ISOLATION, IDENTIFICATION AND ANTIBIOGRAM STUDY OF BACTERIA FROM DIARRHOEIC GOAT IN SPECIFIC AREA OF SYLHET

A Thesis
By
Dr. SULTANA JAHAN

MASTER OF SCIENCE (MS)
IN
MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
FACULTY OF VETERINARY AND ANIMAL SCIENCE
SYLHET AGRICULTURAL UNIVERSITY
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DECEMBER, 2014

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Professor Dr. A. T. M. Mahbub-E-Elahi **Supervisor** **Department of Microbiology and Immunology** **Faculty of Veterinary and Animal Science** **Sylhet Agricultural University** **Sylhet**	**Professor Dr. Md. Abu Bakr Siddique** **Co-Supervisor** **Chairman** **Department of Microbiology and Immunology** **Faculty of Veterinary and Animal Science** **Sylhet Agricultural University** **Sylhet**

Professor Dr. Md. Abu Bakr Siddique
Chairman
Defence Committee
&
Chairman, Department of Microbiology and Immunology
Faculty of Veterinary and Animal Science
Sylhet Agricultural University
Sylhet

DECEMBER, 2014

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The Author
November, 2014

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LIST OF ABBREVIATIONS AND SYMBOLS

Appox.	=	Approximately

LIST OF ABBREVIATIONS AND SYMBOLS

A	=	Acid
AG	=	Acid and gas
BA	=	Blood agar
BGA	=	Brilliant green agar
PBS	=	Phosphate buffer saline
PM	=	Post-mortem
S.	=	Salmonella
Sl.	=	Serial
SS	=	Salmonella-Shigella
TSI	=	Triple sugar iron
µl	=	Micro liter
VRB	=	Violet red bile agar
VP	=	Voges proskaur
%	=	Percentage
*****	=	Most important
+	=	Growth
-	=	No growth
(-ve)	=	Negative
(+ve)	=	Positive
PBS	=	Phosphate buffer saline
S.	=	Salmonella
Sl.	=	Serial
spp	=	Species
SS	=	Salmonella-Shigella
TSI	=	Triple sugar iron
µl	=	Micro liter
VRB	=	Violet red bile agar
VP	=	Voges proskaur
%	=	Percentage
*****	=	Most important
+	=	Growth
-	=	No growth
(-ve)	=	Negative

ABSTRACT

The research work was conducted with 100 faecal samples collected directly from the rectum of diarrhoeic goats during the period of January/2014 to June/2014 of Sylhet govt. goat farm, sylhet, Govt.veterinary hospital, Mirjajangal Sylhet, Govt.veterinary hospital, jaintapur,Sylhet , Kajir bazar, Sylhet, Shah poran region, Sylhet *to find out the causal agents of goat diarrhoea and antibiotic sensitivity against the goat diarrhoea. Isolation and identification of bacteria were confirmed on the basis of their morphology, staining, cultural and biochemical properties. Antibiotic sensitivity test was studied by disc diffusion method of Kirby-Bauer technique. Out of 100 faecal samples 76 samples (76%) were found positive for Escherichia coli, 80 samples (80%) for Salmonella spp. and 41 samples (41%) for Staphylococcus spp. Sensitivity test was conducted by 11 commonly used antibiotics with the test faecal samples. The results showed that out of 11 antibiotics, 5 antibiotics (Penicillin-G, Erythromycin, Amoxycillin, Bacitracin and Cefalexin) were resistant as these did not show any inhibition zone. Remaining 6 antibiotics were sensitive in which Ceftriaxone showed highest inhibitory zone about 34.8 mm in average of 5 samples. Cotrimoxazole showed 2nd highest inhibitory zone about 34 mm in average of 5 samples. Gentamycin showed 3rd highest inhibitory zone about 29.8 mm in average of 5 samples. The findings of the experiment speculate that the use of Ceftriaxone, Cotrimoxazole and Gentamycin might have the preference in clinical control of goat diarrhoea.This test provided the guideline for the veterinarian to select appropriate antibiotics to reduce the economic loss through selecting the sensitive antibiotics.*

CHAPTER 1

INTRODUCTION

Diarrhoeal disease seems to be one of the major community health hazards both for men and animals in most countries of the world. It is resulted from the enteritis, which is inflammation of intestinal mucosa, characterized by abdominal pain, loose faeces, increase in stool mass, stool frequency, vomiting tendency, or stool fluidity (dehydration) that contain 70-95% water. More than 14 L of fluid may be lost per day in severe cases of diarrhoea. The chronic form of diarrhoea may last for days or week and may culminate in death .

Diarrhoea is defined as an increased frequency, fluidity, or volume of fecal excretion. The feces may contain blood or mucous and be smelly. The color of the feces may be abnormal. However, it is not possible to definitively determine the infectious organism by looking at the color, consistency, or odor of the feces. A definitive identification requires a sample for microbiological analysis. In livestock, diarrhoea is called scours. There are many causes of diarrhoea: bacterial, viral, parasites, and diet. Before treating a goat for diarrhoea, it is essential to determine why the animal is scouring.

Diarrhoea causes serious losses with high morbidity and mortality in goats owing to acute loss of fluid, essential minerals and nutrients. The agents responsible for most goat diarrhoea are mainly bacteria, viruses, fungi, protozoa, helminthes, chemical agents, nutritional factors (deficiency) and also conditions like indigestion and faulty management, hepatic cirrhosis and other toxic factors.

Among all other above mentioned causal agents, bacteria are considered to play a significant role in the frequent development of goat diarrhoea. Different bacterial species were isolated from diarrhoeic goats, mainly enterotoxigenic *Escherichia coli, Salmonella spp, Staphylococcus sp.*, and *Bacillius spp.* Incomplete course of treatment and continuous indiscriminate uses of antibacterial drugs against diarrhoeal infection of man and animal might have influenced to produce a new generation of virulent and resistant type of bacteria. Although routine laboratory isolation and drug sensitivity testing are expensive and somewhat impractical, the periodical check on the pattern of the drug sensitivity of organisms is more significant. It is, therefore, important that sensitivity of different bacteria

isolated from diarrhoeic goats needs to be studied from time to time in order to formulate appropriate therapeutic measures.

E. coli scours is an opportunistic disease associated with sloppy environmental conditions and poor sanitation. It is seen in lambs and kids less than 10 days of age, but is most common at 1 to 4 days of age. It usually presents itself as an outbreak in lambs and kids between 12 and 48 hours of age.

The bacteria *Salmonella* has thousands of serotypes and all can potentially cause diarrhoea in animals. *Salmonella* can cause diarrhoea in lambs and kids of any age. Animals less than 1 week of age are more likely to die without clinical signs, whereas animals older than 1 week are more likely to have diarrhoea.

Caprine diarrhoea occurs world wide in goats of any age. In Bangladesh, Diarrhoeal disease remains the most often reported clinical problem in goats. Goat diarrhoea is responsible for poor growth in kids and a significant loss of production both through morbidity and mortality. Some enteropathogens like bacteria, viruses, protozoa and helminthes have been recognized to be associated with diarrhoea. This disease has been reported from UK, USA, Canada, Argentina, France, Australia, New Zealand, Papua New Guinea, South Africa, Switzerland, Iran, India, Algeria, Pakistan, China and Japan. Reports on diarrhoea associated with enteropathogens are very much limited from Bangladesh. Here, it may be mentioned that, research work on diarrhoea in goat is low earlier in Bangladesh. Therefore, an attempt was made to isolate and identify the enterobacteria from goat diarrhoea.

Since 1992, goat rearing has become seriously impaired due to high mortality with diarrhoea like symptoms. The marginal and land-less farmers most easily live on rearing of goats in Bangladesh. So, goat is called the poor man's cow that is the second important livestock in Bangladesh which plays an important role in the rural economy and we can earn substantial amount of foreign currency by exporting skin and other by-products. The estimated goat population in Bangladesh in 1997-98 was 33.50 millions, which was 33.331 millions in 1996-97 and growth rate of goat is about 8.2% yearly whereas cattle growth rate is about 0.80%. The numbers of goat farm at the private sector were 5584 in 1997-98 . So, above information indicates the goat population in our country is increasing day by day.

At present, Asian and other under developing countries, agriculture means crop and cereal production and relatively less importance is given to livestock health and production. As a result, deficiencies of proteins become an unsolved problem. Therefore, enhancement on small ruminants production especially goat in the framework of small holder agriculture assume the great significance. So, present study will be helpful toward goat rearing and enhance dynamism of goat farming which not only alleviate the poverty but also boost up the national economy. Considering all above-mentioned points, the present work was designed with the following objectives:

- Isolation and identification of the bacteria from the diarrhoeic goats.
- To find out the bacterial causal agents responsible for goat diarrhoea.
- To study the antibiogram profile of the isolated bacteria.

CHAPTER 2

REVIEW OF LITERATURE

The purpose of this chapter is to provide a selective review of the past researches in relation to the present study. The numbers of works directly related to the present study were scanty. Literatures on various aspects of bacteria diarrhoea in goats are voluminous. So in this section, only major references having direct relationship with the present work are reviewed with the involvement of the following species:

2.1. *Escherichia coli*

Smith SW (1976) identified important characteristics of *E. coli* strains that cause disease in domestic mammals are determined by transmissible plasmids, these include enterotoxin, haemolysin and K88 antigen in piglet enteropathogenic strains and eterotoxin and K99 antigen production in goat and lamb enteropathogenic strains; most strains that cause generalized infections in young domestic mammals and the results showed that the K88 antigen, probably on account of its adhesive properties, permitted pig enteropathogenic strains of *E. coli* to proliferate in the small intestine of piglets; the K99 antigen performed a similar function in goat and lamb enteropathogenic strains.

Janke BH *et al.,* (1989) observed attaching and effacing *Escherichia coli* (AEEC) adhere to mucosal epithelium in both small and large intestine and induce a distinctive lesion characterized by an irregular scalloped appearance of the epithelial layer. Infection with attaching and effacing *E. coli* was detected in 14 goats, 7 pigs, 2 lambs and 3 dogs in farms and kennels in South Dakota, Minnesota, Iowa, Nebraska and Wisconsin.

Ugochukwu EI *et al.,* (1998) investigated *Enterobacteriaceae* from faecal swabs of West African dwarf goats suffering from diarrhoea in the Nsukka area of Anambra State, Nigeria using a total of 37 enteric bacteria were successfully recovered aseptically from 35 faecal swabs from caprine species and the predominant enterobacteria pathogens isolated from

diarrhoea goats and their relative percentage frequently of isolation were : 27 Escherichia (73%), 7 Proteus (19%) and 3 Shigella (8%). Of the most isolates 73% were *Escherichia coli,* 13% were *Proteus morgani,* 3% *Proteus vulgaris* and 8% *Shigella sonnei.*

Cid D *et al.,* (1996) performed in vitro activities of 14 anti-microbial agents were determined against 92 strains of *E. coli* isolated from lambs (60 strains) and kids (32 strains) affected by neonatal diarrhoea and overall percentage of resistant strains to streptomycin, sulphadimethoxine and tetracycline was very high (above 70%). The *E. coli* strains were highly susceptible to cephalosporins, polymyxin and quinolones.

Munoz *et al.,* (1996) examined faeces samples from diarrhoea and non-diarrhoea lambs and goat kids aged 1-45 days for enteric pathogens. F_{5+} (K_{99+}) and/or F41+ *Escherihia coli* strains were isolated from 26% and 22% of the diarrhoea lambs and goat kids, respectively, although these which did not produce enterotoxins ST I or LT I, were found with similar frequencies in non-diarrhoea animals. Verotoxigenic *E. coli* was isolated from both diarrhoea and non-diarrhoea lambs (4-1% and 8 & 2%, repsectively) and there was no assoication between infection and diarrhoea.

Orden *et al.,* (2003) studied on the faecal samples from 146 diarrhoea lambs and goat kids, and from 511 healthy sheep and goats were screened for the presence of Vero cytotoxin-producing *Escherichia coli* (VTEC). In healthy sheep and goats, VTEC were isolated in 24.4 and 16.2% of the animals, respectively. Moreover, VTEC were detected in 3.1 and 5.9% of the diarrhoea lambs and goat kids, respectively. These data suggest that VTEC seems not to be associated with dirrahoea in lambs and goat kids. Only four VTEC strains were eae-positive. The absence of the eae gene in most of these VTEC strains could indicate that these strains are less virulent for humans that the classical eae-positive enterohaemorrhagic *E. coli* types. However, almost half (42.9%) and 12.2% of VTEC strains isolated from healthy

sheep and goats, respectively, belonged to serotypes associated with severe diseases in humans.

Steven Pao *et al.,* (2005) detected *Salmonella* strains and *Escherichia coli* 0157:H7 in 17 and 5 small ruminants in Virginia, respectively, of 287 tested. Background microflora interfered with the fecal analysis. The combination of *Salmonella* enzyme immunoassay (EIA) detection and xylose-lysine-deoxycholate agar isolation was satisfactory. Modifying enrichment to a 1:100 dilution enabled effective *E. coli* 0157:H7 detection by EIA and isolation by sorbitol-MacConkey agar with cefixime-tellurite.

Al-Majali AM *et al.,* (2007) studied on the distribution of the enterotoxigenic *E. coli* STa-specific receptors on enterocytes and brush border membrane vesicles (BBMVs) prepared from the anterior jejunum, posterior jejunum, ileum and colon of newborn goat kids were investigated. The density of STa-receptors on enterocytes and BBMVs was higher in the posterior jejunum than that in other segments of the kid's intestines and finally findings suggested that the posterior jejunum is a major target for STa within the intestinal tract of newborn goat kids.

La Ragione *et al.,* (2009) investigated that cattle and small domestic ruminants (sheep and goats) have emerged as important sources of *E. coli* 0157: H7 human infection and found similarities to the bovine host, the pathobiology of *E. coli* 0157:H7 in small domestic ruminants does appear to differ significantly from that described in cattle. This review aims to critically review the current knowledge regarding colonization and persistence of *E. coli* 0157:H7 in small domestic ruminants, including comparisons with the bovine host where appropriate.

Abdulaziz *et al.,* (2012) studied on a total of 153 animals at Taif governorate were included 55 diseased sheep, 22 dead lambs, 42 diseased goats and 34 dead kids and the

bacteriological examination revealed that *E. coli* was isolated from diarrhoeic sheep in percentage of 36.36%. Regarding to dead lambs *E. coli* was isolated from lung, liver, spleen and intestine in a percentage of 12 (54.55%), 15 (68.18%), 16 (72.73%), 18 (81.82%), respectively. *E. coli* was isolated from diarrhoeic goats in percentage of 35.71%. Regarding to dead kids *E. coli* was isolated from lung, liver, spleen and intestine in a percentage of 15 (44.12%), 20 (58.82%), 21 (61.76%) respectively.

2.2. *Salmonella spp*

Stableforth and Galloway (1959) described an epidemic pattern of *Salmonella typhimurium* among goats and concluded that the infection was spread from area to area by carnivorous birds which remain infective for 27 days after feeding on contaminated material.

Khan (1961) isolated *Salmonella salford* from sheep and goat and carried out pathogenicity tests with the isolated organisms on rabbits, guinea-pigs and mice. The inocula were prepared from 24 hours old broth cultures which had viable count of 13,330,000 organisms per milliliter. Two rabbits, 29 guinea-pigs and 10 mice were inoculated each with 0.5 ml of the culture subsequently. Faces of all the surviving animals were tested by culturing fecal sample in SS agar, BGA agar and MC agar twice a week for the presence of *Salmonella* and all of the surviving animals were killed 8 weeks after the inoculation. Detail postmortem examinations were carried out and culture were made from liver, bile, intestinal contents and mesenteric lymph nodes of the experimental animals. Rabbits and guinea-pigs showed no symptoms or lesions on postmortem examinations. Nothing was recovered from the live or dead laboratory animals. However, in case mice, six and three died within 24 and 48 hours of inoculations respectively and one mouse died 7 days after inoculation. All those found

dead within 24 and 48 hours of inoculation showed septicemic changes. The same organisms were isolated from heart blood, liver, bile and intestinal contents of the mice.

Kumar *et al.*,(1973) reported the isolation of 11 types of *Salmonella* as serovars from 60 goats and sheep from different outbreaks in India. The most frequently incriminated types were *Salmonella dublin, Salmonella typhimurium, Salmonella choleraesuis* and *Salmonella enteritidis*.

Buxton and Fraser (1977) stated that the *Salmonellae* did not ferment lactose, sucrose or salicin but did ferment glucose, maltose, mannitol, dulcitol and dextrin with production of acid and gas. These were also characterized by their ability to reduce nitrates tonitrites and to produces H_2S. The organisms could not decompose urea, liquefy gelatin or produce indole.

Freeeman (1985) stated that Gram's stain of a pure culture of *Salmonella* revealed the rod shape, short chain forming organism.

Ghossh *et al.*,(1987) has reported the *Salmonella Virchow* infection in goat farm in North-easter Couture et al, (1987) isolated 444 Salmonella isolates from animals during 1979-1986 of which 233 were *Salmonella typhimurium* (mainly from cattle), 138 *Salmonella cholerasuis,* 12 *Salmonella anatium* and 11 *Salmonella enteritidis*. Fifteen other serotypes were identified. Sensitivity was generally high to trimethoprim- sulfamethoxazole and to nitrofurazone. Only 27% of isolates were sensitive to ampicillin.

Tanabe *et al.,* (1988) observed numerous outbreaks of Salmonellosis caused by multi drug resistant *Salmonella typhimurium* occurred in feedlot goats in Tokachi district, Japan during 1979-1982. Salmonella strains isolated in 11 years from 1975 to 1985 in this area were examined for analysis of the epidemic. Of the total 574 strains, 569 (99.1%) were resistant to

one or more antibiotics (ampicillin, streptomycin, sulphamethoxine and tetracycline with or without kenamycin) and resistant to 4 or 5 antibacterial agents.

Adesiyun *et al*., (1988) carried out a study on 200 goats, where 19 (9.5% were positive for Salmonella. Salmonella were isolated from bile (5%), from intestinal contents (2.5%) and from mesenteric lymph nodes (3.5%) . Serotypes isolated from goats were *Salmonella poona, Salmonella kintambo, Salmonella sanktgeorgvar I, Salmonella staneleville* and *Salmonella hull.*

Siddique *et al.,* (1989) carried out an antibiotic sensitivity study on 111 strains of Salmonella isolated in Pakistan during 1982 and 1983 from which they found that 78% of them were sensitivity to ampicillin, gentamycin, kenamycin and neomycin while 83% of them were resistant to tetracycline, tylosin, trimethoprim-sulphametoxazole and streptomycin in area of India for the first time causing disease in goats in India.

Diaz *et al.,*(1989) isloated six *Salmonella* strains from the ileum of 300 goats sent for slaughter from four states in Mexico during January-July 1986. Among those isolates. 2 were *Salmonella anatum,* 2 were *Salmonella montevideo,* 2 were *Salmonella Newport* and 1 was *Salmonella derby.*

Janke BH *et al.,* (1989) observed attaching and effacing *Escherichia coli* (AEEC) adhere to mucosal epithelium in both small and large intestine and induce a distinctive lesion characterized by an irregular scalloped appearance of the epithelial layer. Infection with attaching and effacing *E. coli* was detected in 14 goats, 7 pigs, 2 lambs and 3 dogs in farms and kennels in South Dakota, Minnesota, Iowa, Nebraska and Wisconsin.

Das *et al.,* (1991) conducted a study comprising 200 goat meat samples. They isloated 3 serotypes of *Salmonella.* The commonest serotypes were *Salmonella newport* (24%) followed by *Salmonella anatum* (12%) and *Salmonella typhimurium* (30%).

Mallison *et al.,* (1991) studied on a new selective medium for isolating *Salmonella* using pure culture. They found that XLT4 medium significantly improved isolation rate of *Salmonella* from chickens (swab samples) over the other selective medias evaluated.

Ruiz *et al.,* (1992) inoculated a total of 4,284 H_2S-positive colonies isolated on Salmonella-Shigella agar and 4,350 isolates on Hektoen agar. Out of them all of the 794 colonies isolated on Salmonella-Shigella agar and 752 isolated on Hektoen agar were identified as *Salmonella spp.* with conventional biochemical tests and were found positive with the MUCAP test.

Ekkehard and Velaudapillai (1995) carried out an investigation for the isolation of *Salmonella* form faces of healthy animals from the Colombo slaughter house. Nine types of *Salmonellae* identified. Those were as *Salmonella paratyhi, Salmonella saintpaul, Salmonella typhimurium, Salmonella virchow, Salmonella newport, Salmonella dublin, Salmonella gallinarum, Salmonella poona*and *Salmonella urbana*. Isolation of *Salmonella* was possible in 1 of the 61 cattle, 1 of the 33 dogs, 1 of the 62 chickens, 3 of the 48 goats. 7 of the another set 58 goats and 1 of the 60 pigs.

Visser *et al*., (1995) described a Salmonella dublin infection in young goats. In a period of two week time, 16 of 70 female kids died within one day, because of septicemial bad hygienic and management measures were thought to be the main reason for this outbreak.

Sharma and Katock (1996) reported an acute outbreak of *Salmonella typhimurinm.* On SS agar culture media the isolates grows as whitish or slight grayish colonies with dark central spot reflecting production of H_2S gas.

Ekperigin and Nagaraja (1998) described that all food animals are susceptible to infection with *Salmonella* a genus of Gram negative, non spore forming, usually motile, facultative anaerobic bacilli belonging to the family Enterobacteriaceae. *Salmonellae* are differentiated into over 2200 serologically distinct types (serotypes) based on differences in somatic, flagellar and capsular antigens. Infection with *Salmonella* may or may not lead to a sometimes fatal *Salmonellosis,* a disease that can remain localized in the gastrointestinal tract as gastroenteritis of become generalized as a septicemia and affect several organ systems. Infected food animals that do not develop *Salmonellosis* and those that recover from the disease, become carries of and serve as sources of infection to humans and other animals.

Zhang *et al.,* (1998) isolated 25 strains of Salmonella like organisms form liver, spleen and heart blood specimens collected from infected chickens from poultry farms in Shaanxi, China. In drug sensitivity tests 86% of the isolates were very sensitivity to kenamycin, gentamycin and amikacin.

Dhruba *et. al.,*(1999) isolated *Salmonella* organisms using five basic sugar fermentation reactions. Nearly all isolates were positive for mannitol, maltose and dextrose with the production of acid gas while lactose and sucrose were not fermented.

Abou *et al.,(2000)* reported sudden death after a short period of illness in 153 (62.83) of 376 newborn lambs and goat kids in 3 herds located in EI-Ta-EI Kabir, Ismailia Governorate, Egypt in October and November 1998. *Salmonella typhimurium, Salmonella dublin* and other *Salmonella spp.* were isolated from rectal swabs of diseased animals and

also from the heart blood, liver, gall bladder ad intestinal contents of dead animals. They also detected necrotoxigenic (NT) *E. coli* only in rectal swabs of diseased animals.

Gyurov and Dyakov (2000) used different drugs available in market for the determination of sensitivity against *Salmonella* isolates. They found that 64% of the isolates of Salmonella strain were highly sensitive to gentamycin and colistin and 53% were intermediately sensitive to flumequine and enrofloxacin.

Roy *et al.*, (2002) isolated a total of 569 Salmonella spp. out of 4745 samples from poultry products, poultry and poultry environment in 1999 and 2000 from the Pacific Northwest (USA). Among the isolates 91 *Salmonella spp.* were tested for antibiotic sensitivity. All of the tested isolates were resistant to erythromycin, lincomycin and penicillin except for one sample, *Salmonella* bert, which was moderate sensitive to penicillin. All of the tested Salmonella spp. were susceptible to sarafloxacin and ceftiofur. The percentage of Salmonella spp. susceptible to Sulphamethoxazole-trimethoprim, gentamycin, triple sulfa and tetracycline were 97.83%, 92.39%, 86.96% and 82.61% respectively.

Hossian (2002) isolated *Streptococcus aureus, Eshcerichia coli* and *Salmonella spp*. from diarrheic goats. The author stated that the *Salmonella spp.* produced small round and smooth colonies on Nutrient agar and opaque, translucent and colorless colonies on Salmonella-Shigella (SS) agar. They also produced colorless, pale, transparent colonies on MacConkey (MC) agar and small, round, low, convex, translucent, pale red color colonies on Brilliant Green Agar (BGA) against pinkish background which was initially green in color.

Gene (2002) reported the rod and short to long chain forming Salmonella organisms, which were isolated and identified from liver, spleen, intestinal contents of animals and birds.

Sung *et al.,* (2002) performed an antibiogram study of 72 Salmonella organism isolated from poultry in South Korea, against 13 antimicrobial drugs available in the market. About 57% of the isolates were resistant to nalidixic and (NA). 38.9% to amipicllin (AMP). 34.7% to streptomycin (SM), 27.8% each to carbanicillin (CB) and tetracyline (TE) and 18.1% to kenamycin (KE). There were less than 10% of the strains that were resistant to Sulphamethoxazole-trimethoprim (ST) and cefalotin (CF). The most frequent multiple resistant pattern was against AMP, CB, KF, SM, TE and NA, accounting for (26.4%).

Banani *et al.,* (2003) isolated 111 *Salmonella* samples from commercial chickens having yolk sac infection. The isolates were serogrouped and serotyped using specific antisera. The Salmonella serotypes isolated from chickens were Salmonella enteritidis, *Salmonella typhimurium, Salmonella nigeria, Salmonella montevideo* and *Slamonella blegdam.* These organisms when tested for antibiotic sensitivity. 100% were found to be susceptible to amikacin, chloramphenicol, gentamycin, lincospectin, sulphamethoxazole-trimethoprim, cephalexin, nalidixic acid, tetracycline, amipicillin, furaltadoone and colistin. All of the tested organisms i.e. 100% of the Salmonella isolates were resistant to lincomycin, novobiocin, erythromycin, penicillin, bacitracin, tylosin and taimulin.

Khan (2004) tested 24 Salmonella isolates for antibiotic sensitivity against eight commonly used antibiotics belonging to different groups. All i.e. 100% of the isolates were highly sensitive to cifrofloxacin; moderately snesitive to chloramphenicol, nalidixic acid, and ampicillin; less sensitive to cephalexin, kenamycin and resistant to cloxacillin, erythromycin.

Rahman (2005) isolated 5 *Salmonella spp.* from chickens and tested for antibiotic sensitivity belonging to different groups. All (100%) of the isolates were highly sensitive to cifrofloxacin, sulphamethoxazole-trimethoprim, tetracycline, and chloramphenicol;

moderately sensitive to gentamycin and amipicllin; less sensitive to streptomycin and resistant to cephradine.

Haburn *et al.,* (2006) reported an epidemic of abortions in goat caused by *Salmonella abortusovis* which occurred in Dalmatia, south Croatia, in winter 2003-2004. Five goat flocks with the rate of abortion ranging from 22% to 38% during the last-third of gestation were examined.

Haburn *et. al.,* (2006) carried out the sensitivity testing of the isolates by disc diffusion method and determined the minimum inhibitory concentrations, resulted in a high sensitivity to almost all microbial agents used. Only two isolates were moderately sensitive to oxytetracyclin, whereas one isolate was resistant to streptomycin.

Mulvey *et al.,* (2006) identified multi-drug resistant Salmonella enterica serotype typhimurium phage type DT104, resistant to ampicillin, Chloramphenicol/florphenicol, streptomycin, sulphonamides and tetracycline that has dissminated worldwide. The resistance genes, according to them, reside on the 43-kb Salmonella genomic island 1 (SGI1), which is transferable. Drug-resistant variants of SGIl have been identified in numerous serotypes. Strains harboring SGIl may be more virulent and have a tendency to rapidly disseminate.

Molla *et al.,* (2006) isolated 22 Salmonella in apparently healthy slaughtered sheep and goats in central Ethiopia belonged to 9 different serovars. The common serovars isolated were *Salmonella typhimurium*, followed by Salmonella *nigeria*, Salmonella *blegdam* . Seven of the 22 isolates (31.8%) were found to be mutlidrug-resistant to various antibacterials.

Yadav *et al.,* (2006) investigated bacterial load and antibiogram in ready-to-sale sheep meat with special reference to Salmonella. Samples were collected from 100 sheep carcasses from

retail meat shops in domestic markets. All the samples (n=100) were found positive for coliforms 49.0% were positive for E. coli and 3.0% were positive for Salmonella. The isolates were serotyped as Salmonella infants having antigenic structure 6, 7: r: 1, 5. Antibiogram reveled highest (100%) sensitivity towards amikacin, cetriaxone, ciprofloxacin, chloramphenicol, colistinsulphate, gentamycin and nalidixic acid followed by cefuroxime and tetracycline (66.67% each) and Cotrimoxazole (33.33%). All the strains were resistant to amipicillin.

Kam (2007) isolated 134 isolates of *Salmonella typhi* and reported their susceptibility to six antibiotics such as ampicillin, trimethoprin-sulfamethaxole, tetracycline, chloramphenicol, nalidixic acid and ciprofloxacin. Of the 134 Salmonella typhi isolates, 29 (21.6%) were resistant to at least one and up to five of the antibiotics tested.

Kobayashi *et al.,* (2007) collected a total of 180 swab samples from buffaloes which were investigated for the presence of Salmonella. All the 19 isolates from rectal swabs were serotyped as Salmonella enteritidis and susceptible to all five conventional antibacterial agents (ampicillin, chloramphenicol, streptomycin, oxytetracycline and nalidixic acid) tested. In contrast, 15 Salmonellae that were isolated from foot pads included Salmonella enteritidis, Salmonella dublin and showed multiple drug resistance.

Haque *et al.,* (2007) conducted a study to isolate and determine the etiologic agents causing diarrhoea in goats. Fecal samples were cultured in basic medium to obtain pure cultures of separate genera and were identified based on morphology (in case of solid media) and color and gas productions (in case of liquid media) in different selective media. Biochemical tests (catalase, V-P, oxidase, coagulase, oxidation-fermentation and carbohydrate fermentation test) were performed to confirm the specific bacterial genera. Results revealed that out of 20 experimented fecal samples, *Salmonella spp.* (5.0%), *Staphylococcus spp.* (10%), *E. coli*

(25%), *Bacillus spp.* (85%) and *Clostridium spp.* (65%) were identified in single or mixed infection. Non-bacterial infection was observed in 5% of the isolates. It is suggested that the characterization of the different bacterial isolates will help in the prevention of diarrhoea in goat farms.

McOrist and Millert (2008) investigated an outbreak of acute diarrhoea and deaths in a group of 1,016 feral goats of varying ages, on a 400 hectare Gippsland pine plantation. Within 1 to 2 weeks of release many goats developed acute, severe diarrhoea, weakness and recumbance. 38% of all goats died in the first 2 months on the farm. Autopsy findings were characteristics of *Salmonellosis* in 13 (43%) of the 30 goats examined and *Salmonella spp.*

Cauteren *et al.*, (2009) reported in France, a nation wide out break of gastrointestinal illness due to *Salmonella muenster* occurring during March and April 2008. Twenty questionnaires. Four patients were admitted to hospital and no death was recorded. Among the 21 interviewed cases, 16 reported consumption of goat's cheese. The investigation incriminated goat's cheese as being the most likely source of the outbreak. *Salmonella muenster* was isolated from both cases.

2.3*Staphylococcus spp*

***Franc¸oisvandenesch et al.*, (1995)** examined and strains were identified as *S. caprae*i, they were gram-positive, catalase-positive, oxidase-negative cocci, and if the results of the biochemical tests were characteristics of *S. caprae*. Twenty-three isolates were identified as *S. caprae*by the API database, one isolate was identified as *S. intermedius* because of the production of b-ga- lactosidase, and the two isolates from Japan could not be identified.

Abera *et al.*, (2010) The results of antimicrobial susceptibility testing revealed that *S. aureus* was highly susceptible to chloramphenicol (100%) followed by gentamycin (91.7%),

kanamycin (88.9%) and streptomycin (86.1%). In contrast, isolates were highly resistant to penicillin (94.4%), trimethoprim-sulfamethoxazole (58.3%) and amoxicillin (36.1%).

Alian *et al*., (2012) studied on the prevalence rate of antimicrobial resistance of *S. aureus* isolated from goat cow, sheep and goat milk in Iran. He observed Multi resistance in 34.8% of *S. aureus* isolates. Resistance (resistance and intermediate resistance) to ampicillin was the most common finding (54.3%), followed by resistance to oxacillin (28.3%), tetracycline (26.1%), penicillin G (23.9%), erythromycin (23.9%), trimethoprim-sulfamethoxazole (17.4%) and cephalotin (2.2%). All isolates tested for antibiotic sensitivity were susceptible to methicillin, vancomycin, chloramphenicol and ciprofloxacin. Furthermore, impacts and dynamics of genetic antibiotic determinants should also be investigated using molecular methods.

Md. Ershaduzzaman et al., (2013) examined Hundred diarrhoeic kids faecal samples of which 64% and 98% kids are affected with parasites and bacteria respectively. Around 59.7% and 61.22% had single, 31.34% and 37.76% had dual and only 4.48% and 1.02% had triple concurrent infection respectively in parasite and bacteria associated diarrhoeic kids. The rate of *Bacillus* infection (87%), was found highest followed by *Escherichia coli* (37%), *Staphylococcus* (9%), and lowest of *Salmonella* (5%) infection. Although *Bacillus* and *E. coli* recorded first time at 10 days and but *Staphylococcus* at 30 days of age.

CHAPTER 3

MATERIALS AND METHODS

The present study was undertaken to isolate and identify bacteria from the faecal samples of diarrhoeic goats in selected areas of Sylhet. The whole work was performed in the laboratory of the Department of Microbiology and Immunology, Faculty of Veterinary and Animal science, Sylhet Agricultural University (SAU), Sylhet during the period of January-June, 2014. The study was performed systematically which is laid down below:

The detailed outline of materials and methods are given below:

3.1 Materials:

3.1.1 Media and reagents used for bacteriological study:

3.1.1.1 Solid media for culture:

The sample used for bacteriological analysis were Nutrient agar (NA), Salmonella-Shigella (S-S) agar, Violet Red Bile (VRB) agar, MacConkey agar (MCA), Blood agar (BA), Eosin-Methylene Blue (EMB) agar, and Brilliant Green (BGA) agar, Mannitol salt agar (MSA).

3.1.1.1.1 Preparation of solid media:

3.1.1.1.1.1 Nutrient agar:

Based on the direction of manufactor, 28 grams of nutrient agar powder was suspended in 1 liter of distilled water taken in a conical flask. It was then gently heated with gentle agitation and brought just to the boiling point to dissolve the medium completely. Sterilization by autoclaving for 15 minutes at 15 pounds pressure per square inch (121°C), the medium was cooled at 50°C, mixed properly and poured into sterile petridishes (10 ml in each petridish) and allowed to solidify. Then incubated at 37°C for overnight to check the sterility and used for cultural characterization or stored at 4°C in refrigerator for future use.

3.1.1.1.1.2Mannitol salt agar:

According to the direction of manufacturer, 111 grams of mannitol salt agar powder was suspended in 1 liter of distilled water taken in a conical flask. It was then heated to the boiling point to dissolve the medium completely. Sterilization by autoclaving for 15 minutes at 15 pounds pressure per square inch (121°C), the medium was cooled at 50°C, mixed

properly and poured into sterile petridishes (10 ml in each petridish) and allowed to solidify. Then incubated at 37°C for overnight to check the sterility and used for cultural characterization or stored at 4°C in refrigerator for future use.

3.1.1.1.1.3 Eosin methylene blue agar (EMB):

As per direction of manufacturer, 36 grams of EMB agar agar powder was suspended in 1 liter of distilled water taken in a conical flask. It was then heated to the boiling point to dissolve the medium completely. Sterilization by autoclaving for 15 minutes at 15 pounds pressure per square inch (121°C), the medium was cooled at 50°C, mixed properly and poured into sterile petridishes (10 ml in each petridish) and allowed to solidify. Then incubated at 37°C for overnight to check the sterility and used for cultural characterization or stored at 4°C in refrigerator for future use.

3.1.1.1.1.4 MacConkey agar:

According to the direction of manufacturer, 52 grams of MacConkey agar powder was suspended in 1 liter of distilled water taken in a conical flask. It was then heated to the boiling point to dissolve the medium completely. Sterilization by autoclaving for 15 minutes at 15 pounds pressure per square inch (121°C), the medium was cooled at 50°C, mixed properly and poured into sterile petridishes (10 ml in each petridish) and allowed to solidify. Then incubated at 37°C for overnight to check the sterility and used for cultural characterization or stored at 4°C in refrigerator for future use.

3.1.1.1.1.5 Violet red bile agar:

Based on the direction of manufacturer, 41.53 grams of violet red bile agar powder was suspended in 1 liter of distilled water taken in a conical flask. It was then gently heated to the boiling point to dissolve the medium completely. The medium was cooled at 40°C, mixed properly and poured into sterile petridishes (10 ml in each petridish) and allowed to solidify. Then incubated at 37°C for overnight to check the sterility and used for cultural characterization or stored at 4°C in refrigerator for future use.

3.1.1.1.1.6 Brilliant Green Agar:

About 58 gram of the powder was dissolved in 1L of purified water and mixed thoroughly. The solution was then heated with frequent agitation and boiled for one (1) minute to completely dissolve the powder. After that, it was sterilized by autoclaving at 121^0C for 15 minutes. The medium was distributed into different petridishes and allowed several minutes

for solidification. Petridishes were then allowed to keep into incubator in a inverted position at 37^0C for 24 hr for sterility test. After sterility test, agar plate was streaked with a small pinpoint colony selected from nutrient agar plate. The total streaking procedure was completed into UV lamp after proper sterilization of hand with hexisol hand rub (Buxton and Fraser, 1977).

3.1.1.1.1.7 Salmonella-Shigella (S-S) agar media:

This medium was also used as for the growth of the E. *coli* organisms. About 6gms of dehydrated Salmonella-Shigella agar (Himedia, India) was suspended in 100 ml of cold distilled water taken in a conical flask and heated upto boiling to dissolve the medium completely. After sterilization by autoclaving, the medium was poured in 10 ml quantities in sterile glass petridishes (medium sized) and in 15 ml quantities in sterile glass petridishes (large sized) to form a thick layer therein. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the petridishes partially removed. The sterility of the medium was judged by keeping the petridishes in the incubator at 37°C for overnight and then used for cultural characterization or stored at 4°C in refrigerator for future use (Carter, 1979).

3.1.1.1.1.8 Muller Hinton agar:

According to the direction of manufacturer, 38 grams of Muller Hinton agar powder was suspended in 1 liter of distilled water taken in a conical flask. It was then heated to the boiling point to dissolve the medium completely. Sterilization by autoclaving for 15 minutes at 15 pounds pressure per square inch (121°C), the medium was cooled at 50°C, mixed properly and poured into sterile petridishes (10 ml in each petridish) and allowed to solidify. Then incubated at 37°C for overnight to check the sterility and used for cultural characterization or stored at 4°C in refrigerator for future use.

3.1.1.1.1.9 Blood agar base:

As per direction of manufacturer, 40 grams of blood agar powder was suspended in 1 liter of distilled water taken in a conical flask. It was then heated to the boiling point to dissolve the medium completely. Sterilization by autoclaving for 15 minutes at 15 pounds pressure per square inch (121°C), the medium was cooled at 50°C. Finally 5% sterile defibrinated sheep blood was added to make blood agar.

3.1.1.2 Liquid media for culture:

The liquid media used for the study were Nutrient broth & MR-VP broth.

3.1.1.2.1 Preparation of liquid media:

3.1.1.2.1.1 Nutrient broth:

According to the direction of manufacture, 13 grams of nutrient broth powder was suspended in 1 liter of distilled water taken in a conical flask. The medium was heated slightly to dissolve completely. Then the medium was distributed in test tube. Sterilization by autoclaving for 15 minutes at 15 pounds pressure per square inch (121°C), the medium was cooled at 50°C.

3.1.1.2.1.2 MR-VP broth:

Based on direction of manufacturer, 17 grams of MR VP broth powder was suspended in 1 liter of distilled water taken in a conical flask. Then the conical flask was kept in a water bath to dissolve the medium completely. Then the medium was distributed in test tube at 10 ml volume. Sterilization by autoclaving for 15 minutes at 15 pounds pressure per square inch (121°C), the medium was cooled at 45- 50°C.

3.1.1.2.1.3 Motility Indole Urea (MIU) medium:

Eighteen grams powder of MIU agar base (HiMedia, India) was added to 950 ml of distilled water in a flask and heated until boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at 121'C maintaining a pressure of 15 pounds/sq. inch for 15 minutes. After autoclaving, the medium was put into water bath of 45°C to decrease its temperature. After this the medium in the test tubes were allowed for incubating at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

3.1.1.3 Chemical reagents:

The reagents used for the study were phosphate buffer saline (PBS), reagents for Gram`s staining (crystal violet, gram`s iodine, acetone alcohol, safranine), 3% hydrogen peroxide Methylene blue and other common laboratory chemical and reagent.

3.1.1.3.1 Chemical reagents preparation:

3.1.1.3.1.1 Methyl red solution:

The indicator Methyl red (MR) solution was prepared by dissolving 0.1 gram of Bacto Methyl red (Difco) in 300 ml of 95 percent alcohol and diluting this to 500 ml with the addition of 200 ml of distilled water.

3.1.1.3.1.2 Alpha-naphthol solution:

Alpha-naphthol solution was prepared by dissolving 5 grams of l-naphthol in 100 ml of 95 percent ethyl alcohol.

3.1.1.3.1.3 Potassium hydroxide (KOH) solution:

Potassium hydroxide (KOH) solution was prepared by adding 40 grams of Potassium hydroxide crystal in 100 ml of cold distilled water.

3.1.1.3.1.4 Normal saline solution:

It was prepared by adding 0.85 grams of crystalline Sodium chloride in 100 ml of cold distilled water in a sterilized flask and then sterilized in the autoclave machine for 15 minutes at 15 pounds pressure per square inch 121°C (Cheesbrough, 1985).

3.1.1.3.1.5 Physiological saline solution:

A 0.85 percent Physiological saline solution (PSS) was prepared by dissolving 8.5 grams of chemically pure Sodium chloride (NaCl) in 1000 ml of distilled water in a conical flask. The solution was then sterilized by autoclaving at 121°C maintaining a pressure of 15 pounds pressure per square inch for 15 minutes. After sterilization, the saline was cooled and then kept at refrigerator until use (Cheesbrough, 1985).

3.1.1.3.1.6 Phosphate buffered saline:

For preparation of Phosphate Buffered Saline (PBS) solution, 8 grams of Sodium chloride, 2.89 grams of Di-sodium phosphate, 0.2 grams of Potassium chloride, and 0.2 grams of Potassium hydrogen phosphate were suspended in 1000 ml of distilled water. The solution was heated to dissolve completely. The solution was then sterilized by autoclaving at 121°C maintaining a pressure of 15 pounds pressure per square inch for 15 minutes and stored at refrigerator until use. The pH of the solution was measured by a pH meter and maintained at 7.0-7.2 (Cheesrough, 1985).

3.1.1.4 Glass wares and other necessary appliances:

The different types of glassware`s and appliances used during the course of the experiment were: Test tubes (with or without Durham`s fermentation tubes and stopper), petridishes (small, medium and large), conical flask, beaker, pipette (1ml, 10 ml), hanging drop slides, glass road spreader, test tube stand, blender machine, water bath, incubator, refrigerator, freeze, sterilizing instruments, Platinum loop, hot air oven, electronic balance, compound microscope, slides, cover slips, Laminar air flow, autoclave machine, Distilled water plant, Digital camera, jar, sterilized cotton, immersion oil, beaker, hand gloves, spirit lamps, match lighter, bacteriological loop, glass spreader and forceps, scissors etc.

3.2 Methods:

3.2.1 Preparation of samples for bacteriological examination:

The preparations of different samples for bacteriological examination were performed as follows:

3.2.2 Study area:

Samples were collected from five different areas located in Sylhet region

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Sl. No.	Name of areas
01.	Sylhet govt. goat farm, Sylhet.
02.	Govt.veterinary hospital, Mirjajangal, Sylhet.
03.	Govt.veterinary hospital, jaintapur,Sylhet.
04.	Kazir bazar, Sylhet.
05.	Shah poran region, Sylhet.

3.2.3 Study sample:

The study sample was the faeces collected from rectum of diarrhoeic goats. Both male and female goats were considered.

3.2.4 Number of samples:

About 100 faecal samples from diarrhoeic goats were collected from the above mentioned areas. The samples were collected in four steps as for requirement during research work which is given below:

Table 1: Number of faecal samples of diarrhoeic goats from different areas in Sylhet

SL. No.	Faecal samples Collection from diarrhoeic goats.	Sylhet govt. goat farm, Sylhet.	Govt.veterinary hospital, Mirjajangal Sylhet.	Govt.veterinary hospital, jaintapur,Sylhet	Kazir bazar, Sylhet.	Shah poran region, Sylhet	Total
01.	First Step	05	05	05	05	05	**25**
02.	Second Step	05	05	05	05	05	**25**
03.	Third Step	05	05	05	05	05	**25**
04.	Fourth Step	05	05	05	05	05	**25**
05	**Total**	**20**	**20**	**20**	**20**	**20**	**100**

3.2.5 Collection and Transportation of samples

The faecal samples were collected from diarrhoeic goats directly from rectum by spatula and stored in polythene bags. Each sample was collected separately. Then the collected samples were taken in icebox. Then the samples were transported from the Farms to the laboratory of the department of Microbiology and Immunology, SAU, Sylhet to isolate and identify common bacteria.

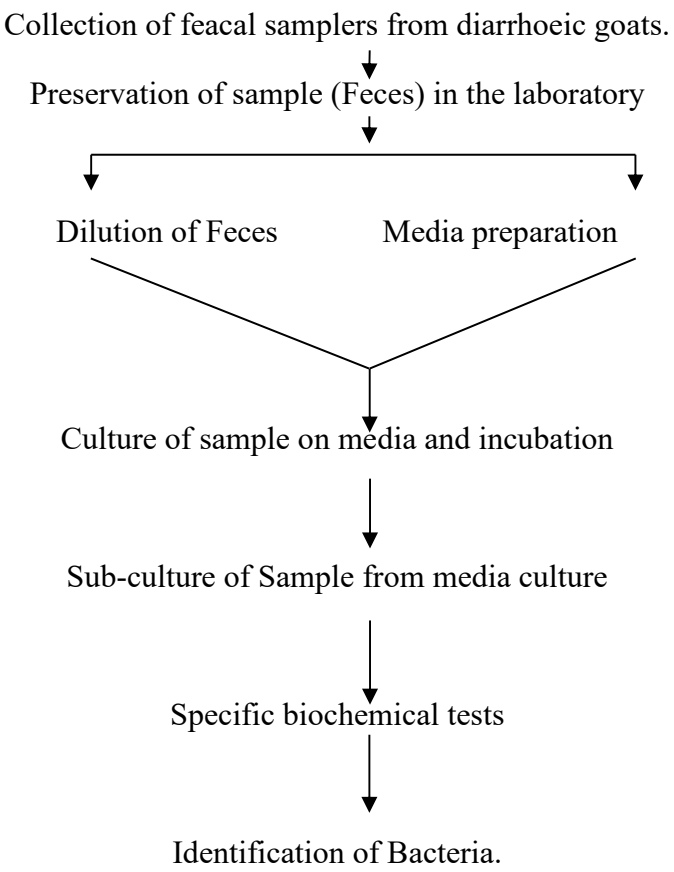
3.2.6 Processing of sample

Each sample was mixed aseptically with sterile distilled water at a ratio of 1:10. Then the sample was shaken properly to make a homogenous suspension. Later on 10 fold serial dilutions (1:10) were prepared ranging from 10^{-2}-10^{-9} according to the recommendation of International Standardization (ISO, 1995). Then the diluted samples were taken in nutrient broth and mixed well. Later on the nutrient broth containing samples were placed into incubator to incubate the sample for 24 hours.

3.2.7 Preservation of sample

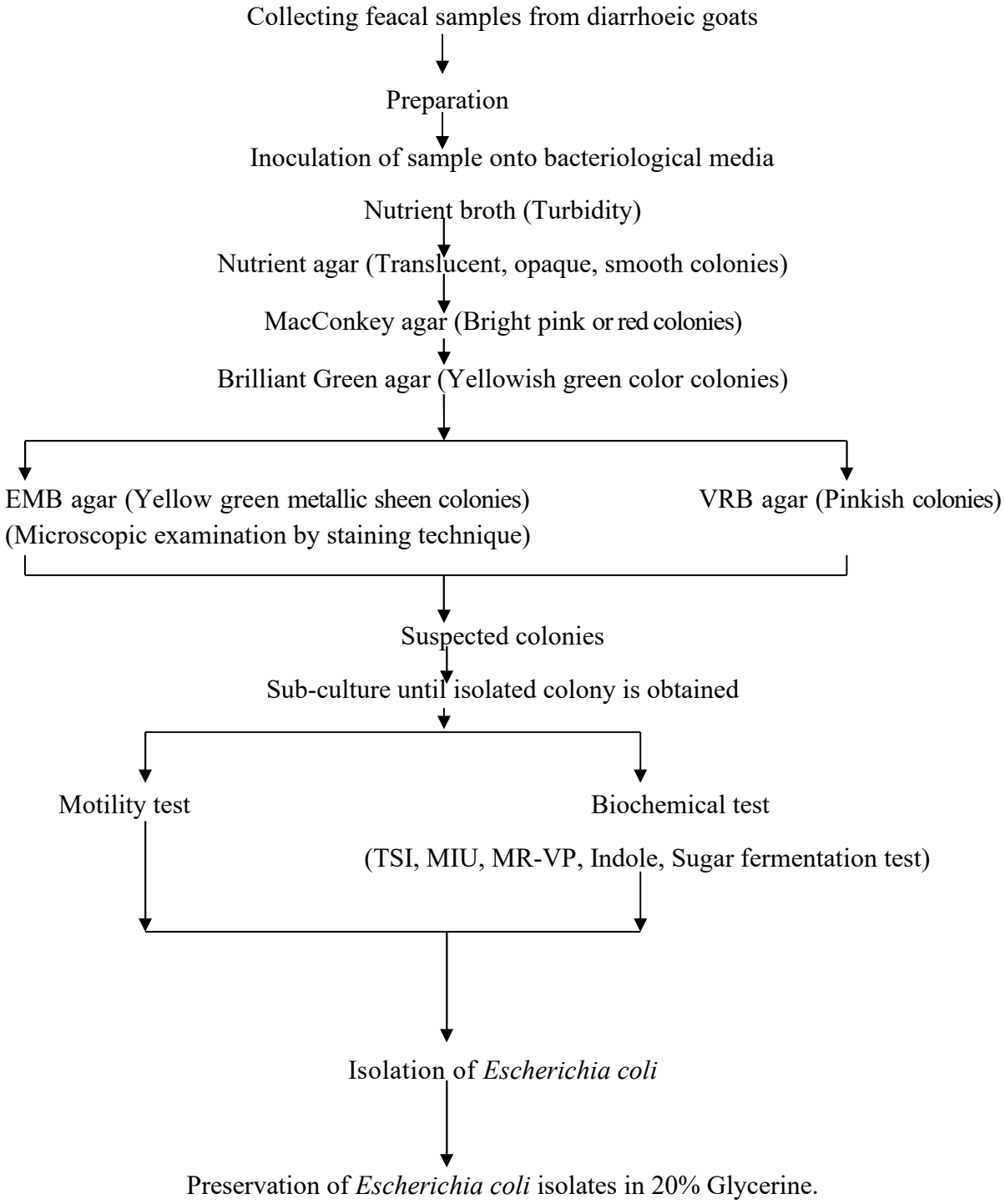
The samples in nutrient broth were placed into freeze to preserve the samples for certain period of time during research work.

3.2.8 Experimental Layout:

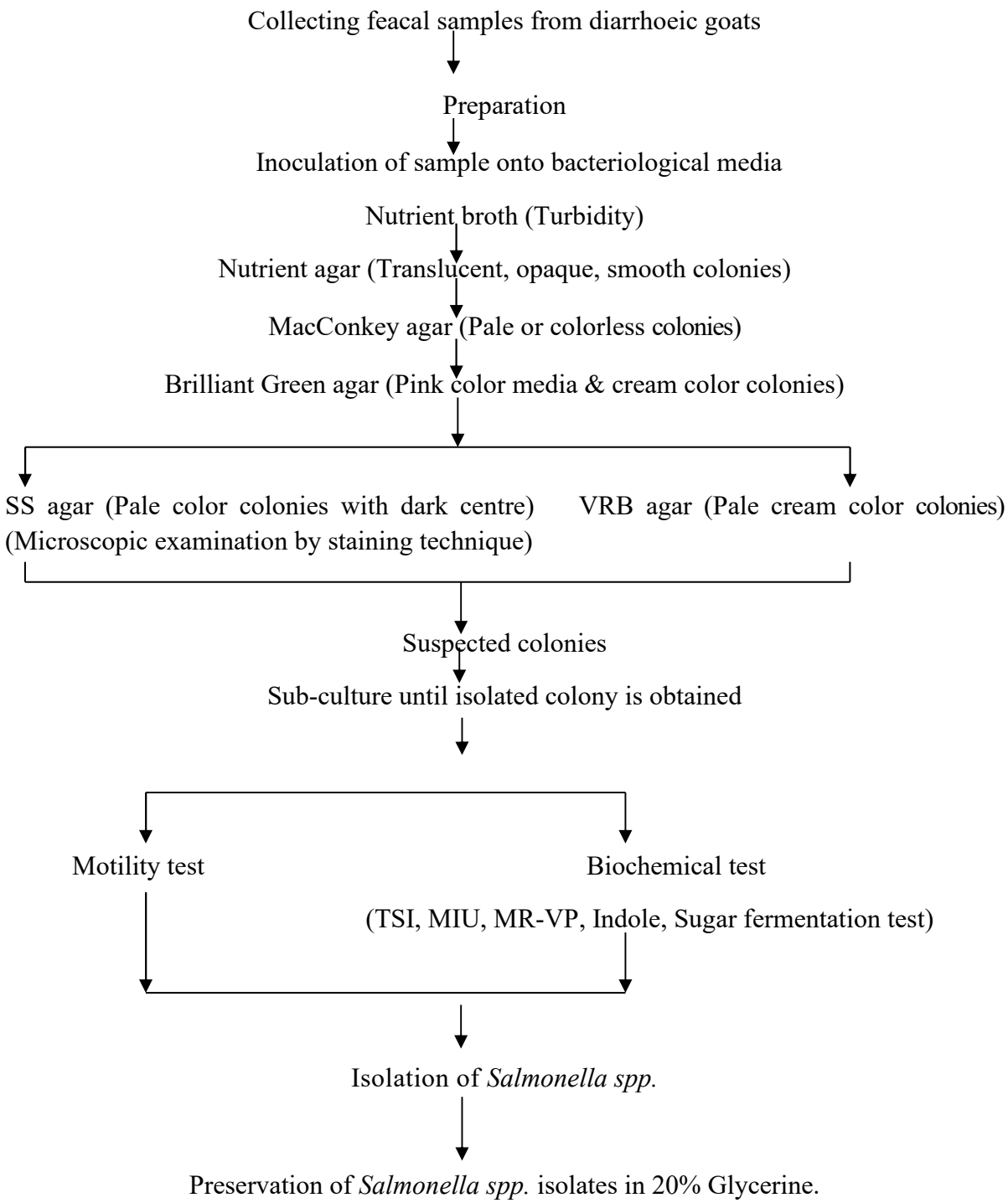


3.2.9 Experimental design:

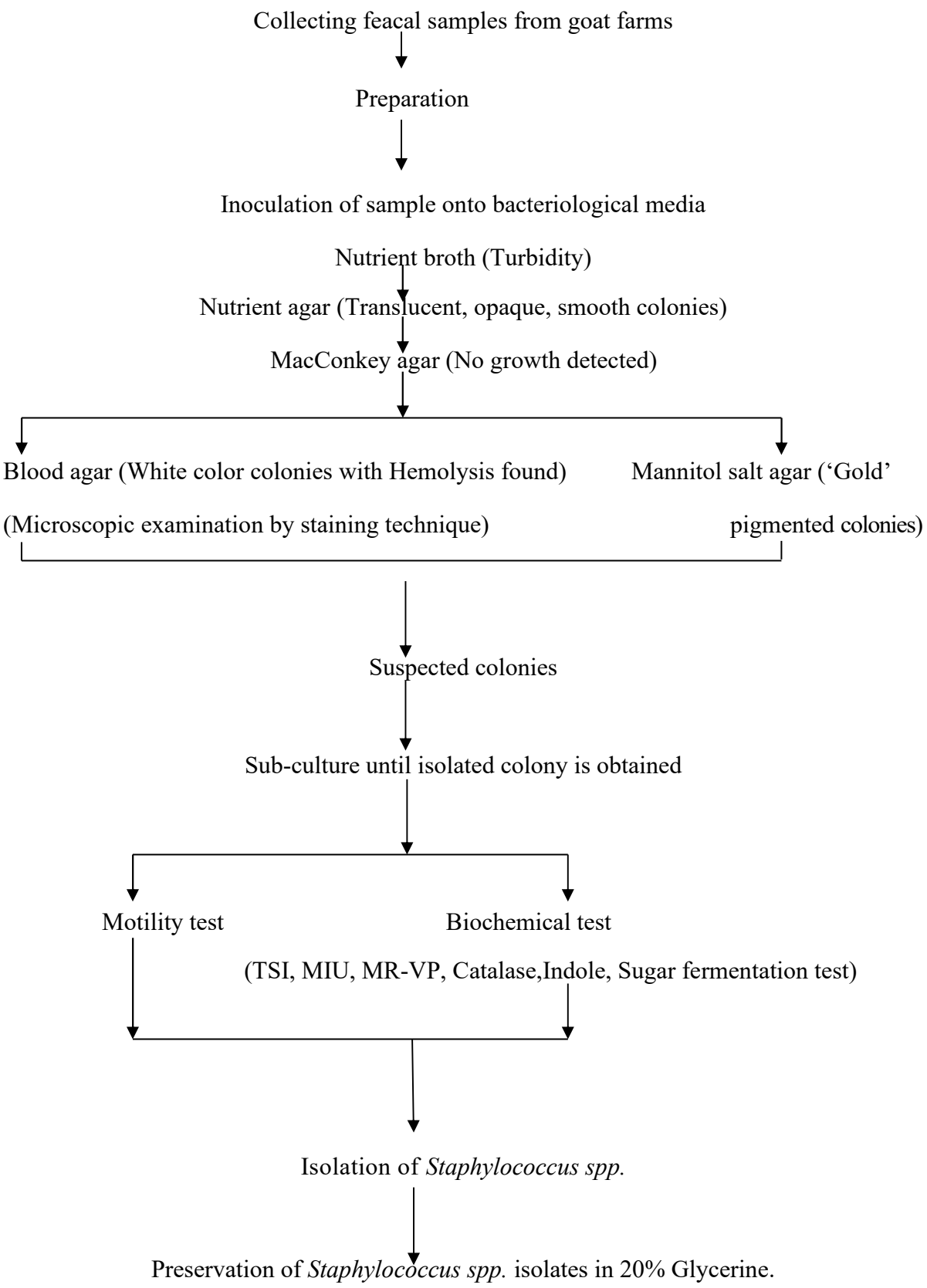
Isolation and identification of *Escherichia coli* from diarrhoeic goats:



Isolation and identification of *Salmonella spp.* from diarrhoeic goats:



Isolation and identification of *Staphylococcus spp.* from diarrhoeic goats:



3.2.10 Cultural and biochemical examination of bacterial isolates obtained from different samples:

The cultural analysis of different samples for bacteriological analysis of different samples was done according to the standard method (ICMSF, 1985). The examination followed detailed study of colony characteristics including the morphological, physiological and biochemical properties. In order to find out types of microorganisms in test samples different kinds of colonies were isolated in pure culture from plate count agar, nutrient agar and Violet red bile Agar and subsequently identified according to the standard method (ICMSF, 1985).

The isolated organisms with supporting growth characteristics on various media were subjected to a number of biochemical tests, such as sugar fermentation test for acid or acid and gas production, catalase test etc. In all cases standard method were followed for conducting these test described by Cowan (1985).

3.2.10.1 Primary culture on nutrient broth

At first samples were incuated in nutrient broth with the help of sterile inoculating loop and incubated at 37º C for 24 hours.

3.2.10.2 Culture on nutrient agar

Culture from nutrient broth were inoculated onto nutrient agar with the help of sterile inoculating loop and incubated at 37º C for 24 hours. The incubated media were then examined for growth of bacteria. Smooth, glistening and opalescent colony were found on nutrient agar.

3.2.10.3 Secondary culture on differential media

Colonies from nutrient agar were subcultured onto MacConkey agar, SS agar and incubated at 37º C for 24 hours. The incubated media were then examined for growth of bacteria.

3.2.10.4 Grams staining

Grams method of staining was followed during the experiment for the morphological study of bacteria to obtain basic information about the presumptive bacterial identification as per recommendation of Cowan (1985).

3.2.10.5 Catalase test

This test is used to differentiate the bacteria that produce the enzyme catalase such as Staphylococcus, from non catalase producing bacteria such as Streptococci. The test was performed by transferring a small amount of growth, preferably a single colony, from solid medium to a microscope slide. A drop of fresh hydrogen peroxide (3%) added, and then a cover slip is applied. The production of gas bubbles constitutes a positive reaction.

3.2.10.6 Sugar fermentation test:

Preparation of sugar solution

For this test sucrose, maltose, glucose, lactose, & mannitol based medium were used. 1% solution in distilled water of the following CHO was prepared.

Maltose, Sucrose, Lactose, Mannitol and Glucose:

Preparation of peptone water

Peptone water (Scharlauchenic S.A Barclelona, Spain) was prepared using base medium. For preparation of 500 ml peptone water 7.5g base powder was dissolved in distilled water & mixes by gentle shaking. Then it was sterilized by autoclaving at 121^0C for 15 minutes.

Durhams tube:

Durhums tubes were applied inversely to tarp the gas. These were previously autoclaved at 121^0C for 15 min.

Organism:

From pure culture in solid medium in agar, loopful of organism was inoculated into nutrient broth and was incubated at 37^0C for 24 hours. Few drops of broth with organisms were used as the source of organisms. The media were inoculated with the organism in aseptic condition under Laminar air flow.

Procedure:

1.5 ml of each of 1% CHO (Maltose, Sucrose, Lactose, Mannitol, Glucose.)mix with liquid medium

↓

Mix 3.5 ml of peptone water with (0.1%) Bromocresol purple indicator.

↓

Mix few drops of broth with organism.

↓

Then the vials were kept into incubator at 37^0C for24 hours.

The result was observed for the change in color form purple to yellow. The gas production was observed in durhams tube.

3.2.10.7 Hemolytic activity

A number of bacteria were inoculated onto blood agar (BA) and incubated at 37 ^{0}C for 24 hours for the determination of their hemolytic property. 5 % sheep blood was used to made blood agar. The colony developed on the blood agar was examined for various types of hemolysis. Hemolysis pattern of the bacteria were categorized according to the types of hemolysis they produced on BA and this was made as per recommendation of carter (1986) and was listed as mentioned below:

1) Alpha (α) hemolysis: a zone of greenish discoloration around the colony manifested by partial hemolysis
2) Beta (β) hemolysis : complete clear zone of hemolysis around the colony
3) Gamma (γ) hemolysis: no detectable hemolysis.

3.2.10.8 Indole, Methyl red, Voges-Proskaur, Citrate utilization Test for enteric bacteria

Indole test:

This test is used to identify the bacteria that possess the enzyme tryptophanase, capable of hydrolyzing and deaminating tryptophan with the production of indole.

Indole test reagent

1. *p*dimethylaminobenzaldehyde 2.0gm
2. 95% Ethyl alcohol 190 ml
3. Conc. HCl 40 ml

Procedure

About 1 ml of ether or xylene was added to 5 ml of 48 hours culture. After shaking well, it was allowed to stand until the ether or xylene rises to the top. Gently run 0.5 ml of the reagent down the side of the tube.

Interpretation

Indole had been accumulated by the ether or xylene, a brilliant red ring was developed just below the ether or xylene layer.

Methyl red test

This test is used to identify bacteria that produce stable acid end products by means of mixed acid fermentation of glucose.

Media and Reagents

MR/VP broth

1. Polypeptone 7 g
2. Glucose 5 g
3. Di-potassium phosphate 5 g
4. Distilled water 1 L
5. Final p^H 6.9

Procedure

MR/VP broth was inoculated with a pure culture of the test organism kept to incubate the broth at 35°C for 48 to 72 hours. At the end of this time, 5 drops of the methyl red reagent was directly added to the broth.

Interpretation

Stable red color was developed in the surface of the medium indicating sufficient acid production to lower the p^H to 4.4 and constitutes a positive test.

Voges Proskauer Test

Reagents

10% KOH

Procedure

Five ml of 10% KOH was added to 5 ml of culture. Mix well and was allowed standing exposed to air. That was observed at intervals of 2, 12 and 24 hours.

Interpretation

A positive test was indicated by the development of an eosin pink color.

Citrate Utilization Test (CUT)

The citrate utilization test is used to determine the ability of an organism, using the enzyme citrase, to use citrate as its sole carbon source.

Media and Reagents

The citrate medium most commonly used was the formula of Simmons. The medium was poured into a tube on a slant.

Procedure

A well-isolated colony was picked from the surface of a primarily isolation medium and inoculated as a single streak on the slant surface of the citrate agar tube. The tube was incubated at 35°C for 24 to 48 hours.

Interpretation

A positive test is represented by the development of a deep blue color within 24 to 48 hours, indicating that the test organism were able to utilize the citrate contained in the medium, with the production of alkaline products. But we found no color change of the medium which indicate negative for citrate utilization.

3.2.10.9 Triple Sugar Iron (TSI) medium

A quantity of 65.0 gm of Bacto TSI medium (HiMedia) was dissolved in 1000 ml of distilled water dispensed in 5 ml amount in each test tube and then the tubes were autoclaved at 121'C maintaining a pressure of 15 lb/sq. inch for 15 minutes. After autoclaving, the medium was put into hot water of 45°C to decrease itstemperature. On solidification of the medium in the test tubes, these were allowed for incubation at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

3.2.10.10 Motility test by Using Hanging Drop Slide

The motility test was performed to differentiate the motile bacteria from non motile ones. Before the test, a pure culture of the organism was allowed to grow in nutrient broth. One to two drops of broth culture was placed in the special slide used for hanging drop method. The slide was examined carefully examined under 100 power objective of the microscope with emersion oil. The motile organism was identified (Wilson *et al.,* 1979).

3.2.11 Procedures for bacterial isolation and identification

The criteria employed for the isolation and identification of bacteria form the mentioned samples were based the following (Wilson *et al.,* 1979; Cowan, 1985 and Carter, 1986). Morphological(size, shape, arrangement, motility) study were made by Gram's staining reaction, colony characteristics, Biochemical reaction, Hemolytic activity, catalase test, IMViC test, motility test. Then antibiotic sensitivity test was also done.Firstly TVC (Total Viable Count) & TCC (Total Coliform Count) were determined by using plate count agar and Violet red bile agar. The suspected colony from these media was subcultured in Nutrient agar, EMB, MacConkey, SS, MSA, Blood agar and BGA to promote the growth of a particular type of bacterium. Finally the pure culture was obtained from the selective media. Staining with "Gram's staining" method along with other tests. Strict aseptic measures were maintained during the period of study. Striking on different solid agar was done under laminar air flow. After performing the above mentioned tests, the results were analyzed and the isolated bacteria present in samples were identified.

3.2.11.1 Techniques for the isolation and identification of *Escherichia coli.*

For the isolation and identification of coliform organisms, the samples were first inoculated on Violet red bile agar for total coliform count. Then the colonies from VRB agar were sub

cultured on EMB, MacConkey, and BGA. Colonies on EMB agar with metallic sheen and colonies on MacConkey with pink color were suspected as positive for E. coli and were confirmed by the IMViC test. The *Escherichia coli* were characterized by positive to indole test, catalase test, Motility test and MR tests and negative to VP and citrate test.

3.2.11.2 Techniques for the isolation and identification of *Salmonella spp.*

Salmonella grow on nutrient agar, MacConkey agar, Brilliant Green agar, Salmonella-Shigella (SS) agar and Violet Red Bile agar. On nutrient agar the *Salmonella* colonies were translucent, opaque, and smooth. On MacConkey agar colonies were Pale or colorless. On Brilliant Green agar media was pink color & colonies were cream color. On SS agar colonies were of black color with dark centre. On VRB agar colonies were pale cream color. Salmonella produced Hydrogen sulphide (H_2S) which is of black color on TSI (Triple Sugar Iron) slant and SS-agar. So the slant became black color and black colonies grown on SS-agar. *Salmonella* were positive to MR test, motility test, catalase test, citrate utilization test, TSI test and negative to Indole test, VP tests.

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

Colonies of Staphylococci are usually round, glistening, convex, smooth, and opaque have a golden colour on Mannitol salt agar (MSA). They are gram positive cocci arranged in cluster mostly. *Staphylococci were* differentiated from Streptococci by catalase test. They are catalase positive. Beta (β) hemolysis was produced by most strain on blood agar (Cowan 1985). *Staphylococci* were positive to MR test, VP test, catalase test and negative to Indole test, Motility test.

3.2.12 Morphological characterization by Gram's staining method

A representative *Escherichia coli Salmonella spp., Staphylococcus spp.* colonies were characterized morphologically by using Gram's staining technique according to the method described by Merchant and Packer (1967).

The procedure was as follows:

A small colony was picked up from MCA, EMB and MSA plates with a bacteriological loop. Smear was prepared on separate clean glass slides and was fixed by gentle heating. Crystal Violate was applied on each smear to stain for two minutes and then washed with running tap water. Few drops of Gram's iodine (act as mordent) was then applied on each

smear to stain for one minute and again washed with running tap water. Acetone alcohol (act as decolorizer) was then added for few seconds and washed with running tap water. Safranine (act as counter stain) was applied on each smear to stain for two minutes and then washed with running tap water. The slides were then blotted with blotting paper and dried in air. Then the stained slide was examined under microscope with high power objective (100X) using immersion oil.

3.2.12.1 Examination of Plates (Identification of the isolates):

Study on gross colony

Morphological characteristics (shape, size, surface texture, edge, elevation, colour, opacity etc.) developed after 24 h of incubation were carefully studied as described by Marchant and Packer (1967) and recorded.

3.2.12.2 Morphological study (Gram's staining)

- A loopful of sterile distilled water was placed in the center of a clean sterile slide.
- A Small colony was picked up with a bacteriological loop and was mixed with distilled water of slide.
- The colony was made to thin smear on a slide.
- The smears were fixed by air drving.
- 0.5% crystal violet solution was then applied on the smear for one minute.
- Lugol's iodine was then added to act as mordant for One minute.
- Acetone alcohol was then added to decolorize for 1-2 seconds.
- Then the slide was washed with water.
- Safranine was added as counter stain and allowed for one minute.
- The slide was then washed with water.
- Then the slide was blotted with blot paper and was allowed to dry. The slide was examined under microscope with high power.

Gram negative (pink coloured), small rod shaped, single or paired arranged organisms were found.

3.2.13 Preservation of Isolated Culture

3.2.13.1 20% Sterile Buffered Glycerin Method

Twenty percent sterile buffered glycerin was made by mixing 20 parts of pure glycerin and 80 parts of PBS. Then a loopful of thick bacterial culture was mixed with 20% sterile buffered glycerin in small vials and was preserved at -20°C. This method is more appropriate for preserving bacteria with no deviation of their original characters for several years (Buxton and Fraser, 1977).

3.2.14 Antibacterial sensitivity test against causal agents of goat diarrhoea.

The antibacterial sensitivity test against causal agents of goat diarrhoea was performed by disc diffusion method to determine the drug sensitivity pattern. This method allowed the rapid determination of the efficiency of a drug by measuring the diameter of the zone of inhibition that result from diffusion of the agent into the medium and their antibacterial activity surrounding the disc. The antibacterial discs used were Ampicillin, Penicillin-G, Nalidixic Acid, Erythromycin, Cotrimozazole, Amoxycillin, Chloramphenicol, Gentamycin, Ceftriaxone, Bacitracin and cefalexin. The processed sample was incubated overnight into Nutrient Agar and poured on Muller-Hinton agar and spreaded uniformly with the help of a glass spreader. Antibacterial discs were applied aseptically on to the surface of the plate at an appropriate arrangement with the help of sterile forceps and incubated at 37^0 C aerobically for 24 hours.

Table 2. Antibacterial agents with their disc concentration

Antibacterial agents	Disc potency/ disc.
Ampicillin	25 µg
Penicillin-G	10 i/u
Nalidixic Acid	10 µg
Erythromycin	15 µg
Cotrimozazole	25 µg
Amoxycillin	30 µg
Chloramphenicol	30 µg
Gentamycin	10 µg
Ceftriaxone	30 µg
Bacitracin	10 µg
Cefalexin	30 µg

Antibacterial sensitivity test against the isolated organisms were performed by 11 commonly used antibacterial agents of different groups. A total of 10 samples were tested for antibacterial sensitivity by the Kirby-Bauser disc diffusion method according to Manual for Laboratory Investigation of Acute Enteric Infection by WHO. After incubation, the plates were examined and diameters of the zone of inhibition for individual antimicrobial agents were measured by mm scale and was compared with sensitivity of control strain. The zone diameters for individual antimicrobial agents were designated as sensitive, intermediate and resistant categories by referring to an interpretation table 3.

Table 3 Sensitivity of control strain (From Manual For Laboratory Investigation Of Acute Enteric Infections, World Health Organization CDD/83.3,1983)

Antibacterial agents	**Disc potency/ disc.**	**Interpretation of result.**			
		Highly sensitive	**Moderately Sensitive**	**Less Sensitive**	**resistant**
Ampicillin	25 µg	Zone diameter is wider than 12 mm.	Zone diameter is wider than 12 mm to 3 mm.	Zone diameter is less than 3mm.	No zone of inhibition.
Penicillin-G	10 i/u				
Nalidixic Acid	10 µg				
Erythromycin	15 µg				
Cotrimozazole	25 µg				
Amoxycillin	30 µg				
Chloramphenicol	30 µg				
Gentamycin	10 µg				
Ceftriaxone	30 µg				
Bacitracin	10 µg				
Cefalexin	30 µg				

CHAPTER 4

RESULTS

4.1 Results of growth of various bacteria on differential media:

Most of the bacteria grow in nutrient broth and produce turbidity and heavy sedimentation in the test tube of nutrient broth after incubation of 24 hours in incubator. On nutrient agar bacteria grow and in most cases produce round, smooth, colorless, dew drop like colonies after incubation of 24 hours.

The bacterial colonies from Nutrient agar were cultured on differential media (selective media) for isolation and identification of specific bacteria.

In nutrient broth *E coli* produces turbidity, cloudiness, heavy sediment at the bottom of the test tube. In nutrient agar it produces translucent, circular, opaque, smooth and colorless colonies. In Macconkey agar it produces large bright pink color colonies. In EMB agar it produce specific Metallic sheen and VRB agar (pink color colonies).

Salmonella spp. were grown on nutrient agar (isolated circular, low convex , smooth, opaque, colorless colonies). SS-agar (colorless colonies with black center), in EMB agar (colorless, pale colonies). *Staphylococcus spp.* were grown on nutrient agar (isolated, circular, low convex, smooth, opaque, colorless colonies), in Mannitol salt agar (Round, smooth, Glistening and 'Gold' pigmented colonies) and Blood agar (Whitish colored, round, smooth colonies with characteristic Hemolysis preferably double zone hemolysis. The colonies grown on differential media were sub-cultured until getting isolated colonies.

The results of growth of these bacteria on selective media are given below in Table-4:

Table 4: Growth Characteristics of Isolated Organisms into Differential Media

Name of isolated Bacteria	Bacterial Colony Characteristics on different Media							
	Mannitol Salt Agar (MSA)	Blood Agar(BA)	MacConkey Agar (MCA)	Eosin Methylene Blue Agar (EMBA)	Nutrient Agar (NA)	Brilliant Green Agar (BGA)	SS-Agar	VRB Agar
Staphylococcus spp.	Round, smooth, Glistening and 'Gold' pigmentation colonies appeared.	Whitish colored, round, smooth colonies with characteristic Hemolysis preferably double zone hemolysis.	No characteristic growth on it.	Colorless growth observed.	Round, smooth, Dew drop like colonies observed.	No characteristic growth detected.	No characteristic growth detected.	No characteristic growth detected.
Escherichia coli	No characteristic growth observed.	Discolored around the growth, slight hemolysis.	Large, mucoid, pink colored colonies.	Heavy metallic sheen in the medium.	Circular, low convex, smooth, colorless colonies.	Yellowish colored colonies.	Slight pinkish colonies.	Isolated large pink color colonies.
Salmonella spp.	No characteristic growth observed.	No characteristic growth, No hemolysis.	Colorless colonies.	Pale colonies without Metallic sheen.	Round, smooth, Dew drop like colonies observed.	Pink coloration of media, pale colonies.	Colorless colonies with black center.	Colorless colonies.

4.2 Result of Biochemical Test:

The suspected organisms were *Escherichia coli*, *Salmonella spp*., *Staphylococcus spp.*and based on the growth characteristics these bacteria were then subjected to various biochemical tests for more confirmation. Results of these tests are shown in **Table-5**.

Table-5: Result of different biochemical tests.

Sl. No.	Name of the Test	*Escherichia coli*	*Salmonella spp.*	*Staphylococcus spp.*
01.	Indole Test	+	-	-
02.	MR Test	+	+	+
03.	VP Test	-	-	+
04.	Catalase Test	+	+	+
05.	Citrate test	-	+	N/A
06.	TSI Test	Slant-Red Butt-Yellow	Butt-Black	N/A
07.	Motility Test	+	+	-
08.	Cellular Morphology	Straight Rods	Rods	Round arranged in grape like structure
09.	Gram's Staining	-ve	-ve	+ve

Legends:

N/A = Not applicable, (-) = No growth,

(+) = Growth, (+ve) = Positive,

(-ve) = Negative.

4.2 .1 Result of Carbohydrate Fermentation Test:

The *Escherichia coli* produced acid and gas in glucose, maltose, sucrose and lactose fermentation but in mannitol fermentation they produced acid only. *Salmonella spp. and Staphylococcus spp.* produced gas only in carbohydrate fermentation. The result of carbohydrate fermentation test is given below:

Table 6: Result of Carbohydrate Fermentation Test

Sl.No.	**Parameter**	***Escherichia coli.***	***Salmonella spp.***	***Staphylococcus spp.***
01.	Glucose	A & G	A	A
02.	Maltose	A & G	A	A
03.	Sucrose	A & G	A	A
04.	Lactose	A & G	A	A
05.	Mannitol	A	A	A

Legends:

A & G = Acid production and gas production,

A = Acid production only and no gas production.

4.3 Frequency distribution of Bacteria (*Escherichia coli, Salmonella spp., Staphylococcus spp.*) in diarrhoeic goats from specific areas of sylhet :

Most of the faeces samples of diarrhoeic goats contained the *Escherichia coli, Salmonella spp., Staphylococcus spp.* and. But the percentage of the presence of *Escherichia coli, Salmonella spp, Staphylococcus spp* bacteria in the faeces samples of diarrhoeic goats was variable in different area.

The highest percentage (90.00%) of *Escherichia coli* was found in Shah poran region, Sylhet. The highest percentage (85%) of *Salmonella spp.* was found in Govt.veterinary hospital, jaintapur, goats from Kazir bazar, Sylhet.. The highest percentage (45%) of *Staphylococcus spp.* was found in Govt.veterinary hospital, Mirjajangal Sylhet and goats from Shah poran region, Sylhet.

Area basis frequency distribution of bacteria in diarrhoeic goats are given below:

Table 7: Area basis frequency distribution of Bacteria in faecal samples.

Name of the area	*E Coli*			*Salmonella spp*			*Staphylococcus sp*		
	Total no of sample	No of positive sample	Percentage (%)	Total no of sample	No of positive sample	Percentage (%)	Total no of sample	No of positive sample	Percentage (%)
Sylhet govt.goat farm	20	14	70	20	16	80	20	8	40
Govt.veterinary hospital, Mirjajangal	20	13	65	20	15	75	20	9	45
Govt.veterinary hospital, jaintapur	20	15	75	20	17	85	20	8	40
Goats at Kazir bazar	20	16	80	20	17	85	20	7	35
goats at Shah poran region	20	18	90	20	15	75	20	9	45

4.3.1 Graphical presentation of farm basis distribution of Bacteria in collected faecal samples.

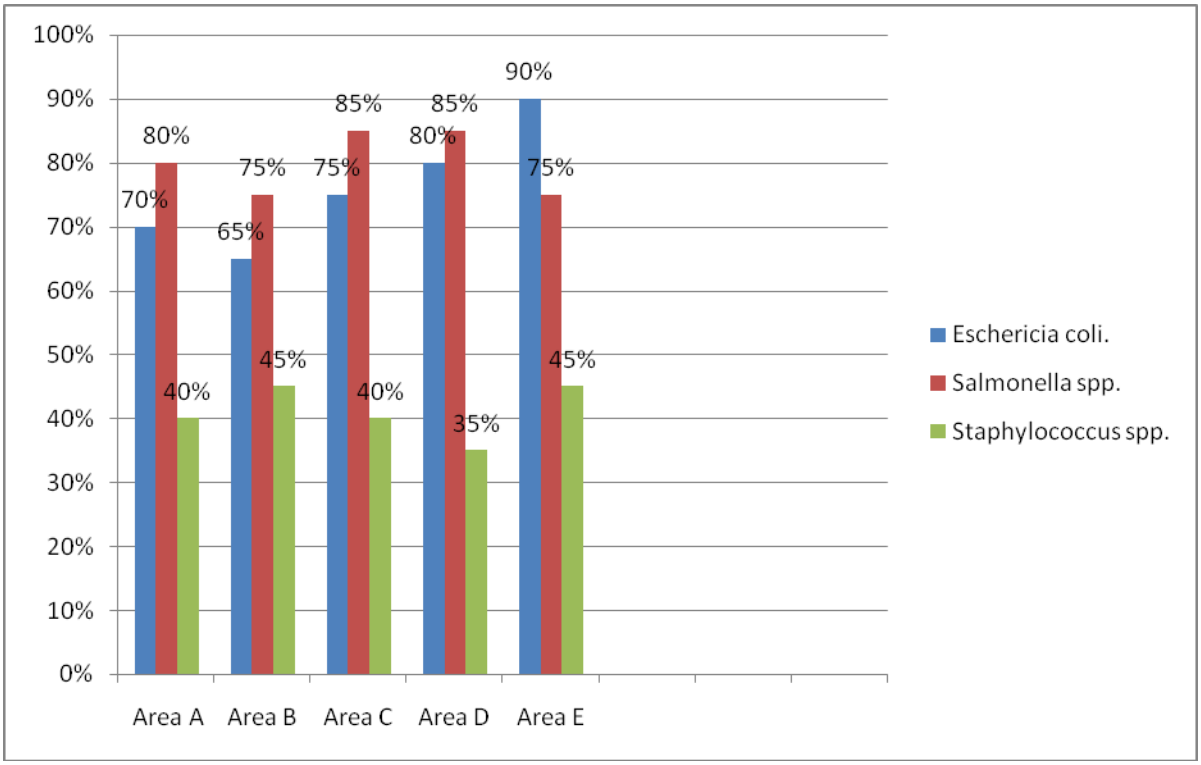


Fig: Bar diagram presentation of percentage of distribution of *Escherichia coli, Salmonella spp., Staphylococcus spp.* from faecal samples of Area A, Area B, Area C, Farm D, Area E.

Area A =Sylhet govt. goat farm, Sylhet.

Area B =Govt.veterinary hospital, Mirjajangal, Sylhet.

Area C =Govt.veterinary hospital, jaintapur, Sylhet.

Area D =Goats from Kazir bazar, Sylhet

Area E =goats from Shah poran region, Sylhet.

4.4Result of Isolation and Identification of Various Bacteria from the faecal samples of diarrhoeic goats.

4.4.1 Result of Isolation of *Escherichia coli*:

The overall Cultural characteristics of *Escherichia coli* in different culture media are summarized in **Table 13**.

Table 8: Cultural characteristics of *Escherichia coli* from faeces of diarrhoeic goats in different culture media

Media used/ Bio-chemical Test	**Colony characteristics/ Bio-chemical Test Result**	**Isolated Bacteria**
Nutrient broth	Turbidity, cloudiness in the broth, heavy sediment at the bottom of the test tube.	*Escherichia coli*
Nutrient agar	Translucent, Circular, opaque, smooth and colorless colonies.	
MacConkey agar	Large bright pink colored colonies.	
Eosin Methyl Blue (EMB) agar*	Heavy 'Metallic Sheen' found in the medium.	
Brilliant green agar	Yellowish green color colonies.	
Violet Red Bile (VRB) agar	Large pink color colonies.	
Salmonella-Shigella agar	Slight pinkish colonies.	
Sugar fermentation test	Acid and gas production in Durham's tube.	
TSI agar*	Butt and Slant are yellow colored.	
Citrate Utilization test	No color change (from green to blue).	
Indole test	Production of Pink color ring in the test tube (positive result).	
Methyl Red (MR) test	Stable red color in the test tube (positive result).	
Voges Proskaure (VP) test	No color change (negative result).	
Catalase Test	Production of gas bubbles (positive result).	

4.4.1.1 Color Plates of Cultural Characteristics (Growth) of *Escherichia coli* on Different Media:

4.4.1.1.1 Liquid Media:

4.4.1.1.1.1 Nutrient broth:

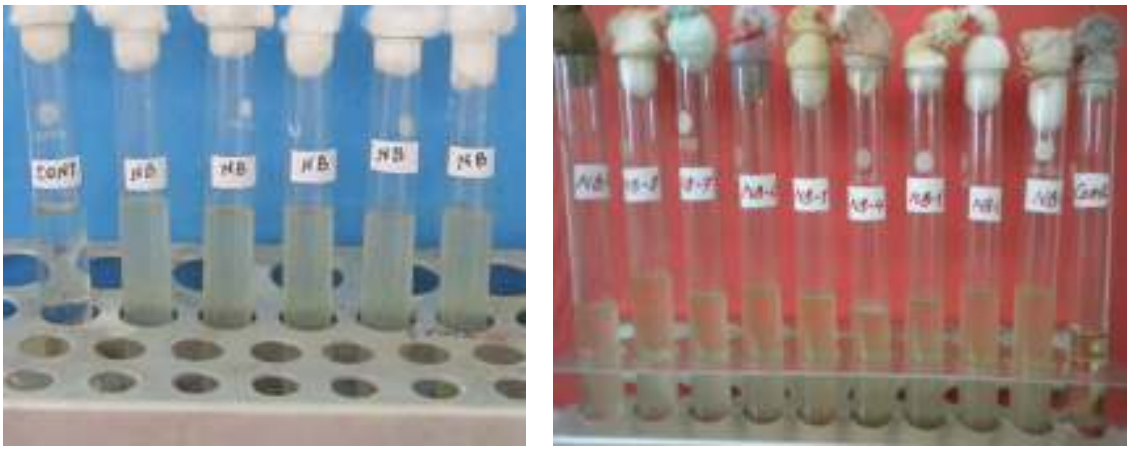


Plate 1: In nutrient broth the isolated *Escherichia coli* organism produced cloudiness, heavy sediment at the bottom of the test tube.

4.4.1.1.2 Solid Media:

4.4.1.1.2.1 Nutrient agar:

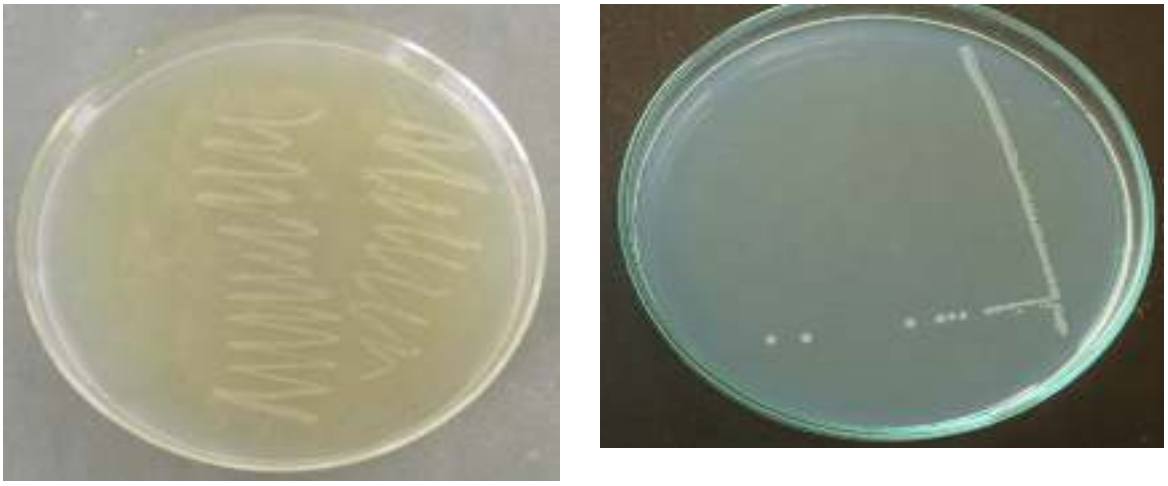


Plate 2: On nutrient agar plate the *Escherichia coli* organism produced isolated circular, low convex, smooth, opaque, colorless colonies.

4.4.1.1.2.2MacConkey agar:

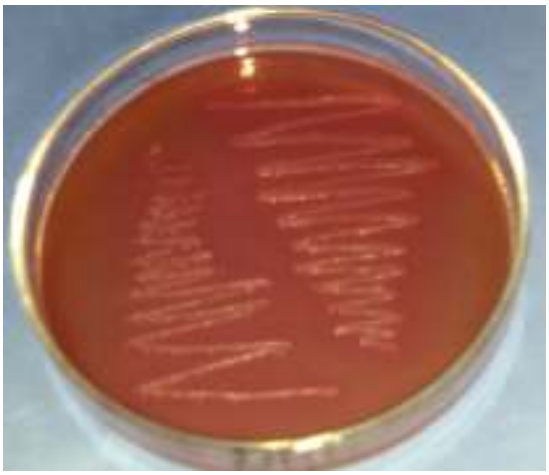



Plate 3: On MacConkey agar plate the *Escherichia coli* organism produced isolated large bright pink colored colonies with lactose fermentation (Characteristics of *Escherichia coli*).

4.4.1.1.2.3Eosin Methylene Blue agar:

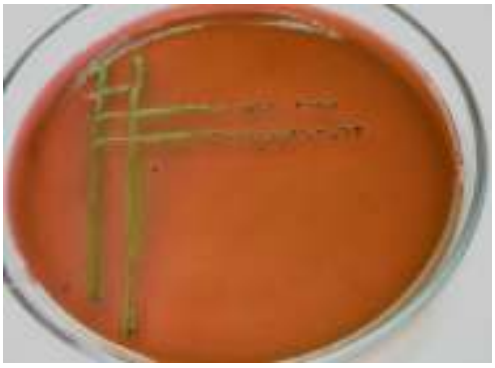
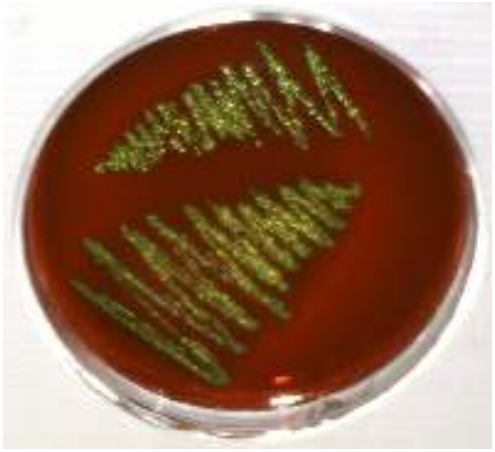




Plate 4: On Eosin Methylene Blue agar plate the *Escherichia coli* organism produced isolated metallic sheen colonies.

4.4.1.1.2.4 Brilliant green agar:

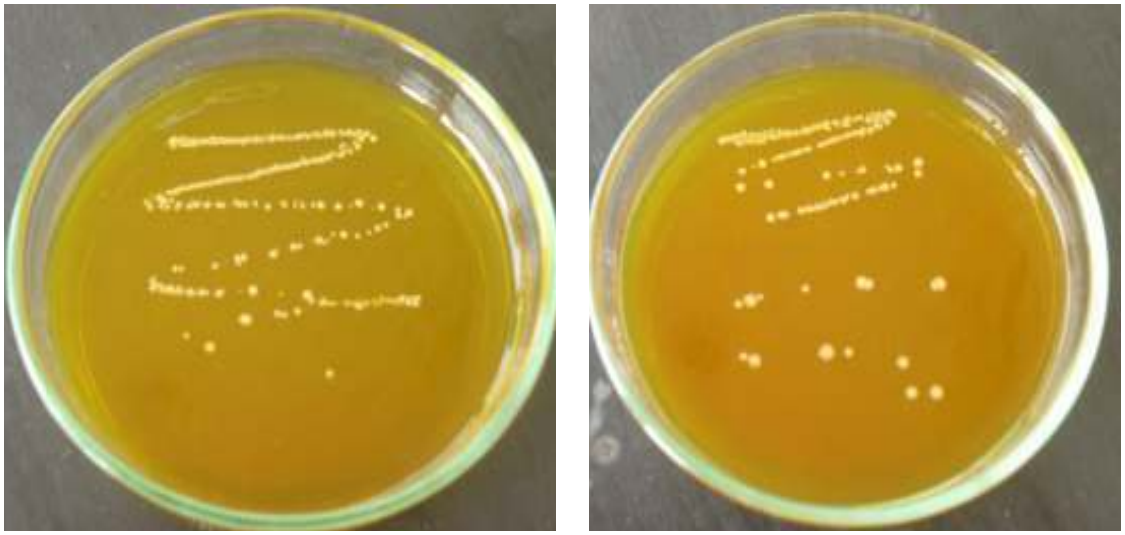


Plate 5: The *Escherichia coli* organism produced yellowish green colonies on Brilliant green agar.

4.4.1.1.2.5VRB (Violet Red Bile agar):

Plate 6: On Violet Red Bile (VRB) agar the *Escherichia coli* organism produced isolated large pink color colonies.

4.4.1.1.2.6 Salmonella-Shigella (SS) agar:

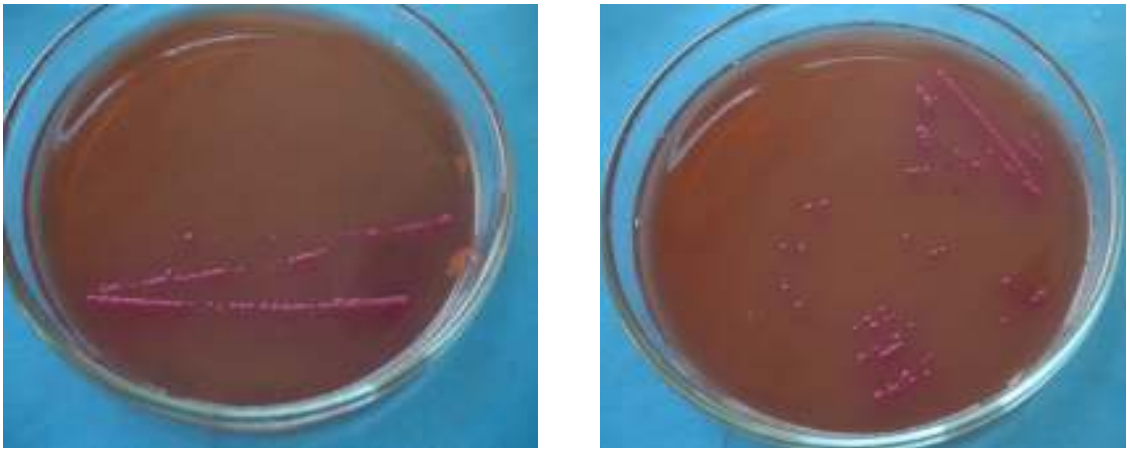


Plate 7: On Salmonella-Shigella agar the *Escherichia coli* organism produced isolated slight pinkish colonies.

4.4.1.2 Biochemical test:

4.4.1.2.1 Sugar fermentation test:

Escherichia coli fermented six basic sugars with production of acid and gas by color change.

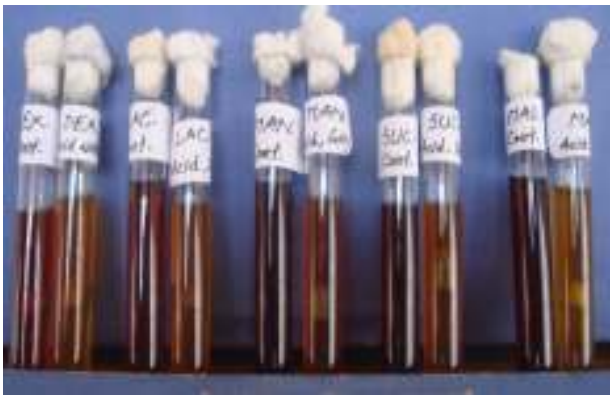


Plate 8: *Escherichia coli* fermented glucose, lactose, sucrose, maltose and mannitol that were designated by color change and production of acid and gas in Durham's tube.

4.4.1.2.1.1 Result of sugar fermentation test:

Table 9: Result of sugar fermentation test

Name of sample	Glucose	Lactose	Sucrose	Dextrose	Maltose	Mannitol
Isolated Escherichia coli	A &G	A &G	A &G	A &G	A &G	A &G

Legends:

A & G = Acid production and gas production,

A = Acid production only and no gas production.

4.4.1.2.2VogesProskaure test:

Plate 9: *Escherichia coli* organism were Voges Proskauer negative (No color change)

4.4.1.2.3 Triple Sugar Iron (TSI) slant Test:

Plate 10: On TSI agar slant, *Escherichia coli* showed positive result that was fermented glucose, lactose and sucrose (butt and slant are yellow colored), gas bubbles in butt and media frequently split.

4.4.1.2.4 Citrate Utilization test:

Plate 11: *Escherichia coli* organisms were negative in Citrate Utilization test (No color change).

Table 10: Citrate utilization test and Triple Sugar Iron (TSI) agar slant reaction of *Escherichia coli*

Name of test	Name of tested bacteria	No. of tested samples	Test results	Indications
Citrate Utilization Test	*Escherichia coli*	11	-	No color changed (Negative test result).
Triple Sugar Iron (TSI) agar slant reaction	*Escherichia coli*	06	+	Slant revealed characteristic red color and Butt-Yellow color (Positive test result).

4.4.1.2.5 Indole test:

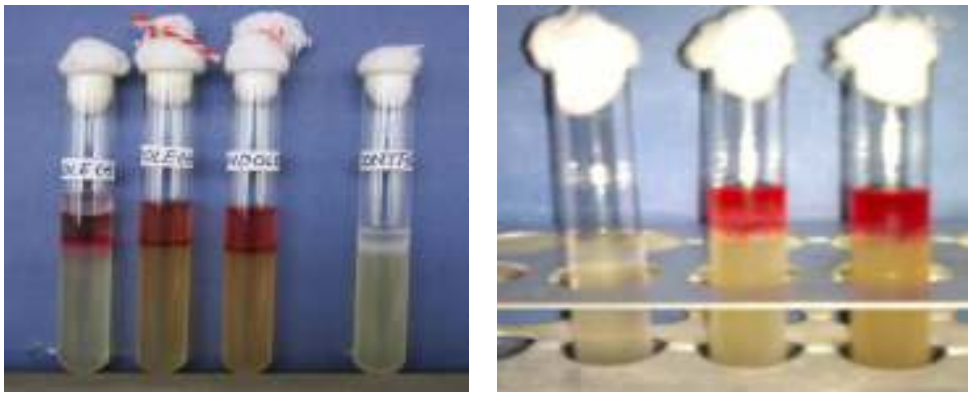


Plate 12: *Escherichia coli* organism were indole positive (Pink color ring).

4.4.1.2.6 Methyl Red test:

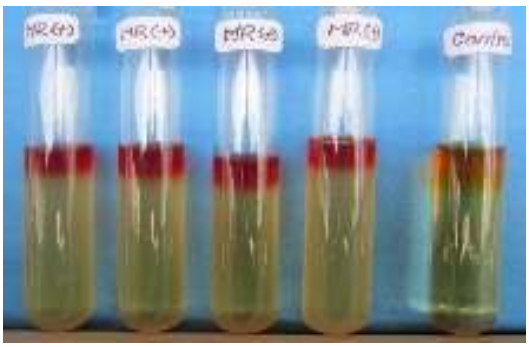


Plate 13: *Escherichia coli* organism were Methyl Red positive (Stable red color).

4.4.1.2.7 Catalase Test:

Palate 14: Catalase test showing gas bubbles of *Escherichia coli.*

4.4.1.3 Gram's staining technique:

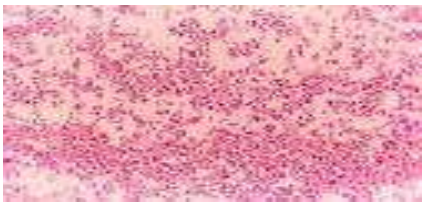


Plate 15: *Escherichia coli* in Gram's staining under microscope revealed Gram-negative, pink color, small rod shaped and arranged in single or paired characteristics.

4.4.1.4 Motility test:

Plate 16: *Escherichia coli* showing motile (forward movement) with hanging drop slide.

4.4.2 Result of Isolation of *Salmonella spp.*:

The overall Cultural characteristics of *Salmonella spp.* in different culture media are summarized in Table 16.

Table 11: Cultural characteristics of *Salmonella spp.* from faeces of diarrhoeic goats in different culture media

Media used/ Bio-chemical Test	**Colony characteristics/ Bio-chemical Test Result**	**Isolated Bacteria**
Nutrient broth	Turbidity, cloudiness in the broth, heavy sediment at the bottom of the test tube.	*Salmonella spp.*
Nutrient agar	Translucent, Circular, opaque, smooth and colorless colonies.	
MacConkey agar	Colorless, smooth, transparent, raised colonies.	
Brilliant green agar	Pale pink color colonies against Pink coloration of media.	
Violet Red Bile (VRB) agar	Colorless (Pale) colonies.	
Salmonella-Shigella agar*	Translucent, Smooth, Small round and colorless colonies with dark/black center.	
Sugar fermentation test	Acid production only and no gas production.	
TSI agar*	Butt is black colored (positive result).	
Citrate Utilization test	Color change from green to blue (positive result).	
Indole test	No color change (negative result).	
Methyl Red (MR) test	Stable red color in the test tube (positive result).	
Voges Proskaure (VP) test	No color change (negative result).	
Catalase Test	Production of gas bubbles (positive result).	

4.4.2.1 Color Plates of Cultural Characteristics (Growth) of *Salmonella spp.* on Different Media:

4.4.2.1.1 Liquid Media:

4.4.2.1.1.1 Nutrient broth:

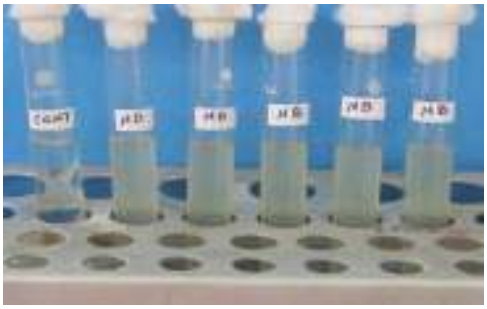


Plate 17: In nutrient broth the isolated *Salmonella spp.*bacteria produced cloudiness, heavy sediment at the bottom of the test tube.

4.4.2.1.2 Solid Media:

4.4.2.1.2.1 Nutrient agar:

Plate 18: On nutrient agar plate the *Salmonella spp.* organism produced isolated circular, low convex, smooth, opaque, colorless colonies.

4.4.2.1.2.2 MacConkey agar:

Plate 19: Growth of *Salmonella spp.*on MacConkey agar showing colorless colonies.

4.4.2.1.2.3 Eosin Methylene Blue agar:

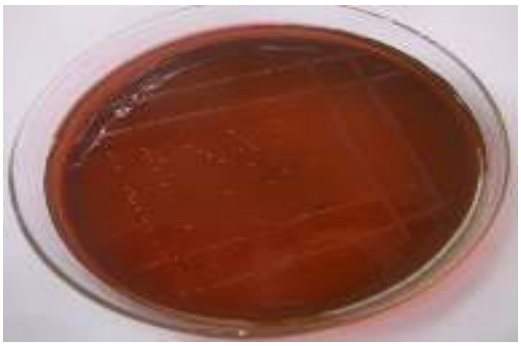


Plate 20: Colorless (pale) colonies of *Salmonella spp.* on Eosin Methylene Blue (EMB)agar.

4.4.2.1.2.4 Brilliant green agar:

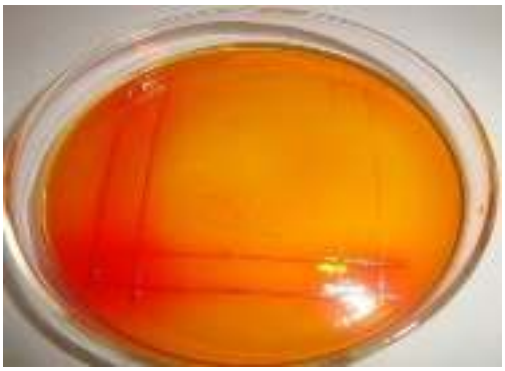


Plate 21: Pink coloration of media and colorless (pale) colonies of *Salmonella spp.* On Brilliant green agar.

4.4.2.1.2.5VRB (Violet Red Bile agar):

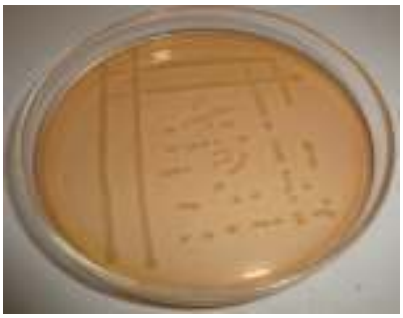


Plate 22: The Colorless growth of *Salmonella spp.*onViolet Red Bile (VRB) agar.

4.4.2.1.2.6 Salmonella-Shigella (SS) agar:

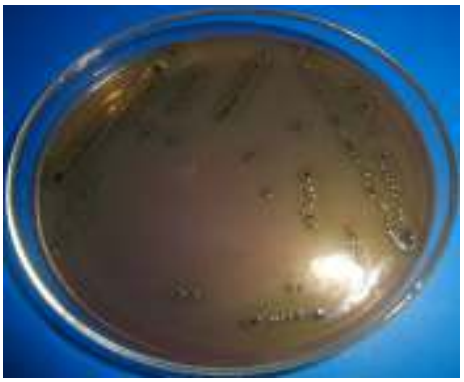


Plate 23: On Salmonella-Shigella (SS) agar *Salmonella spp.*bacteria produced colorless colonies with dark/black center.

4.4.2.1.3 Biochemical test:

4.4.2.1.3.1 Sugar fermentation test:

*Salmonella spp.*fermented six basic sugars with production of acid and gas by color change.

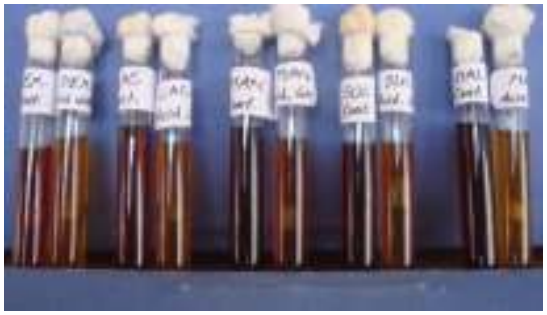


Plate 24: *Salmonella spp.*fermented glucose, lactose, sucrose, maltose and mannitol that were designated by color change and production of acid and gas in Durham's tube.

4.4.2.1.3.1.1 Result of Sugar fermentation test:

Table 12: Result of Sugar fermentation test

Isolated bacteria	Glucose	Lactose	Sucrose	Dextrose	Maltose	Mannitol
Salmonella spp.	A& G	A& G	A& G	A& G	A& G	A& G

A & G =Acid and gas production.

4.4.2.1.3.2 Voges Proskaure test:

Plate 25: In VogesProskauer test *Salmonella spp.*showed negative result (No color change).

4.4.2.1.3.3Triple Sugar Iron (TSI) slant Test:

Plate 26: On TSI agar slant, *Salmonella spp.*showed positive result that was fermented glucose, lactose and sucrose (butt is black colored), gas bubbles in butt and media frequently split.

4.4.2.1.3.4 Citrate Utilization test:

Plate 27: In Citrate Utilization test *Salmonella spp.* showed positive result (color change from green to blue).

4.4.2.1.3.4.1 Results Citrate utilization test and Triple Sugar Iron (TSI) agar slant reaction of *Escherichia coli*:

Table 13: Result of Citrate utilization test and Triple Sugar Iron (TSI) agar slant reaction of *Escherichia coli*

Name of test	Name of tested bacteria	No. of tested sample	Test results	Indications
Citrate Utilization Test	*Salmonella spp.*	03	+	No color changed (Negative test result).
Triple Sugar Iron (TSI) agar slant reaction	*Salmonella spp.*	04	+	Slant revealed characteristic red color and Butt-Yellow color (Positive test result).

4.4.2.1.3.5 Indole test:

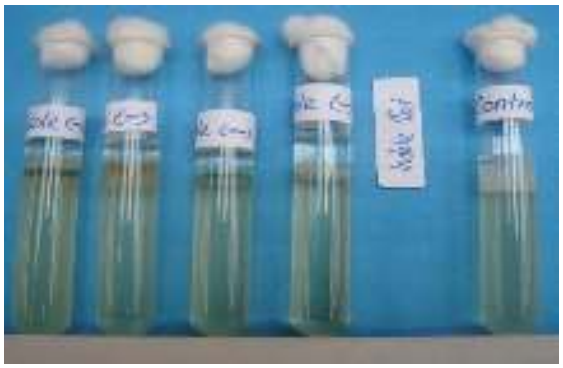


Plate 28: *Salmonella spp.* bacteria showed no color change (indole negative).

4.4.2.1.3.6 Methyl Red test:

Plate 29: *Salmonella spp.* bacteria showed Stable red color (Methyl Red positive).

4.4.2.1.3.7 Catalase Test:


Palate 30: *Salmonella spp.* bacteria showed production of gas bubbles (Catalase test positive).

4.4.2.1.4 Gram's staining technique:

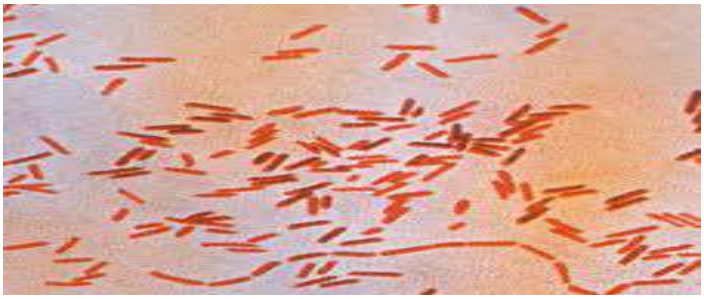


Plate 31: *Salmonella spp.* in Gram's staining under microscope revealed Gram-negative, pink color, small rod shaped and arranged in single or paired or in clusters characteristics.

4.4.2.1.5 Motility test:

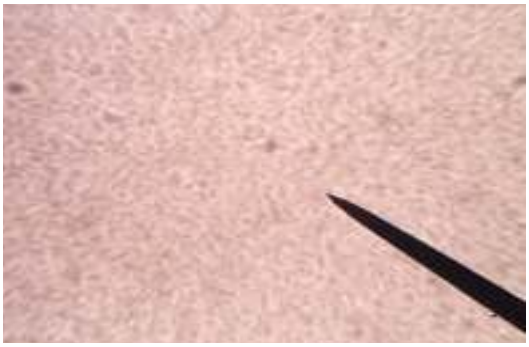


Plate 32: *Salmonella spp.*showing motile (forward movement) with hanging drop slide.

4.4.3 Result of Isolation of *Staphylococcus spp.*:

The overall Cultural characteristics of *Staphylococcus spp.* in different culture media are summarized in **Table 14**.

Table 14: Cultural characteristics of *Staphylococcus spp.* from faeces of diarrhoeic goats in different culture media

Used Media	**Colony characteristics/ Bio-chemical Test Result**	**Isolated Bacteria**
Nutrient broth	Turbidity, cloudiness in the broth, heavy sediment at the bottom of the test tube.	*Staphylococcus spp.*
Nutrient agar	Translucent, Circular, opaque, smooth and colorless colonies.	
MacConkey agar	No Growthdetected.	
Eosin Methylene Blue (EMB) agar	Colorless (pale) colonies.	
Mannitol salt agar (MSA)*	Round, smooth, glistening and 'Gold' pigmented colonies.	
Blood agar*	Whitish colored, round, smooth colonies with hemolysis preferably double zone hemolysis.	

Bio-chemical Test	**Colony characteristics/ Bio-chemical Test Result**	**Isolated Bacteria**
Sugar fermentation test	Acid production only and no gas production.	*Staphylococcus spp.*
Indole test	No color change in the test tube (negative result).	
Methyl Red (MR) test	Stable red color in the test tube (positive result).	
VogesProskaure (VP) test	Eosin pink color (positive result).	
Catalase Test*	Production of gas bubbles on the slide (positive result).	

4.4.3.1 Color Plates of Cultural Characteristics (Growth) of *Staphylococcus spp.* on Different Media:

4.4.3.1.1 Liquid Media:

4.4.3.1.1.1 Nutrient broth:

Plate 33: In nutrient broth the isolated *Staphylococcus spp* .bacteria produced cloudiness, heavy sediment at the bottom of the test tube.

4.4.3.1.2 Solid Media:

4.4.3.1.2.1 Nutrient agar:

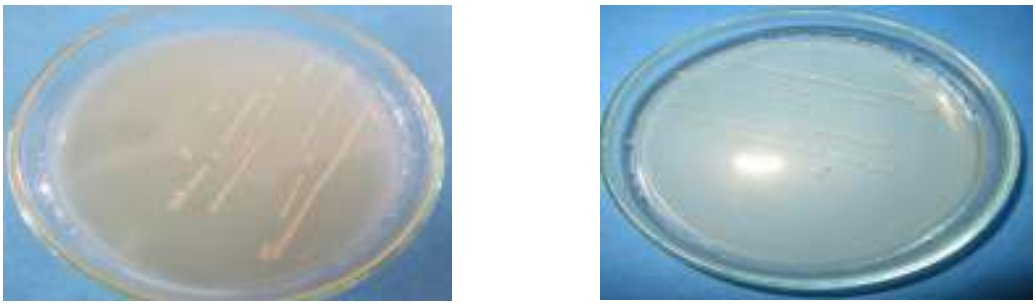


Plate 34: On nutrient agar plate the *Staphylococcus spp.* organism produced isolated circular, low convex, smooth, opaque, colorless colonies.

4.4.3.1.2.2 MacConkey agar:

Plate 35: No Growth of *Staphylococcus spp.*detected on MacConkey agar.

4.4.3.1.2.3 Eosin Methylene Blue (EMB) agar:

Plate 36: Colorless colonies of *Staphylococcus spp.* on Eosin Methylene Blue (EMB)agar.

4.4.3.1.2.4 Mannitol salt agar (MSA):

Plate 37: On Mannitol salt agar (MSA) *Staphylococcus spp.*bacteria produced Round, smooth, glistening and 'Gold' pigmented colonies.

4.4.3.1.2.5 Blood agar:

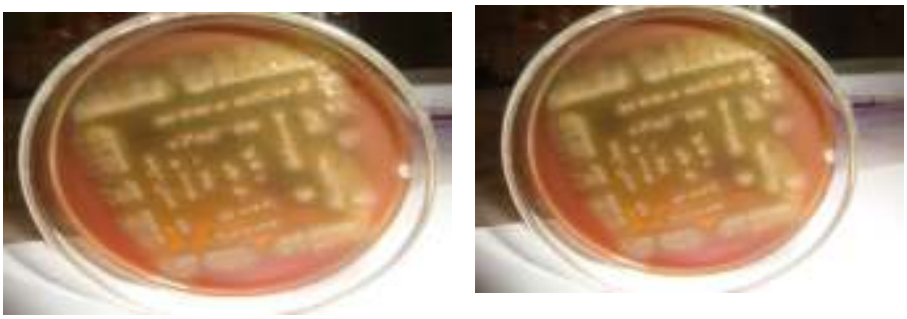


Plate 38: Whitish colored, round, smooth colonies with characteristic Hemolysis preferably double zone hemolysis of *Staphylococcus spp.* on Blood agar.

4.4.3.1.3 Biochemical test:

4.4.3.1.3.1Sugar fermentation test:

*Staphylococcus spp.*fermented six basic sugars (glucose, lactose, sucrose, maltose, fructose and mannitol) with production of acid by color change.

Plate 39: *Staphylococcus spp.*fermented glucose, lactose, sucrose, maltose and mannitol that were designated by color change and production of acid.

4.4.3.1.3.1.1Result of sugar fermentation test

Table 15: Result of sugar fermentation test of *Staphylococcus spp*.

Name of sample	Glucose	Lactose	Sucrose	Dextrose	Maltose	Mannitol
Isolated Staphylococcus spp	A	A	A	A	A	A

Legends: A= Acid production only and no gas production.

4.4.3.1.3.2 Voges Proskaure test:

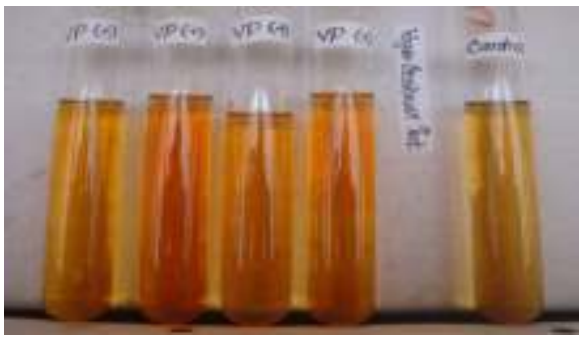


Plate 40: *Staphylococcus spp.* Showed positive result (eosin pink color) of VP (Voges Proskaure) test.

4.4.3.1.3.3 Indole test:

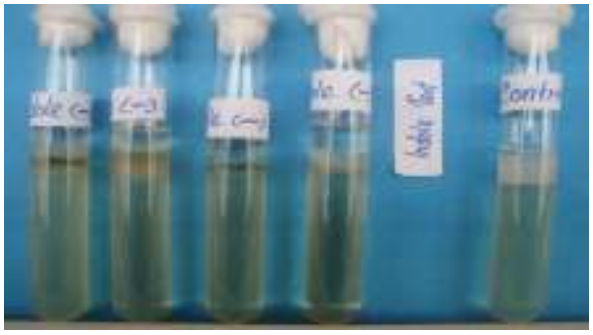


Plate 41: *Staphylococcus spp.* bacteria showed no color change (indole negative).

4.4.3.1.3.4 Methyl Red test:

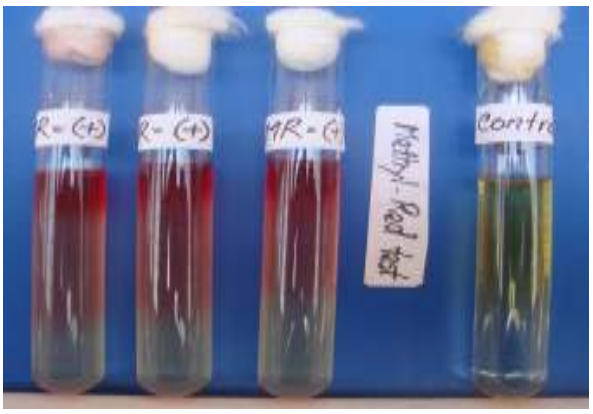


Plate 42: *Staphylococcus spp.* bacteria showed Stable red color in MR test (Methyl Red positive).

4.4.3.1.3.5 Catalase Test:

Palate 43: *Staphylococcus spp.* bacteria showed gas bubbles (Catalase test positive).

4.4.3.1.4 Gram's staining technique:

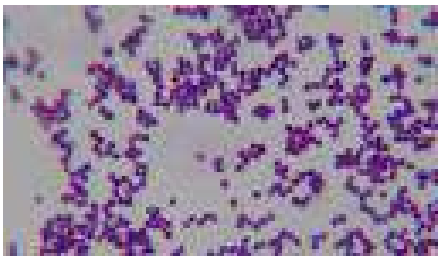


Plate 44: *Staphylococcus spp.* in Gram's staining under microscope revealed Gram-positive, pink color, small round shaped and arranged in grape like structure.

4.5 Results of antibacterial test

The result of the antibiogram study against the goat diarrhoea is shown in Table 16. This study was done to evaluate the most effective drug against the goat diarrhoea. Five samples were subjected to evaluate the results against 11 commonly used antibiotics. Out of 11 antibiotics, 5 antibiotics were resistant (Zone of inhibition 0 mm), remaining 7 antibiotics showed various results in inhibition. Ampicillin inhibited 17 mm, 16 mm, 16 mm, 16 mm, 17 mm, 16 mm in 5 differents samples and its average inhibited zone was 16.2 mm. Penicillin showed no inhibitory zone that is why it is resistant against goat diarrhoea. Nalidixic acid inhibited 28 mm, 30 mm, 30 mm, 30 mm, 29 mm, in 5 differents samples and its average inhibited zone was 29.4 mm. Erythromycin showed no inhibitory zone that is why it is resistant against goat diarrhoea.

Cotrimoxazole inhibited 33 mm, 35mm, 32 mm, 36 mm, 34 mm, in 5 differents samples and its average inhibited zone was 34 mm. Amoxycillin showed no inhibitory zone that is why it is resistant against goat diarrhoea.Chloramphenicol inhibited 22 mm, 24mm, 23 mm, 24 mm, 22mm, in 5 differents samples and its average inhibited zone was 23.2 mm.Gentamycin inhibited 31 mm, 30 mm, 28 mm, 30 mm, 30 mm, in 5 differents samples and its average inhibited zone was 29.8 mm.Ceftriaxone inhibited 36 mm, 34 mm, 36 mm, 34 mm, 34 mm, in 5 differents samples and its average inhibited zone was 34.8 mm. Bacitracin showed no inhibitory zone that is why it is resistant against goat diarrhoea. Cefalexin showed no inhibitory zone that is why it is resistant against goat diarrhoea.

Ceftriaxone inhibited 34.8 mm in average that was the highest inhibitory zone. Inhibitory zone revealed that Ceftriaxone was the 1st choice of drug against goat diarrhoea. This drug suggested against goat diarrhoea on the basis of this study. Results may vary on other studies. To evaluate more accurate choice of drug more studies are needed.

Table.16 Results of inhibitory zone in 5 samples with 11 antibiotics.

Antibacterial agents	**Zone of inhibition (mm)**					**Average. (mm)**	**Remarks.**	**Choice of drug.**
	Sample number							
	1	**2**	**3**	**4**	**5**			
Ampicillin	17	16	16	16	16	16.20	Sensitive	6th
Penicillin-G	-	-	-	-	-	-	Resistant	-
Nalidixic Acid	28	30	30	30	29	29.4	Sensitive	4th
Erythromycin	-	-	-	-	-	-	Resistant	-
Cotrimoxazole	33	35	32	36	34	34	Sensitive	2nd
Amoxycillin	-	-	-	-	-	-	Resistant	-
Chloramphenicol	22	24	23	24	22	23.2	Sensitive	5th
Gentamycin	31	30	28	30	30	29.8	Sensitive	3rd
Ceftriaxone	36	34	36	34	34	34.8	Sensitive	**1st**
Bacitracin	-	-	-	-	-	-	Resistant	-
Cefalexin	-	-	-	-	-	-	Resistant	-

- **mm = milimeter**

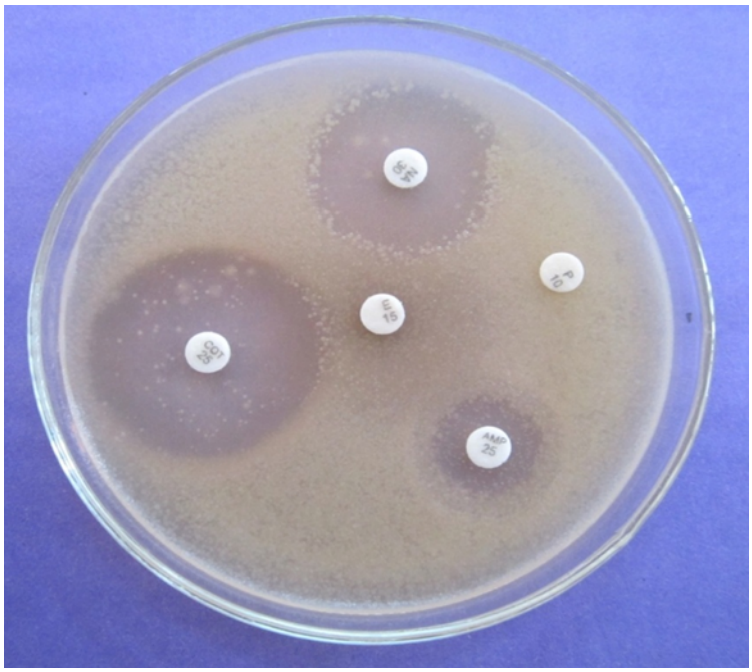


Plate 45. Antibacterial sensitivity test against causal agents of goat diarrhoea showing sensitivity to Nalidixic Acid, Penicillin-G, Erythromycin, Cotrimoxazole andAmpicillin.

Legends

A. NA=Nalidixic Acid

B. P=Penicillin-G

C. E=Erythromycin

D. COT= Cotrimoxazole

E. AMP= Ampicillin

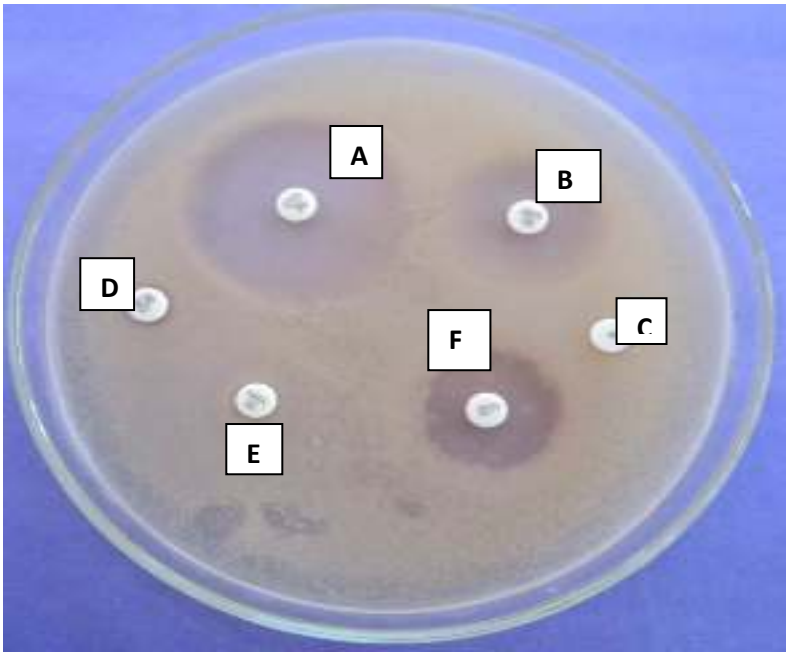


Plate 46. Antibacterial sensitivity test against causal agents of goat diarrhoea showing sensitivity to Ceftriaxone, Gentamycin, Bacitracin, Cefalexin, Amoxycillin and Chloramphenicol.

Legends

A. CTR= Ceftriaxone

B. GEN= Gentamycin

C. B= Bacitracin

D. CN= Cefalexin

E. AMX= Amoxycillin

F. C= Chloramphenicol

CHAPTER 5
DISCUSSION

The economy of Bangladesh is agro based. In the rural areas of Bangladesh more than 70% families rear goat. Goat production particularly in animal husbandry serves a very important role for poor village people. Several diseases are found in goat. Among them diarrhoea causes serious losses with high morbidity and mortality in goats owing to acute loss of fluid, essential minerals and nutrients. The agents responsible for most goat diarrhoea are mainly bacteria, viruses, fungi, protozoa, helminthes, chemical agents, nutritional factors (deficiency) and also conditions like indigestion and faulty management, hepatic cirrhosis and other toxic factors.

The research work was carried out in the laboratory of Microbiology and Immunology Department, Faculty of Veterinary and Animal Science, Sylhet Agricultural University ,Sylhet. The faeces samples of diarrhoeic goats were collected from Sylhet govt. goat farm, Govt.veterinary hospital at Mirjajangal, Govt. veterinary hospital at jaintapur, Goats at Kazir bazar, goats at Shah poran region, Sylhet.

The study was undertaken for the isolation, identification of different types of bacteria from the faecal samples of goat diarrhoea and for antibiotic sensitivity test against goat diarrhoea. A total of 100 faecal samples were collected and examined bacteriologically for the isolation, identification, frequency distribution and the degree of antibiotic sensitivity of various kinds of bacteria isolated from diarrhoeic goats.

In this study, 3 different types of bacteria were isolated from the collected samples. The isolated bacteria were *Escherichia coli, Salmonella spp.* and *Staphylococcus spp.*. Out of 100 faecal samples 76 samples were positive for *Escherichia coli*, 80 samples were positive for *Salmonella spp*, 41 samples were positive for *Staphylococcus spp.*

The frequency distribution of different species of bacterial isolates in different faecal samples were found variable. Result of the present study indicated that all the three different types of bacteria were not present in the same faecal samples collected from diarrhoeic goats.

Ugochukwu and Anukom *et al.,* (1998) investigated *Enterobacteriaceae* from faecal swabs of diarrhoeic goat and found 73% goats suffering from E. coli. Cid D *et al.,* (1996) studied

about overall percentage of resistant strains of E .coli to streptomycin, sulphadimethoxine and tetracycline was very high (above 70%). The *E. coli* strains were highly susceptible to cephalosporins, polymyxin and quinolones. Siddique *et al.,* (1989) carried out an antibiotic sensitivity of Salmonella from which they found that 78% of them were sensitivity to ampicillin, gentamycin. Zhang *et al.,* (1998) isolated 25 strains of Salmonella like organisms and in drug sensitivity tests 86% of the isolates were very sensitivity to kenamycin, gentamycin and amikacin. Roy *et al.,* (2002) isolated a total of 569 Salmonella spp. out of 4745 samples and the percentage of Salmonella spp. susceptible to gentamycin is 92.39%. Sung *et al.,* (2002) performed an antibiogram study of 72 Salmonella organism .About 57% of the isolates were resistant to nalidixic and (NA). 38.9% to amipicllin (AMP). 34.7% to streptomycin (SM). Abera *et al.,*(2010) The results of antimicrobial susceptibility testing revealed that *S. aureus* was highly susceptible to chloramphenicol (100%) followed by gentamycin (91.7%), kanamycin (88.9%) and streptomycin (86.1%). In contrast, isolates were highly resistant to penicillin (94.4%) and amoxicillin (36.1%).

The antibiotic sensitivity test against the pathogens present in goat diarrhoea with commonly used 11 antibiotics such as Nalidixic Acid, Penicillin-G, Erythromycin, Cotrimoxazole, Ampicillin, Ceftriaxone, Gentamycin, Bacitracin, Cefalexin, Amoxycillin and Chloramphenicol were studied. The result of the inhibition of the bacterial growth were variable in different antibiotics. Ceftriaxone inhibited 34.8 mm in average that was the highest inhibitory zone. Inhibitory zone revealed that Ceftriaxone is the 1st choice of drug against goat diarrhoea. Five antibiotics (penicillin–G, Erythromycin, Amoxycillin, Bacitracin and Cefalexin) were resistant to goat diarrhoea. The findings of this study varies with the result of Tripathi and Soni (1982); Joshi *et al* (1986); Panwar *et al* (1990), Joon and Kaura (1993) who reported the antibiotic sensitivity test against isolated bacteria responsible for diarrhoea whereas this study against the overall bacteria causing goat diarrhoea. The variation in the sensitivity of antibiotics against the goat diarrhoea may be due to the out come of choice and also the indiscriminate use of antibiotic in different disease stage to various species of animals.

The results of isolation, identification, biochemical test, frequency distribution and antibiotic sensitivity against goat diarrhoea in the present study indicated that isolated 3 organisms might play an important role for the development of goat diarrhoea. Detailed further study at

molecular level about the extrinsic and intrinsic factors, which might have direct or indirect influence on the development of goat diarrhoea in association with microbes are required.

The results of this test provided the guideline for the veterinarian to select appropriate antibiotics to reduce the economic loss through selecting the sensitive antibiotics .So this study would be so helpful to create public awareness about resistant bacteria and related antibiotics because the same group of antibiotics are used in both man and animal.

CHAPTER 6

SUMMARY AND CONCLUSION

The present research work was conducted for the isolation, identification, determination of biochemical properties, frequency distribution and antibiotic sensitivity of the bacteria from diarrhoeic goats at sylhet region. For this purpose, the rectal swab were collected from goats with the history of diarrhoea from five different areas in sylhet.

A series of tests were performed for the isolation and identification of different types of bacteria and to determine the frequency distribution and antibiotic sensitivity againstgoat diarrhoea to different antibiotics. Different types of ordinary, enriched and selective media such as Nutrient agar, Blood agar, MacConkey agar, Eosin Methylene Blue agar, Salmonella-Shigella agar, Triple Sugar Iron agar, Violet Red Bile agar etc. were used for the determination of the cultural characteristics of the different types of isolated bacteria. Biochemical properties of the isolated bacteria were studied by sugar fermentation, catalase and IMViC utilization tests. As the isolates of the bacteria were different so the patterns of reactivity of the isolates with various biochemical tests were also different (Table 5)

On the basis of morphology, staining, cultural and biochemical characteristics, the isolated organisms were identified as Escherichia coli, Salmonella spp. and Staphylococcus spp. (Table 4). Out of 100 faecal samples 76 samples were positive for Escherichia coli, 80 samples were positive for Salmonella spp, 41 samples were positive for Staphylococcus spp.

The highest percentage (90.00%) of *Escherichia coli* was found in Shah poran region, Sylhet.. The highest percentage (85%) of *Salmonella spp.*was found in Govt.veterinary hospital, jaintapur,Sylhet Kazir bazar, Sylhet. The highest percentage (45%) of *Staphylococcus spp.* was found in Govt.veterinary hospital,Mirjajangal ,Sylhet,Shah poran region, Sylhet.

The antibiotic sensitivity test against the goat diarrhoea with commonly used 11 antibiotics. The result of the inhibition of the bacterial growth were variable in different antibiotics. Ceftriaxone inhibited 34.8 mm in average that was the highest inhibitory zone. Inhibitory zone revealed that Ceftriaxone is the 1st choice of drug against goat diarrhoea.

Further study in connection with this research work might be

1) Comparative bacteriological quality determination of faeces of diarrheic goats at Sylhet region.

2) Serological characterization of E Coli, Salmonella spp, and Staphylococcus spp isolated from diarrhoeic goats..

3) Molecular characterization of E Coli, Salmonella spp, and Staphylococcus spp isolated from diarrhoeic goats.

CHAPTER 7

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