci (catalase producer) from Streptococci (non-catalase producer). Hydrogen peroxide (H_2O_2) was broken down into water and oxygen. Production of oxygen was indicated by bubble formation. All *S. aureus* isolates were catalase positive. A total of 10 samples were found as catalase positive, as described by Cheesbrough (1985). All the isolates of Staphylococci gave positive reaction in coagulase test indicated that the isolates were pathogenic S. aureus. solation of by cultural, morphological and biochemical tests were performed according to methods described by Cheesbrough (1985), Hummerjohann *et al.,* (2014) and Jahan *et al.,* (2015). After overnight incubation,11 samples showed bright pink or red colonies on MC agar, were identified as *E. coli*. For presumptive identification of *E. coli*, the selective colony on Mc Conkey agar for each sample was sub-cultured successively onto Eosin Methylene Blue (EMB) agar giving greenish-black colonies with metallic sheen. All the isolates were catalase positive, and methyl-red positive, as reported by Nazir *et al.,* (2005) and Roy *et al.,* (2012).

Nine antibiotics were used against the isolated bacteria. *Escherichia coli*, the most common causative organism of mastitis. Among these antibiotics, Amoxicillin showed 36.36%, sensitivity, Ampicillin shown 36.36%,Tetracycline shows 54.54% sensitivity, Streptomycine and Cotrimethaxole/ trimethoprim shows 81.81% sensitivity, Gentamycin, Cefttriaxone showed 90.91% sensitivity, Cefuroxime and Cefixime showed 100% sensitivity. It was shown that Cefuroxime and Cefixime was highly sensitive and Ceftriaxone and ,Gentamycin showed 9.09% resistance. Amoxicilin shown higly resistance among these 9 antibiotics. Amoxicilin showed 45.45% resistance. Amoxicillin, Ampicillin and tetracycline shown 18.18%, 27.27% and 9.09% intermediate resistance respectively against *E. coli*. This study was agreement with Moges *et.al., (*2011) who reported that *E. coli* showed less sensitive to Ampicilin (40%), Tertracycline (40%) and highly sensitive to Streptomycin (80%); Bishi (1998) who reported tetracycline were effective only 12% against *E.coli.* Chandrasekaran *et.al.,* (2014) showed that *E. coli* show more sensitivity to Gentamicin (73.1%) and Ceftriaxone (69%). The isolates had highest resistance to Amoxicillin (52.1) and Oxytetracycline (47.95). Gashe *et.al.*, (2018) reported that The majority of *Escherichia coli* isolates 46 (73%) were resistant to Ceftriaxone and 41 (65%) of them were resistant to Ceftazidime. Moges *et.al.,(2011)* reported that Ampicillin is 76%, Tetracycline 18%, Streptomycine and Amoxicillin was only 6% resistance where Ampicilin 24%, Amoxicillin 12%, Tetracycline 21% intermediate resistance, Amoxicillin and Tetracycline was 82%, Streptomycine 72% sensitive to *E.coli* in human and food animals. Ayatollahi et.al.,(2013) reported that sensitivity agaist Cefixime was 37.2%, Ceftriaxone 52.6% , Gentamycin 66.9%, Cotrimethaxole 40.1% where intermediate resistance against Cefixime was 5%, Ceftriaxone 5.8% , Gentamycin 10.3%, Cotrimethaxole 5.1% and resistance against Cefixime was 57.9%, Ceftriaxone 41.6% , Gentamycin 22.8%, Cotrimethaxole 54.7% to *E. coli* in children respectively.

Nine antibiotics were used against the isolated *S. aureus*. Among these Cotrimethaxole/ Trimethoprim shows 60% sensitivity, Tetracycline shows 70% sensitivity, Amoxicillin, Ampicillin, Streptomycine showed 80% sensitivity, Gentamycin, Cefttriaxone shown 90% sensitivity, Cefuroxime and Cefixime shown 100% sensitivity. It was shown that Cefuroxime and Cefixime is highly sensitive and Ceftriaxone showed 10% resistance

Cotrimethaxole shown higly resistance among these 9 antibiotics. Cotrimethaxole/ trimethoprim showed 30% resistance. Amoxicillin, tetracycline, streptomycine and Cotrimethaxole/trimethoprim shown 10% intermediate resistance against *S. aureus.*

Moges *et.al*.,(2011) who reported that *S. aureus* showed less sensitive to Ampicilin (18.5%), Streptomycin (51.8%) and highly sensitive to tertracycline (70.4%). S. aureus showed more sensitivity to Gentamicin (71.2%) and Ceftriaxone (69.2%). The isolates had highest resistance to Amoxicillin (61.5%) and Oytetracycline (49%). Gashe et.al., (2018) reported that *Staphylococcus aureus,* which accounted 19% of the total bacterial isolates, showed 23.4% and 34% resistance to Ceftriaxone and Ceftazidime, respectively. Unakal and Kaliwal (2010) reported that *Staphylococcus aureus* were susceptible to been identified Ceftriaxone 80.88% followed by Cefotaxime 79.41%, Gentamycin 52.94%, Amoxicillin 36.76%, Ampicillin 29.41%, Sharma et.al(2015) reported that Cefixime 66.67% Colistin 55.56% , Streptomycin 44.44%, Ampicillin 33.33%, Cefuroxime, Gentamycine and Tetracycline 22.22% resistance to *S aureus.* Intermediate sensitivity drugs could not be compared due to lack of relevant literature.

CHAPTER 5
SUMMARY AND CONCLUSION

The present study was conducted at different dairy farm in Amtali, Barguna. The experiment was performed by clinical examination of dairy cow for mastitis infection and collection of milk from mastitis infected cow. Then laboratory work was performed in Sher-e-Bangla Agricultural University Medicine and public health lab. For isolation and identification of bacteria and Antimicrobial resistance profile was also conducted to know the sensitivity and resistance capacity of certain bacteria against certain antibiotic drug. The specific objectives of this study was undertaken to know the prevalence of mastitis in Amtali, Barguna to Isolate and identify bacteria harbors in mastitis infected cow's milk in Amtali, Barguna to investigate the antibiotic resistance pattern of the isolated bacteria, to know the efficacy of some antimicrobial drug for proper treatment. The sample was collected from different dairy farm and 300 cow was examined to detect clinical mastitis. Among 300 cow, 15 cow were identified for mastitis infection and the prevalence of mastitis was determined as 5%.In 300 dairy cow, 160 local cow were examined and 6 cow were infected and the prevalence was 3.75%, 140 crossbreed cattle were examined and 9 cow were infected and the prevalence was 6.42%. During study time cow were categorized on the basis of age and separate into 3-4 year, 5-6 year, 7-8 year and 9-10 year aged cow group. The prevalence of mastitis in 3-4, 5-6, 7-8, 9-10 years aged cow were 3.04%, 4.29%, 5.88% and 6.25% respectively. On the basis of seasonal influence cow were divided into two seasonal group: Dry season and wet season. A total of 135 cows in dry season, 165 cows in wet season were studied. Among them 5 cows in dry season, 10 cows in wet season were mastitis affected, and the overall prevalence of mastitis in dry and wet season was 3.70% and 6.06% respectively. In wet season prevalence of mastitis was higher than in dry season. The mastitis incidence was 8.75% during 3^{rd}-4^{th} Pairity which was higher than 1^{st}-2^{nd} and $\geq 5^{th}$ pairity where the prevalence of mastitis were 3.33% and 3.84% respectively. Among 15 infected cows, the prevalence of mastitis in cow having peri-parturient disease was 86.67% and Cow without a history of peri-parturient disease was 13.33%. The prevalence of mastitits

was 26.67% at 1st-2nd month of lactation, 53.33% at 3rd-4th month of lactation and 20% at 5th -6th month of lactation. The occurrence of mastitis was 26.67% in cow in farm with brick-block floor, 20% in cow in farm with soil floor and 53.33% in cow when the floor of farm was wet and soiled. The occurrence of mastitis was significantly higher in farm where the floor was partly or completely wet and soiled. Among 15 mastitis infected cattle, (11) 73.33% infected cow reared in dirty farm and (04) 26.67 infected cows were reared in clean farm.

The overall prevalence of *E coli* was 73.33% and *Staphylococcus aureus* was 66.67% among the 15 sample which was identified by bacterial culture and biochemical test. Nine antibiotics were used against the isolated bacteria to know the susceptibility and resistance capacity. Amoxicillin shown 36.36% sensitivity, Ampicillin showed 36.36%,Tetracycline showed 54.54% sensitivity, Streptomycine and Cotrimethaxole/ trimethoprim showed 81.81% sensitivity, Gentamycin, Cefttriaxone showed 90.91% sensitivity, Cefuroxime and Cefixime shown 100% sensitivity agains *E coli*. It was shown that Cefuroxime and Cefixime was highly sensitive and Ceftriaxone and, Gentamycin shown 9.09% resistance to *E coli*. Amoxicilin showed higly resistance among these 9 antibiotics. Amoxicilin showed 45.45% resistance. Amoxicillin, Ampicillin and Tetracycline, showed 18.18%, 27.27% and 9.09% intermediate resistance respectively against *E.coli*. Cotrimethaxole/ Trimethoprim shown 60% sensitivity, Tetracycline shows 70% sensitivity, Amoxicillin, Ampicillin, Streptomycine showed 80% sensitivity, Gentamycin, Cefttriaxone shown 90% sensitivity, Cefuroxime and Cefixime showed 100% sensitivity to *S. aureus*. It was shown that Cefuroxime and Cefixime was highly sensitive and Ceftriaxone showed 10% resistance Cotrimethaxole shown higly resistance among these 9 antibiotics. Cotrimethaxole/ trimethoprim showed 30% resistance. Amoxicillin, Tetracycline, Streptomycine and Cotrimethaxole/trimethoprim showed 10% intermediate resistance against *S. aureus*. The results of the current study indicated that antimicrobial resistance is increased which reduce recovery of the diasese, increase treatment cost and have great impact in dairy farm. As mastitis is one of the most economically devastating diseases in dairy cattle worldwide. It is also considered as one of the most important diseases that affect the welfare of the animal on the farm. Mastitis is

recognized worldwide as one of the most costly diseases affecting dairy industry. Many dairymen do not recognize fully tremendous losses sustained through unrealized milk production. The study showed that higher occurrence of clinical mastitis in large farm, cross breed cattle, higher aged, higher parity, unhygienic dirty farm, weak and wet soiled floor condition, lower parity and lower managent in wet season than dry season. Affected cows suffered from general ill health and poor reproductive performance. Abuse of antibiotics to treat this disease results antibiotic resistance develop and duration of illness is extended. Indiscriminate use of antimicrobial agents should be avoided in order to eliminate health hazards in man and animals caused by *E. coli* & *S. aureus* through preventing the development of multi-drug resistant mutants in nature. A well documented continued research and educational effort is required to increase producer awareness of mastitis to the dairy enterprise. Awareness on Antibiotic Resistance to Mastitis should also need to establish to avoid haphazard use of Antibiotics. Control of this costly disease must be based on a continuing program of elimination and prevention of infection. To improve the general health, welfare and productivity of dairy cows, many therapeutic and prevention strategies should be practiced.

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APPENDIX

APPENDIX-1: Composition of different media

1. Nutrient broth

Peptic digest of animal tissue 5.0 gm

Sodium chloride 5.0 gm

Beef extract 1.5 gm

Yeast extract 1.5 gm

Distilled water 1000 ml

Final pH (at 25ºC) 7.4 ± 0.2

2. Nutrient Agar

Peptone 5.000 gm

Sodium chloride 5.000 gm

HM peptone B# 1.500 gm

Yeast extract 1.500 gm

Agar 15.000 gm

Final pH (at 25°C) 7.4±0.2

3. MacConkey Agar

Peptones (meat and casein) 3.000 gm

Pancreatic digest of gelatin 17.000 gm

Lactose monohydrate 10.000 gm

Bile salts 1.500 gm

Sodium chloride 5.000 gm

Crystal violet 0.001 gm

Neutral red 0.030 gm

Agar 13.500 gm

pH after sterilization(at 25°C) 7.1±0.2

4. Eosin Methylene Blue Agar

Peptic digest of animal tissue 10.000 gm

Dipotassium phosphate 2.000 gm

Lactose 5.000 gm

Sucrose 5.000 gm Eosin -

Y 0.400 gm Methylene

blue 0.065 gm Agar

13.500 gm

Final pH (at 25°C) 7.2±0.2

5. Blood agar

Peptone 10.00gm

Tryptose 10.00

Sodium Chloride 5.00gmAgar 15.00gm

Final Ph7.3±0.2

Disttilled water 1000ml

6. Mannitol Salt Agar

Protease peptone 10.00gm

D-Mannitol 10.00gm

Beef Exttract 1.0gm

Sodium Chloride 75.00gm

Phenol-red 0.025gm

Agar 15.00gm

Disttilled water 1000ml

7. Mueller Hinton Agar

HM infusion B from 300.000

Acicase 17.500

Starch 1.500

Agar 17.000

Final pH (at 25°C) 7.4±0.1

8. Methyl Red Indicator

Methyl red 0.200 gm

Ethyl alcohol 60.000 ml

Distilled water 40.000 ml

10. Phosphate buffer saline

Sodium chloride 8.0 gm

Disodium hydrogen phosphate 2.8 gm

Potassium chloride 0.2 gm

Potassium hydrogen phosphate 0.2 gm

Distilled water to make 1000 ml