MULTIDRUG RESISTANT *SALMONELLA SPP*. AND *STAPHYLOCOCCUS SPP*. ISOLATED FROM MILK IN DHAKA CITY

By

Md. Anamul Hasan Allied

14376001

A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
MS in Biotechnology

Department of Mathematics and Natural Sciences
Brac University
March 2021

Declaration

It is hereby declared that

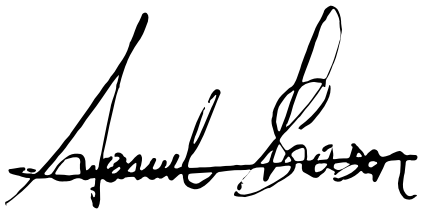
1. The thesis submitted is my/our own original work while completing the degree at Brac University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.

4. I/We have acknowledged all main sources of help.

Student's Full Name & Signature:



MD. ANAMUL HASAN ALLIED

_____

Student Full Name

14376001

Student ID

Approval

The thesis/project titled "[Thesis/Project Title]" submitted by

Md. Anamul Hasan Allied (14376001)

Of Summer 2014 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Masters in Biotechnology on [Date-of-Defense].

Examining Committee:

Supervisor: (Member)	Mahboob Hossain, PhD Professor, Mathematics and Natural Sciences Brac University _____
Joint-Supervisor: (Member)	Dr. Md. Giasuddin Chief Scientific Officer and Head, Animal Health Research Division Bangladesh Livestock Research Institute (BLRI) _____
Program Coordinator: (Member)	_____ Full Name Designation, Department Institution
External Expert Examiner: (Member)	_____ Full Name Designation, Department Institution
Departmental Head: (Chair)	_____ Full Name Designation, Department Institution

Ethics Statement

This material is an original work, which has not been previously published elsewhere. It is my own research and analysis in a truthful and complete manner. The paper properly credits all the sources used (correct citation).

Abstract

The foodborne pathogen *Salmonella spp.* and *Staphylococcus spp.* are responsible for a food-borne disease affecting both humans and animals. To evaluate the presence of these nuisance bacteria, a total of 61 samples comprising 39 raw milk and 22 brand milk samples were collected, using convenience sampling from different points/markets of Dhaka and the local farms at Mohakhali, Hemayetpur, and BLRI. The Samples were examined for identification of *Salmonella spp.* and *Staphylococcus spp*., total viable bacterial count (TVBC), and total coliform count (TCC). The raw milk samples had TVBC with values that ranged between 3–8.2 $\log_{10}$ CFU/mL in contrast brand milk samples had TVBC values ranging between 0-8.6 $\log_{10}$ CFU/mL. A total of 5 (8.2%) Salmonella isolates were confirmed by conventional PCR with the inV gene. Of these, 4 (10%) and 1 (4%) *Salmonella spp.* were isolated from raw milk and commercially packaged brand milk samples respectively. A total of 27 (44.3%) *Staphylococcus spp.* were isolated from 39 (69.2%) raw milk samples. Antibiotic susceptibility testing indicated that all 5 isolates of *Salmonella spp.* were resistant to at least 14 antibiotics especially tetracycline-class antibiotics. Fourteen (51.6%) of the *Staphylococcus spp.* isolates showed resistance to ampicillin and penicillin, which is indicative of the presence of β-lactamase producing gene. In conclusion, the high bacterial load beyond the permissible level in the milk samples, the presence of *Salmonella spp.* in the milk, and also multidrug-resistant strains of *Salmonella* and *Staphylococcus spp.* are of serious public health significance.

Keywords: Total viable bacterial count, Total coliform count, Antimicrobial susceptibility, Beta-lactamase

Dedicated to my beloved parents

Acknowledgment

I would like to acknowledge my respected supervisor, Professor Dr. Mahboob Hossain, joint-supervisor Dr. Md Giasuddin, and everyone who has helped at any point during my thesis.

Table of Content

List of Tables

List of Figures

List of Acronyms

TVBC	Total viable bacterial count
TCC	Total Coliform Count
CFU/ml	Colony-forming unit per ml
MDR	Multi-drug resistant
UHT	Ultra-high-temperature
MRSA	Methicillin-resistant S*taphylococcus aureus*
QMRA	Quantitative microbial risk assessment
CDC	Center for Disease Control
WHO	World Health Organization
TSI	Triple Sugar Iron
RVB	Rappaport Vassiliadis broth
XLD	Xylose Lysine Deoxycholate agar
TBE	Tris Borate EDTA
BLRI	Bangladesh Livestock Research Institute

Chapter 1: INTRODUCTION

1.1 Background

Milk is used as a major source of nutrition all around the world. Cow's milk especially is a source of nutrition in Bangladesh. Traditional milk collectors and sellers in Bangladesh usually collect milk in an unorganized way and are usually being supplied to the consumers from the urban and rural areas by milkman called Goalas. Things have, of course, changed quite a bit nowadays. Several industrial processors have emerged—collecting, processing, and selling milk and milk products in packaged form with the promise of hygiene and quality. There is a potential risk of health hazard if not properly processed and packaged leading to the growth of a large variety of bacteria as milk is an excellent source of food for microorganisms as well.

1.2 Background of *Salmonella*

There are two recognized species in the genus *Salmonella*, namely; *S. enterica* and *S. bongori* (Janda, 2006). About 2541 *Salmonella* serotypes have been recognized currently (Popoff et al., 2004).

Salmonellae are well-known pathogens, highly adaptive and potentially pathogenic for humans and/or other animals (Fluit, 2005). Foodborne infection caused by the *Salmonellae* is known as salmonellosis. Some *Salmonella* serotypes such as *Salmonella Typhi* and *Salmonella Paratyphi*, which are well adapted to man usually cause severe diseases in humans as septicemic typhoid syndrome (enteric fever). *Salmonella* infections in humans range from gastrointestinal infections that are accompanied by inflammation of intestinal epithelia, diarrhea, and vomiting, to typhoid fever, a life-threatening infection (Hensel, 2004). Foodborne diseases caused by nontyphoidal *Salmonella* represent an important public health problem worldwide (Jun et al., 2007).

1.3 Total Viable Bacterial Count (TVBC)

Some studies conducted in Bangladesh on the total bacterial count provided the following data in different sources of milk samples. It has been reported (Alam et al.,

2017) that, for the raw milk marketed in Chittagong 33(18%) of the 186 raw milk samples contain *E. coli* (18.3% and 11.8% markets and dairy farm, respectively) indicator bacteria for any enteric pathogens. The mean viable count of total bacteria was 4.04×10^8 CFU/ml and the mean viable count of *E. coli* in the contaminated raw milk was 1.88×10^6 CFU/ml. (Prodhan et al., 2016)found there was greater than 22000 CFU /ml of total plate count in raw milk which exceeds the BDS standards while in pasteurized milk it was 7000 CFU/ml. Among specific pathogens, *Staphylococcus spp.* was noticed to be the predominant ones and was recovered from 9 samples out of 20 samples in a range of 10^2-10^3 CFU/mL. *Klebsiella spp*. and *Vibrio spp.* were found within 6 and 9 samples, respectively. A study of antibiotic susceptibility test revealed multi-drug resistant (MDR) trait in Bangladesh (Malek et al., 2016).

1.4 *Salmonella spp.* and *Staphylococcus spp.* in Milk

Salmonella typhi 10 (35.71%), *S. aureus* 27 (96.43%), *E. coli* 15 (53.57%) were found in the vendor milk samples. In the brand milk samples, they didn't find *Salmonella typhi* but found *S. aureus* 3 (42.86%), *E. coli* 2 (28.57%) among the sample collected from a different location in Bangladesh (Munsi et al., 2016). Out of the 350 milk samples examined 14 (4.0%) were positive for *Salmonella*. The antibiotic resistance pattern of 14 *Salmonella* isolates from raw and fermented milk showed 9 different resistance patterns of the 11 antimicrobial agents used.

A total of 84 (52.50%) raw milk samples were *Staphylococcus aureus* positive, 72 (45.00%) were *Escherichia coli* positive, 2 (1.25%) were *Salmonella* positive, 2 (1.25%) were *Listeria monocytogenes* positive, and 3 (1.88%) were *Campylobacter* positive in northern china (Lan et al., 2017). Of 486 Bulk Tank Milk samples tested, 12 samples (2.5%) resulted positive for the presence of MRSA in Italy (Parisi et al., 2016). Over the years, bacterial pathogens including *Salmonella* have developed resistance to various antibiotics (Brands et al., 2005); (Gebreyes and Thakur, 2005); (Chao et al., 2007). Antimicrobial resistance in *Salmonella*, which has led to the failure of treatment in salmonellosis and other bacterial infections has been the concern of individual patients and public health (Travers and Michael, 2002a); (Butt et al., 2003); (Okeke et al., 2005)).

1.5 Global Antibiotic resistance scenario

The problem of antimicrobial resistance is particularly pressing in developing countries, where the infectious disease burden is high and cost constraints prevent the widespread application of newer, more expensive agents (Okeke et al., 2005). According to (Bekele and Ashenafi, 2010), a higher frequency of resistance of *Salmonella* to the commonly used antibiotics in human medicine such as streptomycin, amoxicillin, and gentamycin was observed in isolates from cattle and poultry in Ethiopia.

Quantitative microbial risk assessment (QMRA) is an important approach for food safety by which risk and factors that influence food safety are identified. The goal is to provide an estimate of the level of illness that a pathogen can cause in a given population. For QMRA, there is a need for microbiological methods that generate quantitative data (Forsythe, 2008).

Chapter 2: REVIEW OF LITERATURE

2.1 Evolution of *Salmonella*

It is believed that *Salmonella* and *Escherichia coli* are evolved from a common ancestor about the time of the evolution of mammals, as mammalian and avian pathogens (Wray and Wray, 2001). *Salmonella* could have diverged from the genus *Escherichia* 120 and 160 million years ago (Cotter and Dirita, 2000).

2.2 General Characteristics of the Genus *Salmonella*

Salmonella is a heterogeneous bacterial genus consisting of rod-shaped, Gram-negative, non-spore-forming, catalase-positive, oxidase-negative, and predominantly motile bacteria possessing peritrichous flagella. Its members are generally small enterobacteria with a diameter ranging from 0.7 to 1.5 micrometer and a length of 2.0 to 5.0 micrometer. *Salmonella* grows optimally at a temperature of 35 to 37°0 C, pH of 6.5 to 7.5, and water activity of 0.94 to 0.84 (Yan et al., 2004).

Salmonella spp. catabolize carbohydrates such as glucose, mannitol, into acid and gas the genus *Salmonella* produces usually gas from glucose except for S. *Typhi* which ferments glucose and mannitol without gas production. Hydrogen sulfide is usually produced on triple sugar iron agar but some strains of *S. Choleraesuis* and most strains of *S. Paratyphi A* do not (Barbara et al., 2000). Most of them are urease, indole, and oxidase negative but catalase positive.

2.3 Salmonellosis

Salmonellosis is a bacterial disease caused by strains of *Salmonella* (Ohl and Miller, 2001). Salmonellosis remains among the main causes of foodborne illness in developing as well in developed countries. It is known to be an important cause of severe diarrhea among children as well as other age groups of a population.

2.3.1 Causes of Salmonellosis

Salmonella infections include gastrointestinal infections that are characterized by inflammation of intestinal epithelia, diarrhea, and vomiting, to typhoid fever, a life-threatening infection (Hensel, 2004). The severity of *Salmonella* infections is determined by the host immune status and the pathogenicity of the bacterium (van Asten and van Dijk, 2005).

2.3.2 Health and Economic Impacts of Salmonellosis

The Center for Disease Control and Prevention has estimated that *Salmonella* infections were responsible for 1.4 million annual illnesses, resulting in nearly 600 deaths in 2003 in the United States. The estimated annual costs in dollars in 1998 and 2003 of medical care and loss of productivity due to food-borne *Salmonella* infections in the United States were $2.3 and $2.9 billion (Frenzen, 1999).

2.3.3 Symptoms of Salmonellosis in humans

The most common manifestation of nontyphoidal salmonellosis is mild to moderate gastroenteritis, consisting of diarrhea, abdominal cramps, vomiting, and fever. Typically, symptoms of gastroenteritis develop within six to seventy-two hours after ingestion of the bacteria (Pegues, 2005). The symptoms are usually self-limiting and typically resolve within two to seven days. In a small percentage of cases, septicemia

and invasive infections of organs and tissues can occur, leading to diseases such as osteomyelitis, pneumonia, and meningitis (Cohen, 1987).

2.3.4 Prevalence and Incidence of Salmonellosis

Significant outbreaks of Salmonellosis occurred around the world at different times. For instance, in the United States, 164,044 (approximately 32,000 annually) during 1998-2002 (Lynch et al., 2006); in China approximately 70% 80% and during 1992-2005 (Chen et al., 2004, Jun et al., 2007, Liu, 2008), in Germany, a total of 42,851 (Robert Koch Institute) (Safety, 2009) In 2006, a total of 160,649 confirmed cases of human salmonellosis were reported in the EU (Liu, 2010).

2.4 Transmission of *Salmonella*

Salmonella spp. is mainly transmitted by the fecal-oral route. They are carried asymptomatically in the intestines or gall bladder of many animals and are continuously or intermittently shed in the feces.

People are often infected when they eat contaminated foods of animal origin such as meat, eggs, milk, vegetables, and fruits (OIE, 2005).

2.5 Gastroenteritis

The incubation period ranges from five hours to five days, but signs and symptoms usually begin 12-36 hrs. after ingestion of contaminated food. The shorter incubation periods are usually associated with higher doses of the pathogen or highly susceptible persons. Signs and symptoms include diarrhea, nausea, abdominal pain, mild fever, and chills. Diarrhea varies from a few, thin, vegetable soup-like stools to massive evacuations with dehydration. Sometimes vomiting, prostration, anorexia, headache, and malaise occur. The syndrome usually lasts 2-5 days. The excreta of infected persons will contain large numbers of *Salmonellae* at the onset of illness. The numbers decrease over time and few persons excrete non-Typhi *Salmonellae* after 3 months (Gomez, 1997).

2.6 Enteric fever

The incubation period ranges from 7 to 28 days (depending primarily on dose), and on average 14 days. Malaise, headache, high persistent fever, abdominal pain, body aches, and weakness occur, commonly with either pea-like diarrhea or constipation. Nausea, vomiting, cough, perspiration, chills, and anorexia may occur. Rose spots sometimes appear on the trunk, back, and chest. A slow heart rate, a tender and distended abdomen, enlarged spleen, and sometimes bleeding from the bowel or nose are observed. The senses are dulled and patients may become delirious. Relapses sometimes occur (Miller, 1995).

2.7 Prevalence of *Salmonella* in Milk

Different *Salmonella* prevalence has been documented in different parts of the world from milk. For instance, in Bangladesh, 35.71% of vendors milk and none brand milk samples (Munsi et al., 2016), 1.85% of milk (Rahman et al., 2018), in Zaria, 4% of milk (Tamba et al., 2016). A larger segment of the rural and urban population is dependent on livestock for food and generation of income. Thus, many zoonotic bacterial pathogens like *Salmonella* can reach humans through the consumption of contaminated foods and food products of animal origin.

2.8 Antimicrobial Resistance of *Salmonella*

In addition to its pathogenicity, there has been concern about antimicrobial resistance in *Salmonella*, which has led to the failure of treatment of Salmonellosis and other bacterial infectious diseases. (Travers and Michael, 2002b); (Butt et al., 2003).

Drug resistance in bacterial pathogens like *Salmonella* is mainly due to intensive use of antimicrobial drugs in food-producing animals humans (van den Bogaard and Stobberingh, 2000); (Threlfall et al., 2000). Resistance against extended-spectrum cephalosporins and fluoroquinolones are also being increasingly reported after 1991 (Chiu et al., 2004). According to CDC (2002a), there has been also an increase in multi-drug resistant *Salmonella* serotypes.

Increased antibiotic resistance among *Salmonella* is not only in the percentage of isolates resistant to a particular antibiotic but also in the development of resistance against newer antibiotics (Fluit, 2005).

2.9 Global trends of antibiotic resistance

In a study conducted in the USA, *Salmonella* isolated from pre-harvest turkey production sources were resistant to multiple antibiotics (Nayak et al., 2004).

In Indonesia, *Salmonella Enteritidis* isolates recovered between 1995 and 2001 were resistant to most of the antimicrobials tested, except fluoroquinolones. A study in Alberta, Canada, indicated high resistance of *Salmonella* isolates from food and food animals to ampicillin, streptomycin, sulfamethoxazole, and tetracycline (Johnson et al., 2005). There are also reports of *Salmonella* resistant strains isolated from foods of animal origin in other parts of the world such as China (Yan et al., 2010), Iran (Dallal et al., 2010) Netherlands (Van Duijkeren et al., 2003), France (Weill et al., 2006), Portugal (Antunes et al., 2003), Nepal (Khanal et al., 2007), Spain (Carraminana et al., 2004) and Senegal (Stevens et al., 2006).

2.10 Prevention and Control of Salmonellosis

Periodic surveillance of the level of *Salmonella* contamination in the different food animals, food products, and the environment is necessary to control the spread of the pathogen and infection of man (Arumugaswamy et al., 1995, Dawson, 1992). Safe food production requires knowledge of the nature and origin of the animals, animal feed, the health status of animals at the farm. It also needs knowledge on the use of veterinary medicinal products, the results of any analysis of the samples taken at the farm (Snijders and Van Knapen, 2002).

Spoilage of end products, by potential microbiological pathogens, can be reduced by inactivating the pathogen growth. (Farkas, 1998). Enabling rapid identification of microbial contamination to allow rapid response (Doyle, 2006), knowledge and attitude of the consumers (Woteki, 2001), personal hygiene of food handlers (Nowak et al., 2006), consumer perception of food safety (Redmond and Griffith, 2004), and continuous further education are equally important to achieve food safety practices (Fischer, 2006).

2.11 Evolution of *Staphylococcus*

S. aureus is a Gram-positive, non-spore-forming spherical or ovoid non-motile organism arranged in grapes-like clusters. *S. aureus* can grow at temperatures 7°C to 48.5° C, pH- 4.2 to 9.3, and NaCl concentration up to 20% (ICMSF, 1996) and (Stewart, 2003). The pathogen infectiousness is due to a combination of toxin-mediated virulence, invasiveness, and antibiotic resistance.

Food poisoning generally has a rapid onset with an incubation period of 1-6 h Common symptoms include nausea, vomiting, abdominal cramps, and diarrhea. Recovery is usually occurring between 1-3 days (FDA, 2012, Stewart, 2003). The foods frequently implicated in food poisoning are milk and dairy products, meat and meat products, poultry and egg products, salads, bakery products, especially cream-filled pastries and cakes, and sandwich fillings (Hennekinne, 2012). The organism is a commensal and opportunistic pathogen. The *Staphylococcus* genus is widely distributed in nature and is living on the skin and mucous membranes of warm-blooded mammals.

The mortality of *S. aureus* bacteremia remains approximately 20-40% despite the availability of effective antimicrobials (Lowy, 2003). The bacteria problem is increased due to the development of multidrug-resistant strains which increases their virulence profile and pathogenicity.

2.12 Prevalence of *S. aureus* in milk product

(Muehlherr. J.E., 2003) observed a total of 407 samples of bulk tank milk (344 of goat's milk and 63 of ewe's milk) collected from 403 different farms throughout Switzerland. Standard plate counts and Enterobacteriaceae counts were performed along with a prevalence study of *S. aureus*: *Campylobacter spp.*, Shiga toxin-producing *E. coli*, *Salmonella spp.*, and *Mycobacterium avium spp. paratuberculosis*. The median standard plate count for bulk-tank milk from small ruminants was 4.70 $\log_{10}$ CFU/ml (4.69 $\log_{10}$ CFU/ml for goat's milk and 4.78 $\log_{10}$ CFU/ml for ewe's milk), with a minimum of 2.00 $\log_{10}$ CFU/ml and a maximum of 8.64 $\log_{10}$ CFU/ml. Enterobacteriaceae were detected in 212 (61.6%) goat's milk and 45 (71.4%) ewe's milk samples, whereas *S. aureus* was detected in 109 (31.7%) samples of goat milk and 21 (33.3%) samples of ewe's milk. (Gundogan, 2005) examined 180 samples of raw milk,

pasteurized milk, and ice cream and found a 94.5 percent prevalence of *S. aureus* from these samples.

(Kumar, 2010) studied *Staphylococcus* in milk (dairy farm, vendors, and house) and milk products (viz; dahi, ice cream, gulabjamun, burfi, khoa, and butter) and revealed that out of 135 samples, 25 samples were found contaminated with *Staphylococcus spp.* and *E. coli.* The highest rate of contamination was recorded in burfi while the lowest was observed in ice cream.

(De Oliveira, 2011) examined 50 samples of raw milk samples and found that 34 (68%) were contaminated by *S. aureus.* with the average varying from $6.3x10^2$ to $2.8x10^5$ CFU/ ml. In the contaminated samples of raw milk. 6 had levels of *S. aureus* corresponding to 10^2 CFU /ml; 14 had levels of 10^3 CFU /ml; 7 counts of 10^4 CFU /ml; 6 samples of 10^5 CFU /ml and 1 sample of 10^6 CFU /ml.

(Sharma, 2011) conducted a study on 115 cattle milk samples and were found 25 (21.73%) isolates of *S. aureus*.

(Jahan. M. Rahman. M.. Shafiullah. M.. Parvej. S.. Md. Chowdhury, 2015) during a study of 47 raw cow milk samples observed 12 (25.53%) isolates were confirmed as *S. aureus*.

2.13 Antimicrobial susceptibility of *S. aureus* isolates

(Gundogan, 2005) evaluated resistance of *S. aureus* to different antibiotics by the Kirby-Bauer disc diffusion test They observed *S. aureus* strains resistant to penicillin G, methicillin, and bacitracin. Few numbers of the strains were resistant to erythromycin All strains were susceptible to vancomycin, ciprofloxacin, and cefoperazone-sulbactam.

(Nema, 2007) during an investigation of enterotoxigenic staphylococcal food poisoning, observed strains were sensitive to oxacillin and vancomycin.

(Hoertle, 2009) examined in vitro antimicrobial susceptibility against the isolates of *S. aureus* and observed 100 percent susceptibility for vancomycin and teicoplanin and resistance to at least one drug was 96%, 97%, and 100% for the years 2001, 2003, and 2004 respectively. Except for ampicillin and penicillin, antimicrobial resistance decreased from 2001 to 2004.

(Sharma, 2011) studied antimicrobial resistance of 25 cattle milk isolates and observed 80-90 percent of isolates were showing multiple drug resistance to the majority of antimicrobial agents tested. Isolates were found resistant to nalidixic acid, amoxicillin+sulbactam. cloxacillin, erythromycin, kanamycin, and sensitive against ofloxacin, ampicillin. oxacillin, streptomycin, tetracycline, sulphafurazole ciprofloxacin.

(Thaker, 2013) during a study of 6.25% milk and milk products, isolated strains of *S. aureus* showed the highest sensitivity towards cephalothin, co-trimoxazole, cephalexin, and methicillin as 100%, followed by gentamicin (90%), ciprofloxacin (80%); oxacillin (70%), streptomycin (60 %) and ampicillin (60%). The pattern indicated that the overall high percent of *S. aureus* isolates were resistant to penicillin-G (100%) followed by ampicillin (40%), oxytetracycline, and oxacillin (20%), and streptomycin and gentamicin (10 %).

(Jahan. M. Rahman. M.. Shafiullah. M.. Parvej. S.. Md. Chowdhury, 2015) examined 25.53% isolates of *S. aureus* in raw cow milk. The strains were resistant to penicillin (100%), erythromycin (75%), and amoxicillin (100%) and sensitive to ciprofloxacin (83.33%), oxacillin (100%), cloxacillin (100%), and neomycin (100%).

2.14 The objectives of this Study

To this end, the general objective of this study was to investigate the prevalence, load, and antibiotic-resistance patterns of *Salmonella* and *Staphylococcus* strain in milk samples collected from the dairy farm and commercially packaged (brand) milk in the study site. Thus, the specific objectives of this study were:

1. To investigate the prevalence of *Salmonella spp.* and *Staphylococcus spp.* in milk samples collected from the dairy farm and commercial packaged milk of the study area.
2. To know the antimicrobial susceptibility of the *Salmonella* and *Staphylococcus* isolates.
3. To determine the total bacterial count and total coliform count from milk samples.

Chapter 3: MATERIAL & METHODS

3.1 Materials

3.1.1 Minor Equipment

Petri-dishes, Iceboxes, bijou bottles, universal bottles, wire loops, straight wire, Bunsen burner, test tube holders, measuring cylinder, polythene bags, scissors, conical flasks, test-tubes, pipettes, masking tape, permanent markers, and milk samples.

3.1.2 Media and Reagents

3.1.2.1 Enrichment Media

- Rappaport Vassiliadis broth (RVB)
- Brain Heart Infusion broth (BHI)

3.1.2.2 Isolation Media

- Nutrient agar (NA)
- Eosin Methylene Blue (EMB)
- Xylose Lysine Deoxycholate Agar (XLD)
- Mannitol Salt Agar (MS)
- Baird Parker Agar (BP)

3.1.3 Characterization of Isolates

3.1.3.1 Biochemical analysis

- TSI – Triple Sugar Iron Agar
- Indole and H_2S production test
- Methyl red
- Voges Proskauer

3.1.4 Gram Staining

3.1.4.1 Gram staining reagents

- Crystal violet
- Lugol‟s iodine
- 70% ethanol
- 1% safranine

3.1.5 Antibiotic susceptibility testing

Amoxicillin/Clavulanic acid (AMC 30µg), Ampicillin (AMP 10µg), Azithromycin (AZM 15µg), Cefixime (CFM 5µg), Chloramphenicol (C 30µg), Ciprofloxacin (CIP 5µg), Ceftriaxone (CTR 30µg), Cefotaxime (CTX 30µg), Doxycycline (DO30µg), Enrofloxacin (ENR 5µg), Erythromycin (E 15µg), Cefepime (FEP 30µg), Gentamicin (CN 10µg), Imipenem (IPM 10µg), Kanamycin (K 30µg), Levofloxacin (LEV 5µg), Nalidixic acid (NA 30µg), Norfloxacin (NOR 10µg), Oxytetracycline (OT 30µg), Pefloxacin (PEF 5µg), Streptomycin (S 10µg), Co-trimoxazole (SXT 25µg), Tetracycline (TE 10µg), Linezolid (LZD 30 µg), Nitrofurantoin (F 300 µg), Penicillin (P 10 µg), Tetracycline (TE 10µg), Trimethoprim/Sulphamethaxazole (SXT 25 µg).

3.1.6 Detection of Salmonella by PCR analysis

- Master mix
- inv A gene
- DNA
- PCR buffer
- Primer
- Taq DNA polymerase
- $MgCl_2$
- Glycerol
- DMSO

3.2 Study area and sample collection

A total of 61 milk samples from 9 different areas were collected from Savar, Hemayetpur, Mirpur, Mohakhali, and Mymensingh. 39 milk samples were collected

from different farms among these areas and also 22 packaged milk samples from different companies. The milk samples were taken in vials and kept in a cold chain till analysis.

3.2.1 Sample Collection Procedure

1. After wearing gloves, brushing off any dirt, debris, or bedding particles from the udder and teats was done.
2. Then Pre-dip with teat dip (0.5% iodine) leaving the pre-dip on the teat for at least 20 to 30 seconds before removal.
3. Drying each teat thoroughly, and removing the pre-dip using a single, dry paper with particular emphasis on the teat end.
4. For 15 to 20 seconds, carefully and vigorously scrubbed the teat end and orifice with cotton (but not dripping wet) with 70% ethyl alcohol. Using a separate swab for each teat being sampled, even within the same cow. Continued to clean the teat end until the swab is completely clean and white, to prevent recontamination of teat ends, clean the teats on the far side of the udder first followed by the teats on the near side of the udder.
5. Double checked to ensure that the teats and udder are clean and dry.
6. After Removing (fore strip) three or four streams of milk from the quarter being sampled to minimize chances of sample contamination from bacteria in the teat end.
7. The collection vial was opened immediately before the sample is taken. The teat end did not touch the container or skin debris or dirt enter the container. Collection vial was kept at 45° angle to keep debris (hair, manure, dirt) from accidentally falling into the collection vial. Teat was turned toward the collection vial, striving for direct streams of milk into the vial.
8. 3 to 5 ml of milk (few streams) was taken. Immediately cap was placed on the container and sealed so it was airtight.
9. Labeled the sample vials using a waterproof marker so that they will not come off during transport to the laboratory.

10. Immediately collection vial was placed on an icebox and kept refrigerated until delivered to the lab.

3.3 Total Viable Bacterial Count (TVBC)

Duplicate plates were prepared for a Hundred-fold serial dilution and the average was taken. The serial dilution was carried out on the milk samples by taking 1 ml into 9 ml normal saline diluents (10^{-1}) using a Pasteur pipette, then 1 ml was taken from the 10^{-1} dilution to another 9 ml normal saline to give 10^{-2} dilution. From the 10^{-2} diluent, 1 ml was taken to another 9 ml normal saline to give 10^{-3} dilution. From the 10^{-3} diluent, 1 ml was taken to another 9 ml normal saline to give 10^{-4} dilution. From the 10^{-4} diluent, 1 ml was taken to another 9 ml normal saline to give 10^{-5} dilution. From the 10^{-5} diluent, 1 ml was taken to another 9 ml normal saline to give 10^{-6} dilution and then 1 ml from 10^{-6} onto nutrient agar plate was inoculated using a surface spread to cover the surface using glass bent rod. From the 9 ml normal saline, it gave a dilution of 10^{-7} on the plate and then incubated at 37°C for 18-24 hrs. After 18-24 hrs. incubation the total aerobic count and coliform count on the nutrient agar, Eosin methylene blue agar plate was carried out. All organisms growing on the agars were considered. Observation of metallic sheen with a dark center demonstrated the presence of *E. coli* in the EMB agar and White, yellowish-white, off-white-colored colonies were observed on nutrient agar media. The count was calculated in coliform forming unit/per ml (CFU/ml) of the milk samples. The standard maintained according to table 1.

Table 1: Milk standard of different countries in the world. (Chanda et al., 2008)

Name of the country/region	Standard plate count	Coliform bacterial count	Grade/Standard
The USA	200,000/ml	Nil	A
	1,000,000/ml	10/ml	B
	No Limit	100/ml	C
New York	Not to exceed 100,000/mL	-	Pre-pasteurized milk for Grade A use

	20,000/ml	Not to exceed 10/mL	Grade A pasteurized milk
	30,000/ml	-	Raw milk
European Union	<100,000/ml	Nil	-
Denmark	<30,000/ml	Nil	Extra superior bacteriological quality
	30,000-100,000/ml	Nil	Satisfactory bacteriological quality
	100,000-300,000/ml	100/ml	Less satisfactory bacteriological quality
	300,000-800,000/ml	-	Non-satisfactory bacteriological quality
	>800,000/ml	-	Very unsatisfactory bacteriological quality
India	Not exceeding 200,000/ml	Nil	Very good
	Between 200,000 and 1,000,000/ml	-	Good
	Between 1,000,000 and 5,000,000/ml	-	Fair
	Over 5,000,000/ml	-	Poor

3.4 Bacterial culture isolation and characterization

3.4.1 Pre-enrichment

Pre-enrichment for all the sample was carried out on Buffered peptone water is inoculated at ambient temperature, then incubated at 37 °C ± 1 °C for 18 h ± 2 h.

3.4.2 Selective enrichment for *Salmonella*

Enrichment for *Salmonella spp.* was carried out on Rappaport Vassiliadis broth (RVB) (SC, Difco USA) on raw and commercial milk samples. 1ml of each raw and

commercial milk sample was aspirated using a 5 ml sterile syringe into 9 ml of RVB and then incubated at 37°C for 24 hrs.

3.4.3 Isolation of *Salmonella*

Following enrichment in RVB, a loop full of the incubated broth was smeared and streaked onto Xylose Lysine Deoxycholate agar plates using a sterile wire loop and incubated at 37°C for 24 – 48 hrs. After incubation, the plates were examined for the presence of typical and suspect colonies. Typical colonies of *Salmonella* grown on XLD-agar have a black center and a lightly transparent zone of reddish color due to the color change of the media (ISO 6579,2002) while H_2S negative variants grown on XLD agar are pink with a darker pink center. Lactose-positive *Salmonella* grown on XLD agar are yellow with or without blackening.

3.4.4 Isolation of *Staphylococcus*

The specimens were directly inoculated by streaking onto mannitol salt agar (MSA) and incubated at 37 ºC for 24 h. All colonies from primary cultures were purified by subculture onto MSA medium and incubated at 37ºC for 24- 48 hrs. Gram stain slides were investigated. The specimens were enriched in Brain Heart Infusion broth at 37°C for 24 hrs., then streaking on to mannitol salt agar (MSA) and Baird parker agar the following day and incubated at 37ºC for 24 hrs. All colonies from primary cultures were purified by subculture onto MSA medium and incubated at 37º C for 24- 48 hrs.

3.4.5 Gram Reaction

After 24 hrs. incubation on XLD, MSA and BPA smear from distinct colonies of *Salmonella* were made on sterile glass slides and flame. Fixed smears were then gram stained using the gram staining procedure as described by (Cheesbrough, 2002). Stained smears were then viewed under a compound microscope at X 100 objective lens for typical *Salmonella* gram reaction and morphology.

3.4.6 Biochemical characterization of isolates

Suspected *Salmonella* isolates on XLD were subjected to biochemical tests based on indole production, Methyl Red (MR), and Voges-Proskauer (VP) using MR-VP medium (Merck, Germany). Presumptive colonies were transferred to tubes of Triple Sugar Iron (TSI) agar, Urea, Methyl Red, Voges Proskauer and incubated at 37°C for

18-24 hrs. Confirmed isolates were stored on nutrient agar slants at -4°C for further studies. Biochemical characterization was done based on standard techniques (Cowan, 2003). All isolates that were typical of *Salmonella spp*. were tested and substrates were considered to belong to the genus *Salmonella*. Typical *Salmonella* reactions such as indole negative, methyl red positive, Voges-Proskauer negative, motile in motility medium.

3.4.6.1 Triple Sugar Iron Agar test (TSI)

In this test, the Triple Sugar iron Agar was prepared according to the manufacturer's instruction and was inoculated with the isolates by stabbing and streaking respectively. This was followed by incubation at 37°C for 24-48 hrs. It was then observed for hydrogen sulfide production (which is indicated by a black precipitate at the butt of the tube) and carbohydrate fermentation indicated by gas production and color change) (Quinn, 2002). Also, the test tube showed yellow at the top, leaving the bottom light red, which indicates alkaline over acid which is a typical *Salmonella* reaction on TSI slants (Carter and Chengeppa, 1991).

3.4.6.2 Methyl red-Voges Proskauer Test

Samples were inoculated into 5 ml of MR-VP broth and incubated at 37°C for 24 hrs. After incubation, 1ml of the broth was transferred to a small serological tube followed by the addition of 2-3 drops of methyl red, and the color on the top of the medium was read immediately. The red coloration on the addition of the indicator signified a positive test for *Salmonella spp.* To the rest of the broth in the original tube 5 drops of 40% potassium hydroxide (KOH) were added followed by 5 drops of 5% of alcoholic (ethanol) alpha Naphthol. The cap of the tube was loosened and placed in a sloping position. The development of a red color starting from the liquid-air interface within 1 hour indicates a positive test. *Salmonella spp.* are reported to be Methyl red positive with an orange to red coloration and Voges-Proskauer negative with no coloration (Cheesbrough, 2002).

3.4.6.3 Indole test

Samples were inoculated into 4 ml tryptophan broth and incubated at 37°C for 24hrs. After incubation three drops of Kovac's indole reagent were then added and shaken gently. Within one minute, the reaction is read added as the presence or absence of the ring was observed. No color change is indicative of a negative result and a red or pink ring is indicative of a positive indole test.

3.4.7 Antimicrobial Susceptibility testing of Isolates

This was performed using a panel of 11 commonly used antimicrobial agents by the Agar Disc Diffusion method following Clinical Laboratory Standards Institute (CLSI, 2016) guidelines (Bauer, 1966) and cultured on Mueller-Hinton agar (Oxoid Basingstoke, U.K). The following antimicrobial agents and concentrations were used: Amoxicillin/Clavulanic acid (AMC 30µg), Ampicillin (AMP 10µg), Azithromycin (AZM 15µg), Cefixime (CFM 5µg), Chloramphenicol (C 30µg), Ciprofloxacin (CIP 5µg), Ceftriaxone (CTR 30µg), Cefotaxime (CTX 30µg), Doxycycline (DO 30µg), Enrofloxacin (ENR 5µg), Erythromycin (E 15µg), Cefepime (FEP 30µg), Gentamicin (CN 10µg), Imipenem (IPM 10µg), Kanamycin (K 30µg), Levofloxacin (LEV 5µg), Nalidixic acid (NA 30µg), Norfloxacin (NOR 10µg), Oxytetracycline (OT 30µg), Pefloxacin (PEF 5µg), Streptomycin (S 10µg), Co-trimoxazole (SXT 25µg), Tetracycline (TE 10µg), Linezolid (LZD 30 µg), Nitrofurantoin (F 300 µg), Penicillin (P 10 µg), Trimethoprim/Sulphamethaxazole (SXT 25 µg). (Oxoid Basingstoke, UK). Colonies (4-5) of the test isolates from overnight cultures on XLD plates were picked and emulsified in sterile normal saline. The turbidity of the suspension was adjusted to match 0.5 McFarland's standard. Ten µl of the suspension was then dispensed and spread on Mueller-Hinton (Oxoid UK) agar plates to create a uniform lawn. The pre-inoculated plates were used for the disc diffusion test. The antibiotic discs were placed on the surface of each of the pre-inoculated Mueller-Hinton plates using a disc dispenser (Oxoid Basingstoke, U.K), and the plates were incubated aerobically at 37°C for 24 hrs. After incubation, the diameters of the antibiotic inhibition zones were measured to the nearest millimeter (mm) using a meter rule and were classified as susceptible (S), intermediate resistant (I), or resistant (R) according to the CLSI criteria (2016).

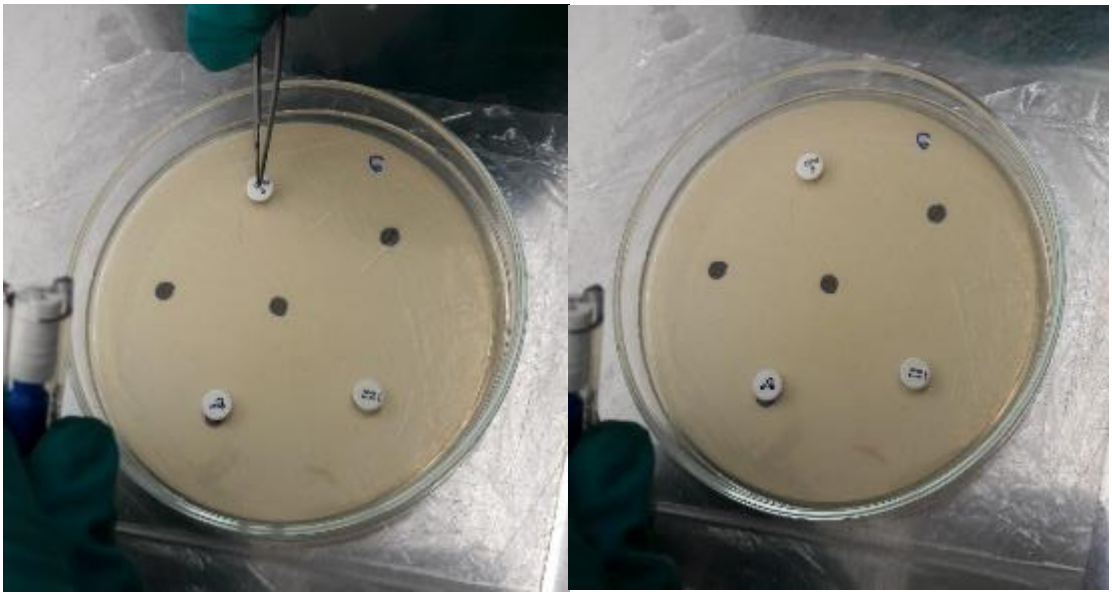


Figure 1: Placement of antibiotic disk for the evaluation of antimicrobial susceptibility

3.4.8 DNA Extraction

From the enrichment media, 1.5 ml of overnight growth bacteria were used. The cultured broth was put in an Eppendorf tube then it was centrifuged for ten minutes at 10000 rpm. After centrifuging the supernatant was removed and the precipitate at the bottom of the Eppendorf tube was dried for some time. After drying, 200 µl of nuclease-free water was added and vortexed to mix the precipitate into the nuclease-free water. After proper mixing, the tube was placed in a water bath at 95°C for 10 minutes, after the completion time the tube was immediately put into ice for 10-minutes. After ten minutes the tube was centrifuged for 10 minutes at 10000 rpm. One hundred µl of the supernatant was used as DNA template for the PCR.

3.4.9 PCR Identification

3.4.9.1 Master mix composition for invA gene Conventional PCR technique

Total DNA (5µl) was subjected to conventional PCR in a 25µl reaction mixture containing 2X PCR buffer (0.025U/µL Taq DNA polymerase, reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP (dATP, dCTP, dGTP and dTTP), 5% glycerol and a variable concentration of specific primer ,0.5 mM $MgCl_2$ and 3% DMSO was added.

3.4.9.1.1 Procedure

• At fast, an iceless cold storage system for 96 well plates or ice cup and PCR tubes was taken, then Primer and the master mix was put into ice.

• PCR tubes were taken and were labeled, put in an iceless cold storage system for 96 well plates.

• Then 25µl reaction mixture per sample was prepared by adding H_2O, primer, and master mix into the PCR tube according to the recipe.

3.4.9.1.2 The mechanical process of conventional PCR

• Five µl extracted DNA template was added to the master mix containing PCR tube and centrifuged at 1000 rpm for a few seconds.

• Then PCR tube was placed into the thermal cycler.

3.4.9.1.3 Thermal Profile of Conventional PCR invA gene

Table 2: Conventional PCR gene for the Salmonella isolates with sequence, primer name.

PCR name	gene	Primer Name	Sequence (5′–3′)	Primer con. µM	Annealing temp (°c)	Amplicon size (bp)	Reference
Con. PCR	invA	139	GTGAAATTATC GCCACGTTCGG GCAA	0.4	64	284 bp	(Rahn et al., 1992)
		141	TCATCGCACCG TCAAAGGAACC	0.4			

invA gene's Conventional-PCR amplification was carried out as follows: initial denaturation at 95°C for 15 min; 35 cycles of 95°C for 30 s, 64°C for 30 s and 72°C for 30 s and final elongation at 72°C for 5 min.

3.4.9.2 Agarose Gel electrophoresis

After mixing 1.5 gm agarose powder with 100 mL 1xTBE in a microwavable flask. Microwave for 1-3 min until the agarose is completely dissolved. After cooling down to about 50 °C, ethidium bromide (EtBr) to a final concentration of approximately 0.3 microgram/mL was added. It varied from time to time because of different amount of agarose was used. Agarose was put into a gel tray with the well-comb in place. After solidification, agarose gel was placed into the gel box (electrophoresis unit). At first molecular weight, the ladder was loaded in the first lane then the sample was loaded. Gel ran at 100 V for 10 minutes. Then the gel was observed under UV light for identification.

Chapter 4: RESULTS

4.1 Total Viable Bacterial Count (TVBC)

The total microbial count for raw milk ranged between 3-8.2 $\log_{10}$ with a mean of 1.20 ± 0.19 standard deviation, while the counts for commercial milk ranged between 0-8.6 $\log_{10}$ with a mean of 2.00 ± 0.50 standard deviation.

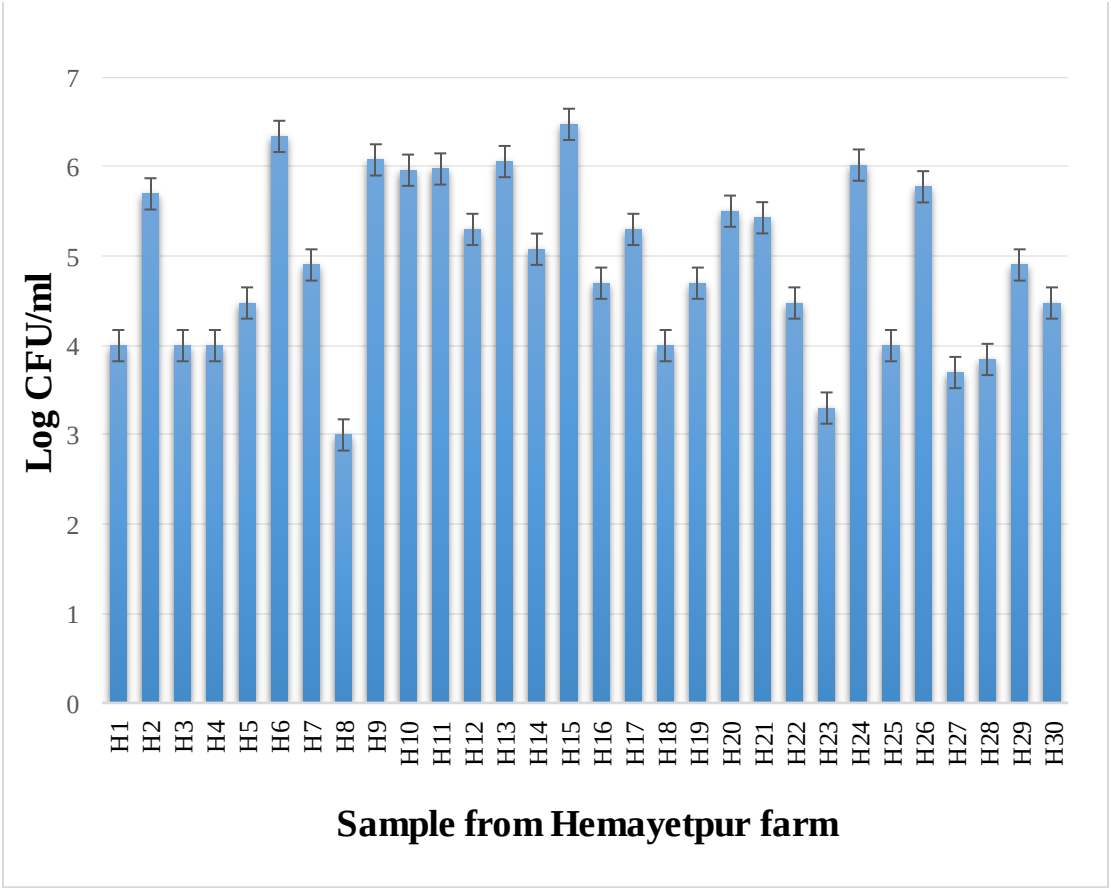


Figure 2: Total viable bacterial count of the sample from Hemayetpur farm.

From Hemayetpur 30 samples were collected, from these samples, the data shows that 2 samples fall in category A of USA standard, 5 sample falls in category B. Seventeen samples meet the criteria of Pre-pasteurized milk for Grade A, 9 samples in Grade A pasteurized milk, 9 samples in Raw milk Category of New York Standards. Eighteen samples in European Union's category of the threshold value. Nine samples in extra superior bacteriological quality, 7 samples in satisfactory bacteriological quality, 4 samples in Less satisfactory bacteriological quality, 3 samples in non-satisfactory bacteriological quality, 7 samples in very unsatisfactory bacteriological quality in Denmark standard. Seventeen samples in very good condition, 8 samples in good condition, 5 samples in fair condition, and no samples were in poor condition according to India standard. According to the BSTI standard of less than 20000 CFU/ml total viable count (Hossain et al., 2011), 9 samples meet the standard.

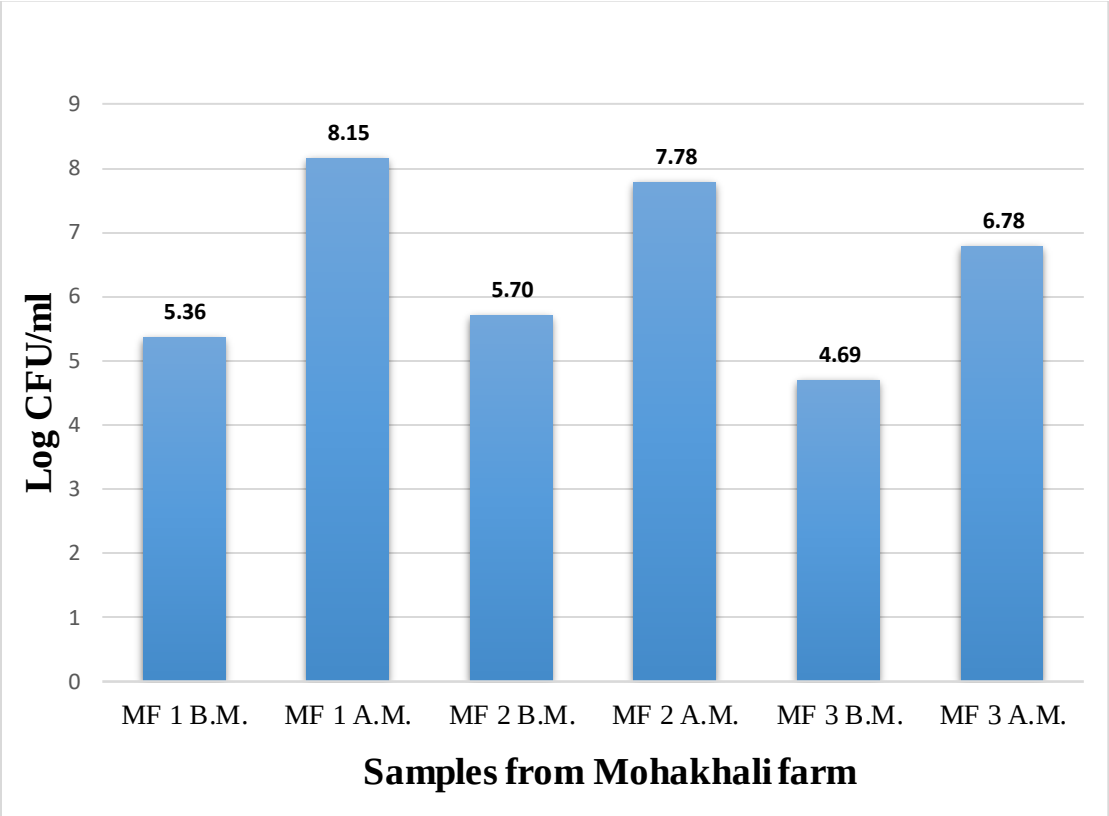


Figure 3: Total viable bacterial count of the sample from Mohakhali farm

Six samples were taken from Mohakhali farm 3 before milking from the tits of pregnant cows and 3 after milking from the milk container. Among them, none of them meet the criteria of the BSTI standard. 1 sample meets the criteria of Grade A, 3 samples in

Grade B of the USA standard. One sample meets the criteria of Pre-pasteurized milk for Grade A of New York standard. One sample meets the criteria of European union standard. 1 sample meets the criteria of Satisfactory bacteriological quality, 1 sample in Less satisfactory bacteriological quality, 1 sample in Non-satisfactory bacteriological quality, and 3 samples in Very unsatisfactory bacteriological quality of Denmark milk standard. One sample is very good, 2 samples in good, and 3 samples in the poor category of Indian standard.

Three sample taken from savar farm shows Grade A in The USA standard, Grade A pasteurized milk in New York standard, Extra superior bacteriological quality in Denmark standard, very good according to Indian standard, and meets the BSTI standard.

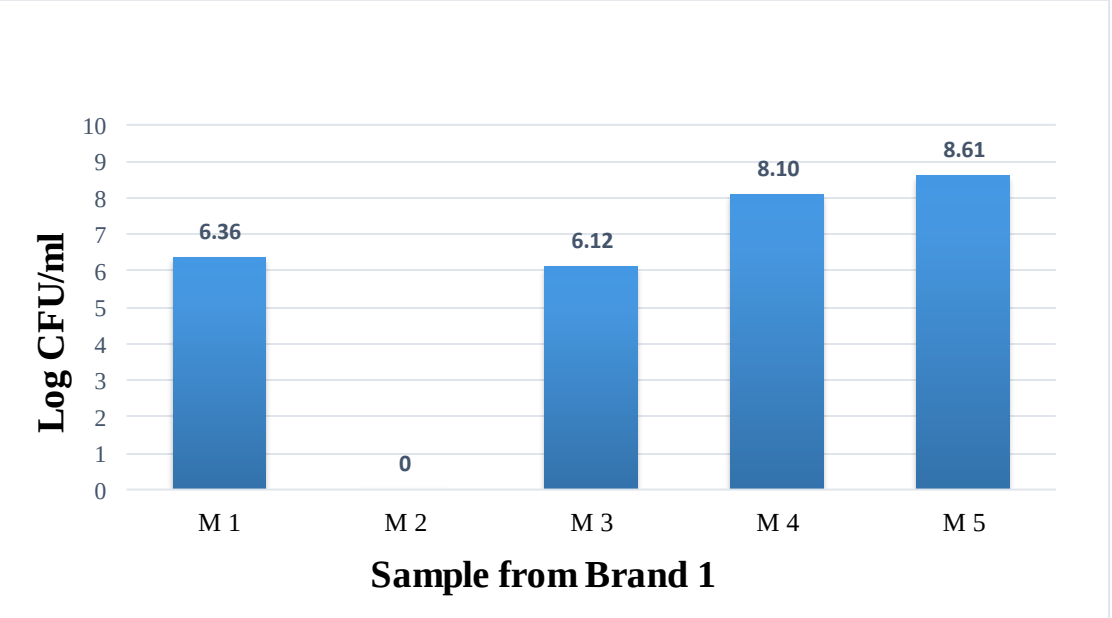


Figure 4: Total viable bacterial count of the sample from Brand 1

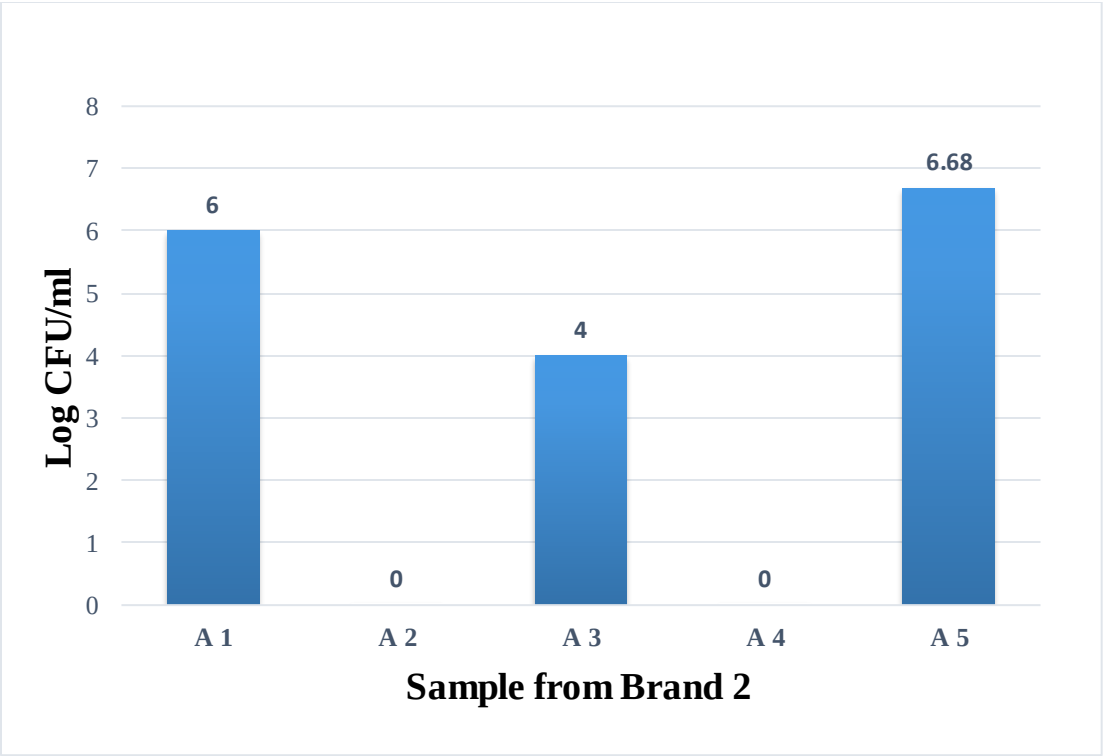


Figure 5: Total viable bacterial count of the sample from Brand 2

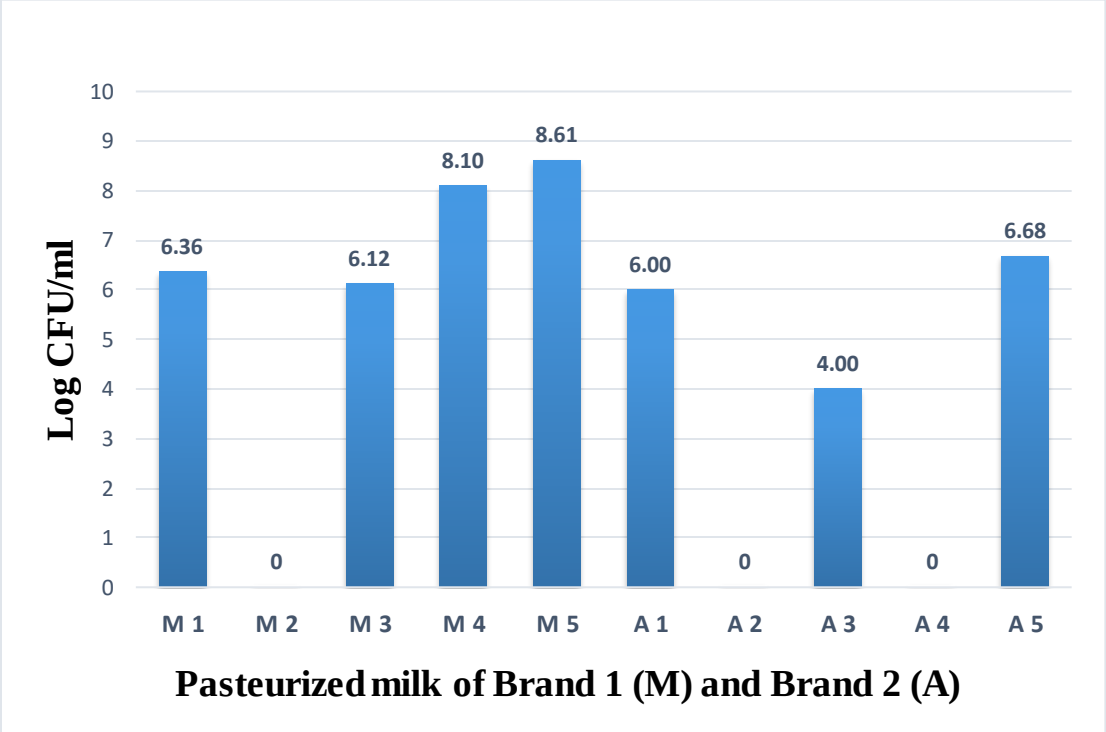


Figure 6: Total viable bacterial count of the sample from Brand 1 & Brand 2

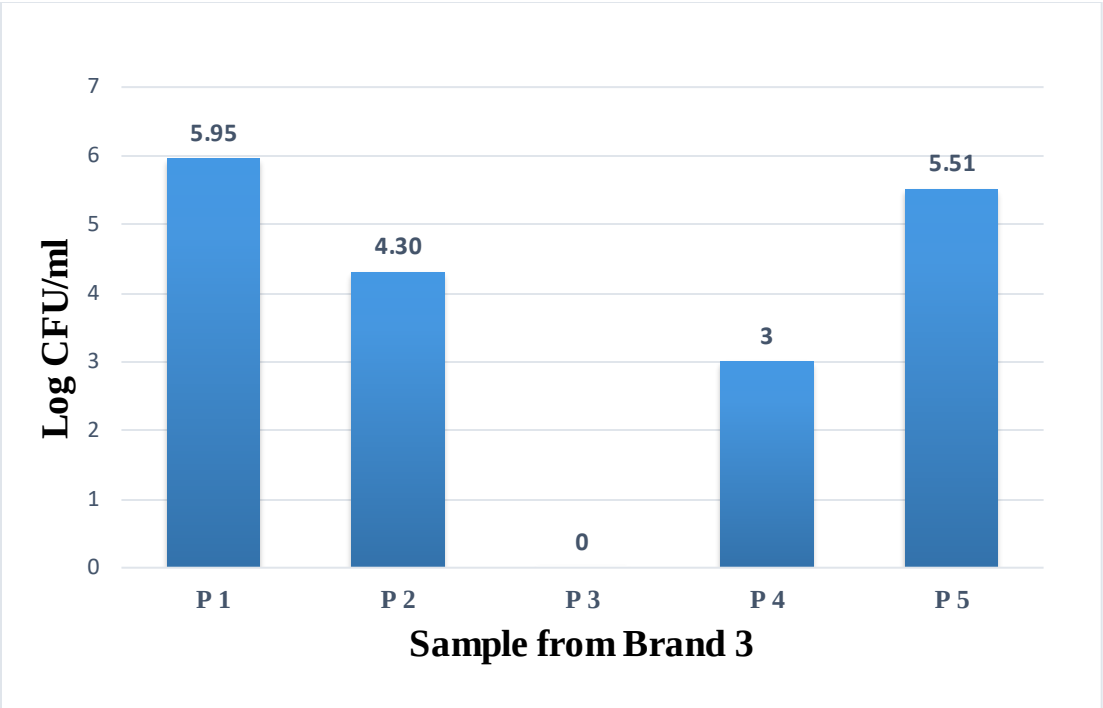


Figure 7: Total viable bacterial count of the sample from Brand 3

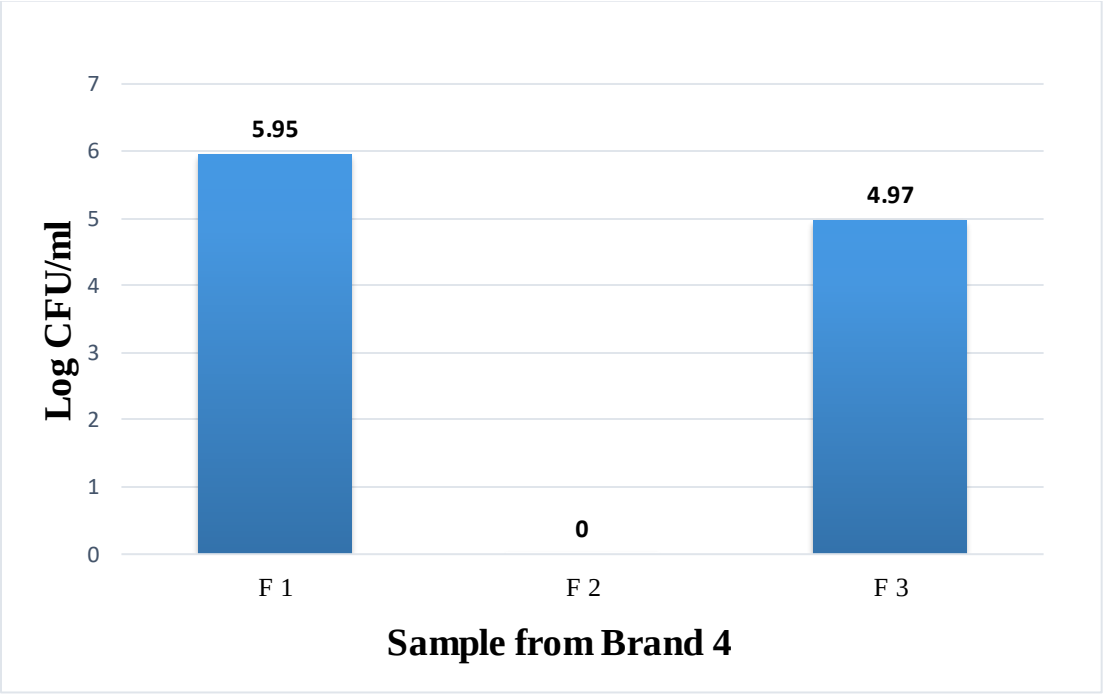


Figure 8: Total viable bacterial count of the sample from Brand 4

Four commercial milk products were evaluated and 2 of them were pasteurized milk, Brand 1 and Brand 2. Two of them was UHT (Ultra High Temperature) processed milk Brand 3 and Brand 4. Two sample from Brand 1 shows fair and 2 samples show poor standard according to Indian standard. And 1 sample shows Grade A in The USA standard, Grade A pasteurized milk in New York standard, Extra superior bacteriological quality in Denmark standard, very good according to Indian standard and meets the BSTI standard. In Brand 2, 1 sample is fair according to Indian standard, 3 sample shows Grade A in The USA standard, Grade A pasteurized milk in New York standard, Extra superior bacteriological quality in Denmark standard, very good according to Indian standard and meets the BSTI standard. In Brand 3, 2 sample meets The USA standard of Grade B, 1 sample in less satisfactory bacteriological quality in Denmark standard, 1 sample in very unsatisfactory bacteriological quality of Denmark standard and 3 samples shows Grade A in The USA standard, Grade A pasteurized milk in New York standard, Extra superior bacteriological quality in Denmark standard, very good according to Indian standard and meets the BSTI standard. In Brand 4, 1 sample is very unsatisfactory bacteriological quality in Denmark standard, 2 samples show Grade A in The USA standard, Grade A pasteurized milk in New York standard, Extra superior bacteriological quality in Denmark standard, very good according to Indian standard and meet the BSTI standard.

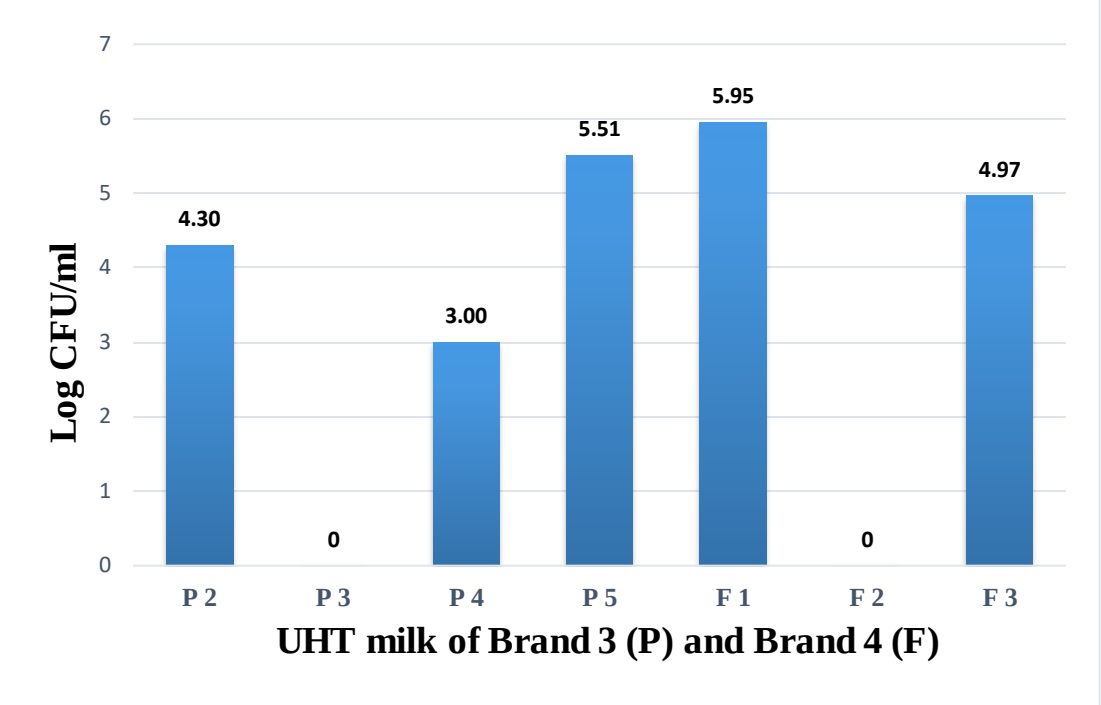


Figure 9: Total viable bacterial count of the sample from Brand 3 & Brand 4

4.2 Total coliform count

The total coliform count of raw milk ranged between 0-7.73 $\log_{10}$ with a mean of 1.23 ± 0.25 standard deviation while the values for commercial milk ranged between 0-6.7 $\log_{10}$ with a mean of 0.87 ± 0.36 standard deviation.

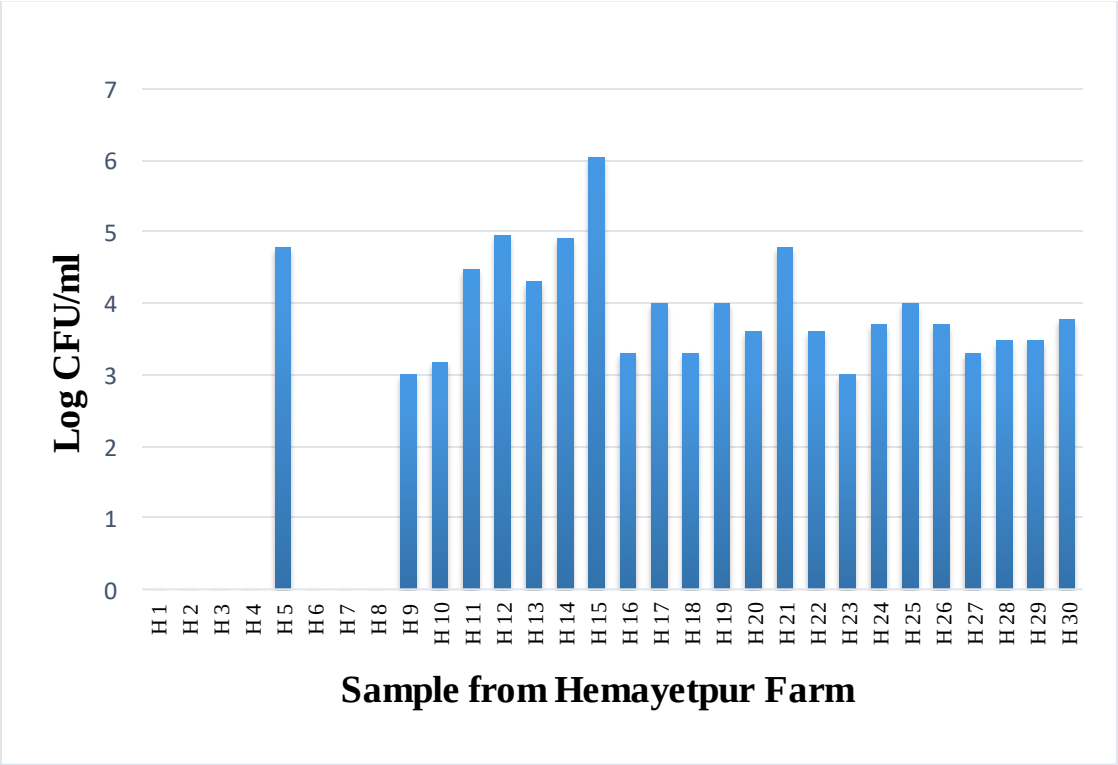


Figure 10: Total Coliform Count of the sample from Hemayetpur farm

From 30 samples, 4 samples show Grade A in The USA standard, Grade A pasteurized milk in New York standard, Extra superior bacteriological quality in Denmark standard, very good according to Indian standard and meet the BSTI standard cumulatively in Standard plate count and coliform bacterial count. Three samples meet the criteria for Grade A in The USA standard, Grade A pasteurized milk in New York standard, Extra superior bacteriological quality in Denmark standard, very good according to Indian standard and BSTI standard in the total coliform count.

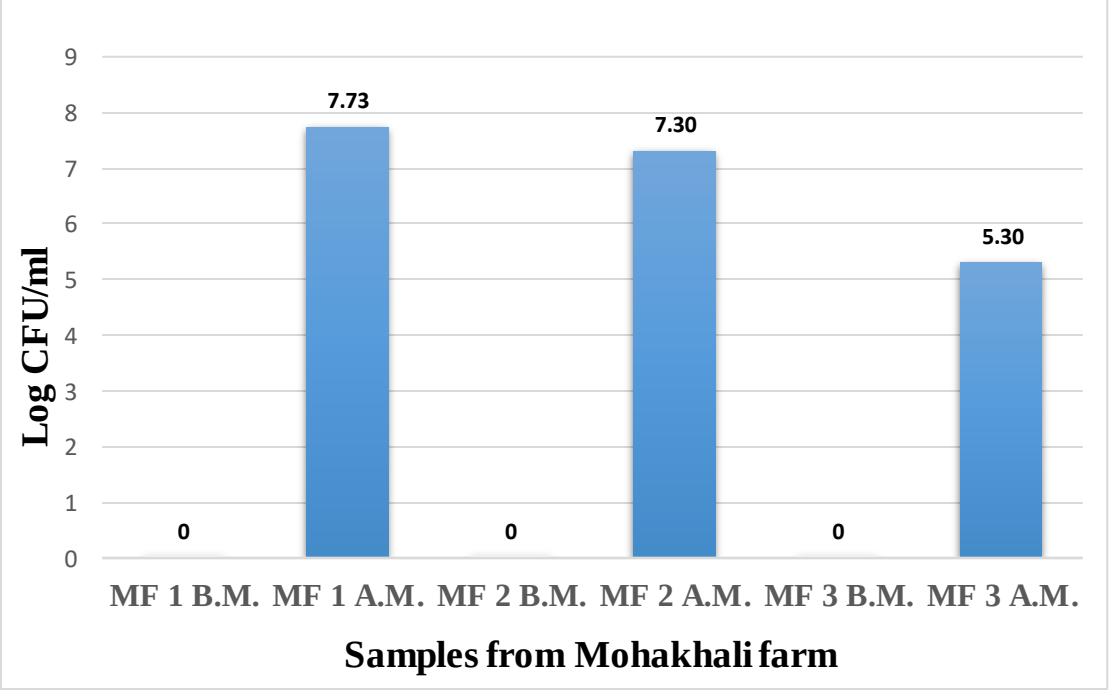


Figure 11: Total Coliform Count of the sample from Mohakhali Farm

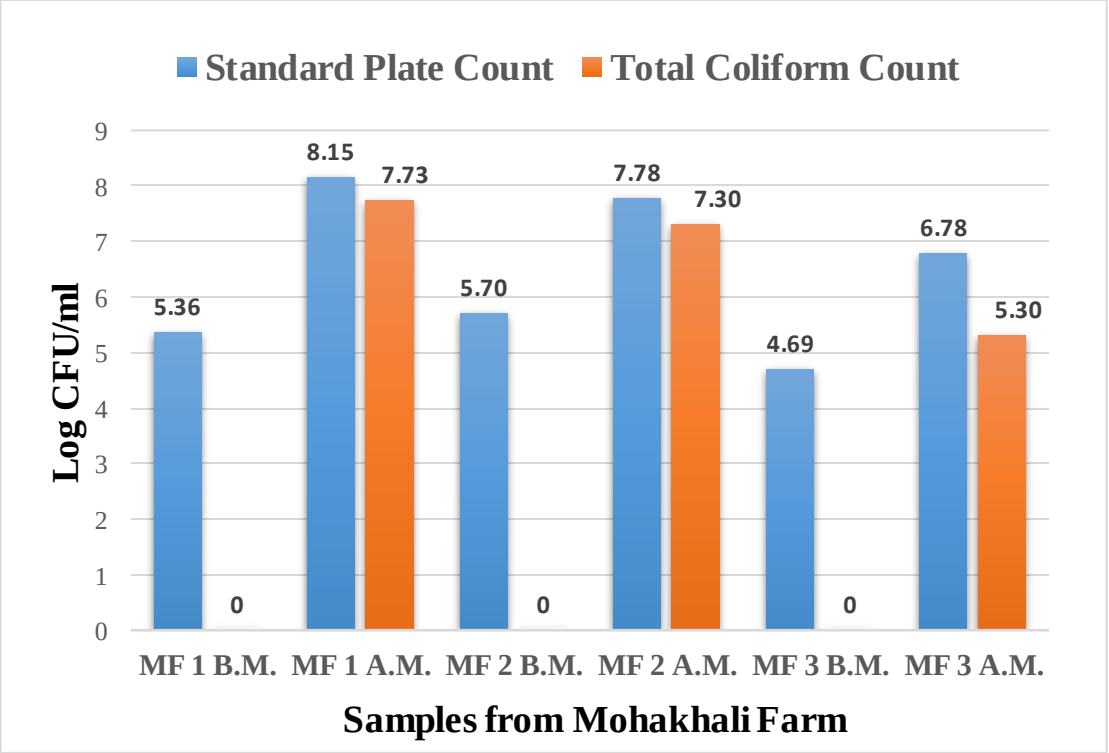


Figure 12: Total viable bacterial count and Coliform Count of the sample from Mohakhali farm

From Mohakhali farm, individually 3 samples show Grade A in The USA standard, Grade A pasteurized milk in New York standard, European Union standard, Extra superior bacteriological quality in Denmark standard, very good according to Indian standard and meet the BSTI standard. One sample shows Grade A in The USA standard, Pre-pasteurized milk for Grade A use in New York standard, European Union standard, Satisfactory bacteriological quality in Denmark standard, very good according to Indian standard and meets the BSTI standard cumulatively with standard plate count and coliform bacterial count. Two samples meet the criteria of Grade B of The USA standard, Less satisfactory bacteriological quality of Denmark standard, and good category of Indian standard.

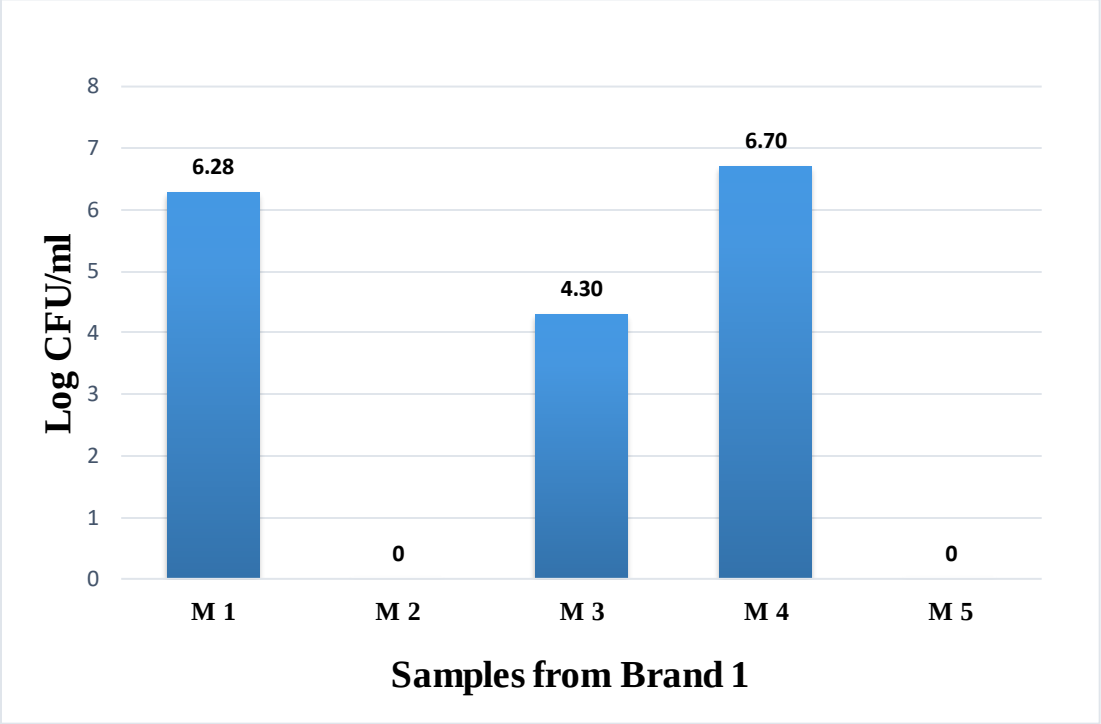


Figure 13: Total Coliform Count of the sample from Brand 1

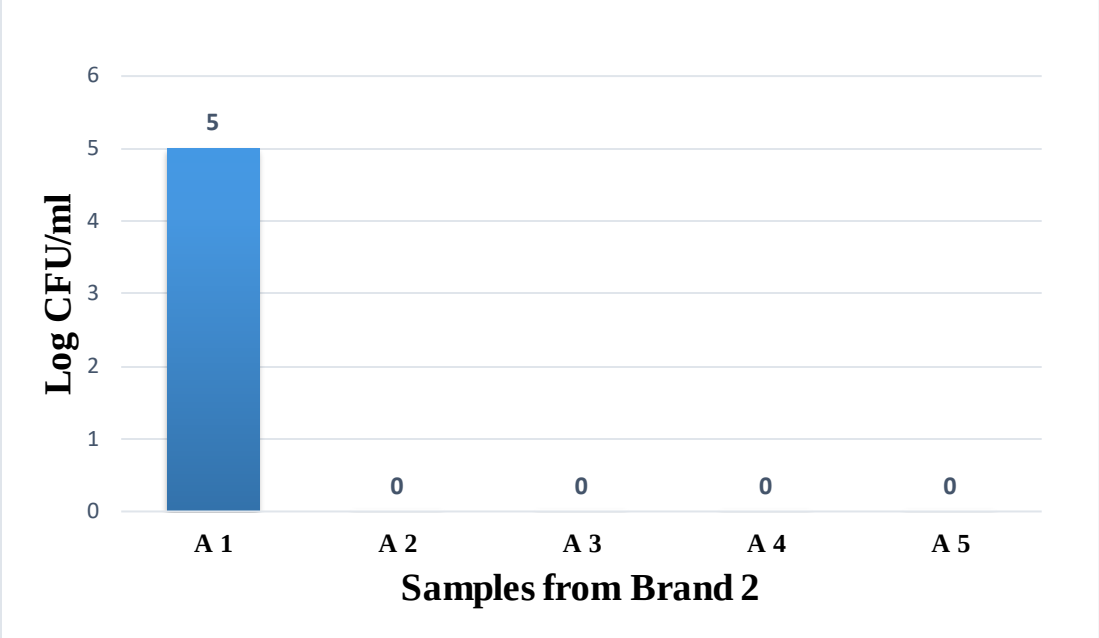


Figure 14: Total Coliform Count of the sample from Brand 2

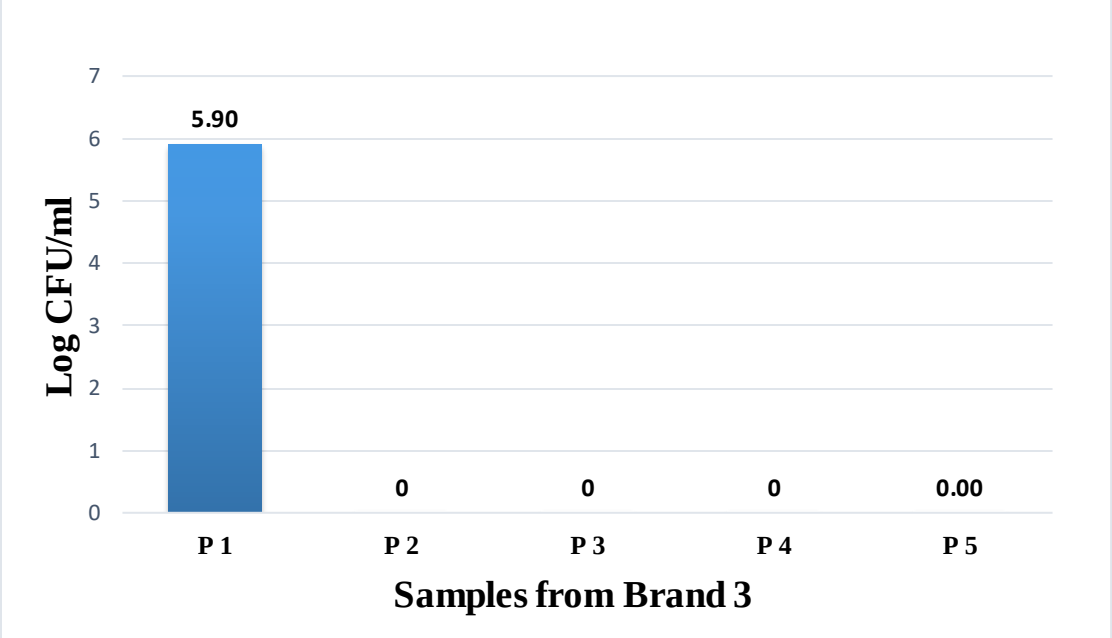


Figure 15: Total Coliform Count of the sample from Brand 3

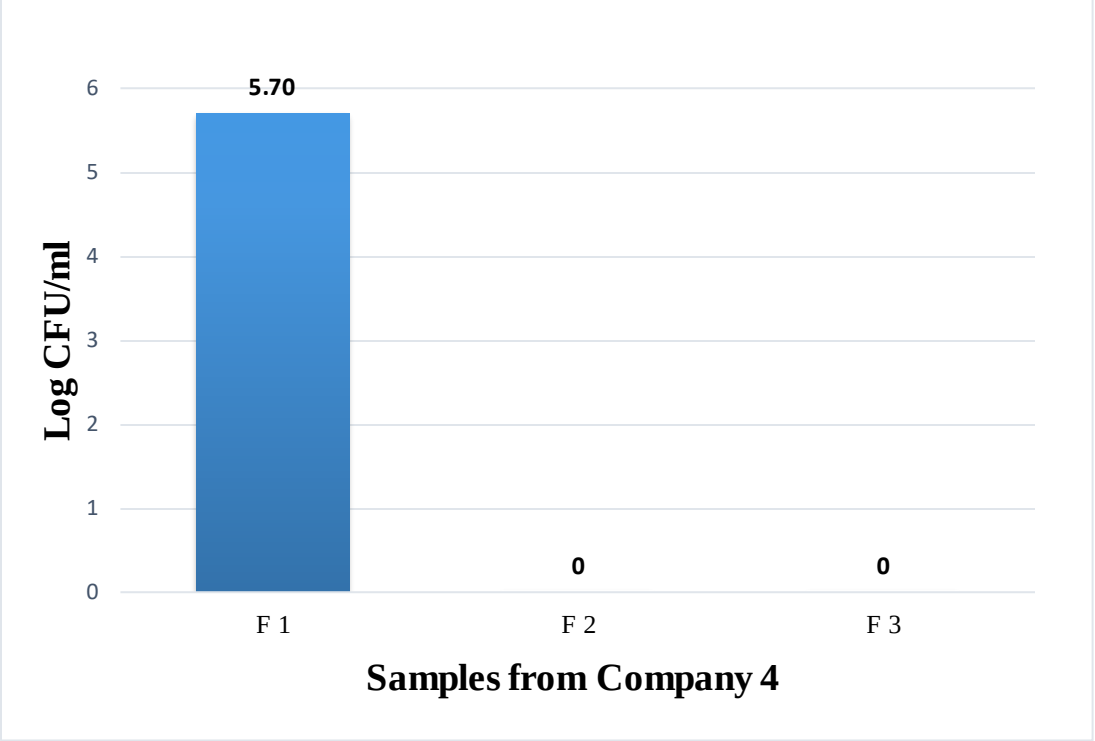


Figure 16: Total Coliform Count of the sample from Brand 4

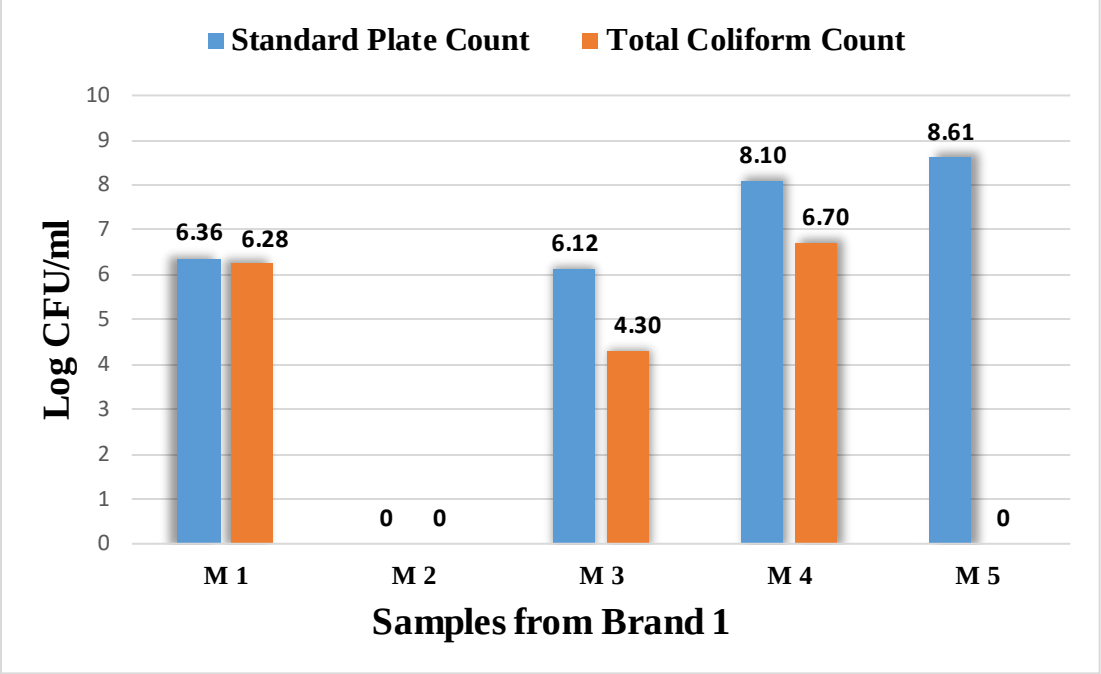


Figure 17: Total viable microbial and Coliform Count of the sample from Brand 1

From Brand 1, 2 samples show Grade A in The USA standard, Grade A pasteurized milk in New York standard, Extra superior bacteriological quality in Denmark standard, very good according to Indian standard and meet the BSTI standard and 1 sample meets Grade A in The USA standard, Grade A pasteurized milk in New York standard, European union standard, Extra superior bacteriological quality in Denmark standard, very good according to Indian standard and meets the BSTI standard cumulatively with standard plate count and coliform bacterial count.

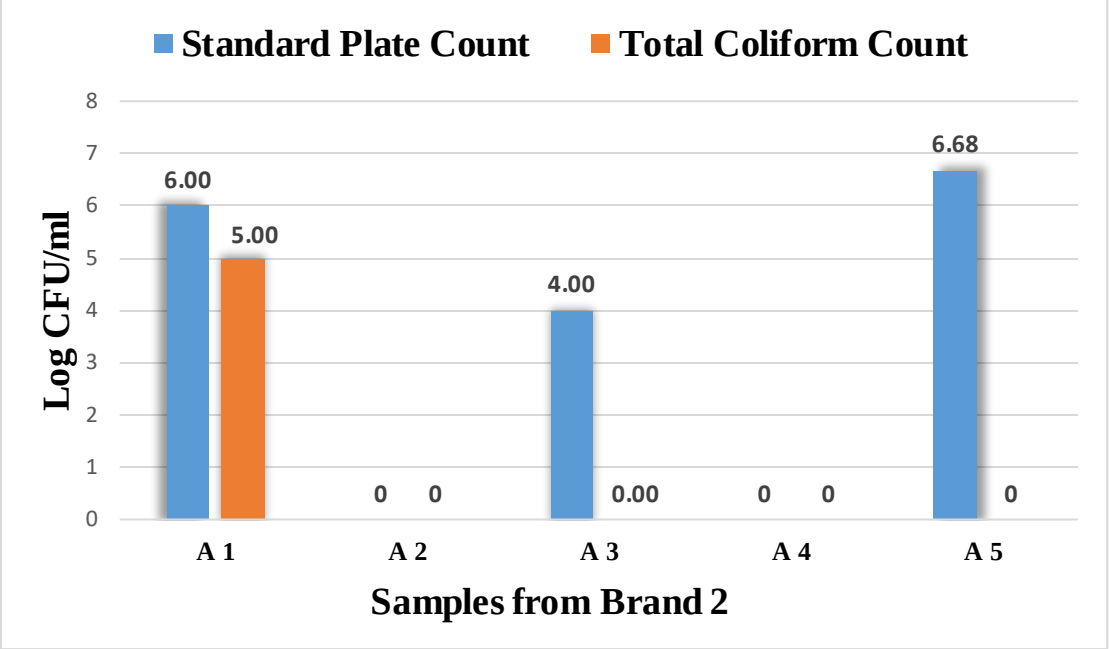


Figure 18: Total viable microbial and coliform Count of the sample from Brand 2

In the case of Brand 2, 3 samples meet Grade A in The USA standard, Grade A pasteurized milk in New York standard, European union standard, Extra superior bacteriological quality in Denmark standard, very good according to Indian standard, and meets the BSTI standard cumulatively with standard plate count and coliform bacterial count. One sample meets Grade C in The USA standard, poor according to Indian standard cumulatively.

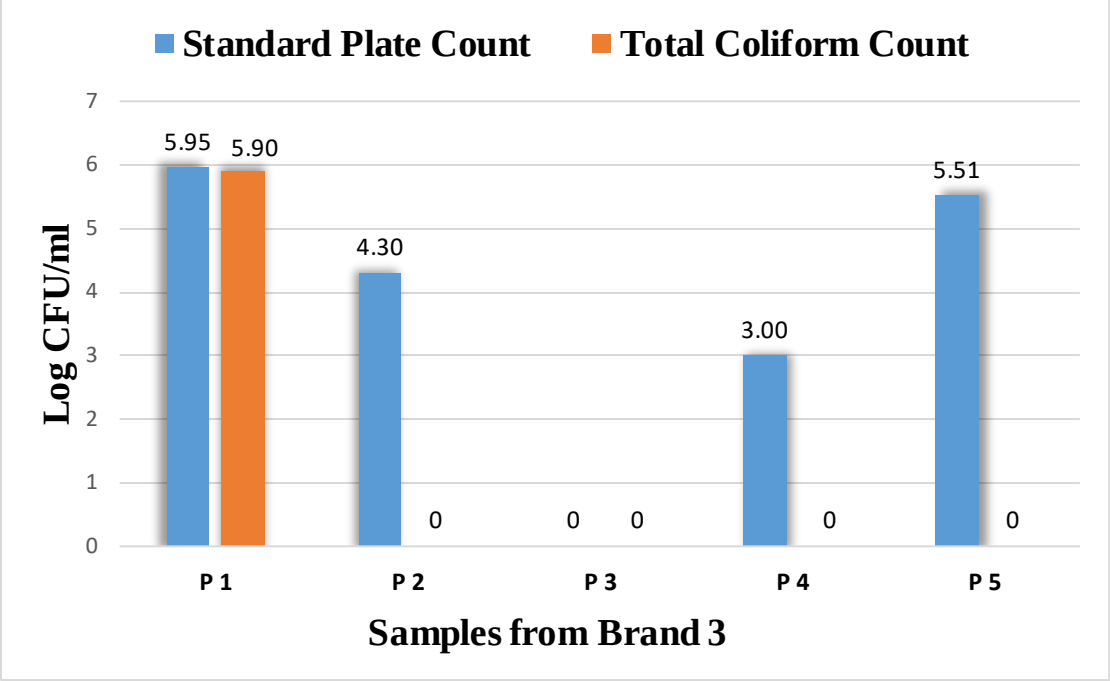


Figure 19: Total viable microbial and coliform count of the sample from Brand 3

For Brand 3, 4 samples meet Grade A in The USA standard, Grade A pasteurized milk in New York standard, European union standard, Extra superior bacteriological quality in Denmark standard, very good according to Indian standard and meet the BSTI standard cumulatively with standard plate count and coliform bacterial count. One sample meets Grade C in The USA standard, non-satisfactory bacteriological quality in Denmark standard, good according to Indian standard cumulatively.

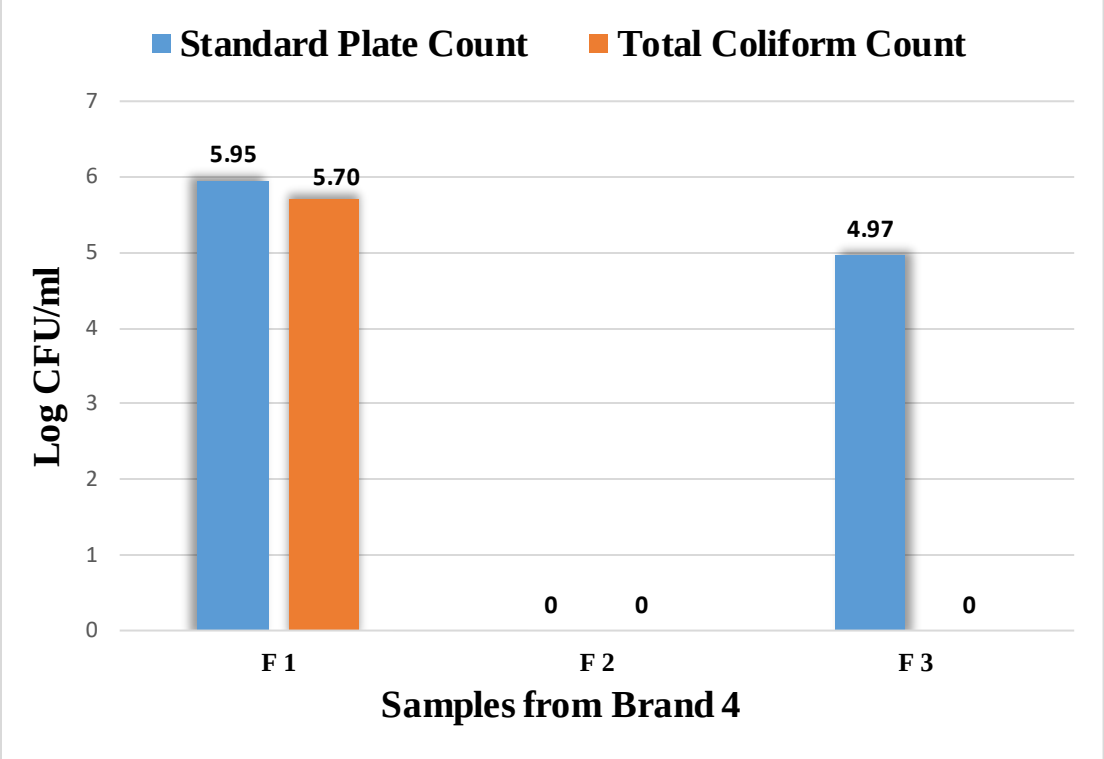


Figure 20: Total viable microbial and coliform count of the sample from Brand 4

For Brand 4, 1 sample meets meet Grade A in The USA standard, Grade A pasteurized milk in New York standard, European union standard, Extra superior bacteriological quality in Denmark standard, very good according to Indian standard and meets the BSTI standard cumulatively with standard plate count and coliform bacterial count. One sample meets Grade A in The USA standard, Pre-pasteurized milk for Grade A use in New York standard, European union standard, satisfactory bacteriological quality of Denmark standard, very good according to Indian standard and meets the BSTI standard cumulatively with standard plate count and coliform bacterial count.

4.3 Identification of *Salmonella*

From all the samples isolated 34 samples showed the characteristic of *Salmonella*. Among them 25 were observed from raw milk gathered from different farms, 8 were from the pasteurized milk, and 1 from the UHT milk.

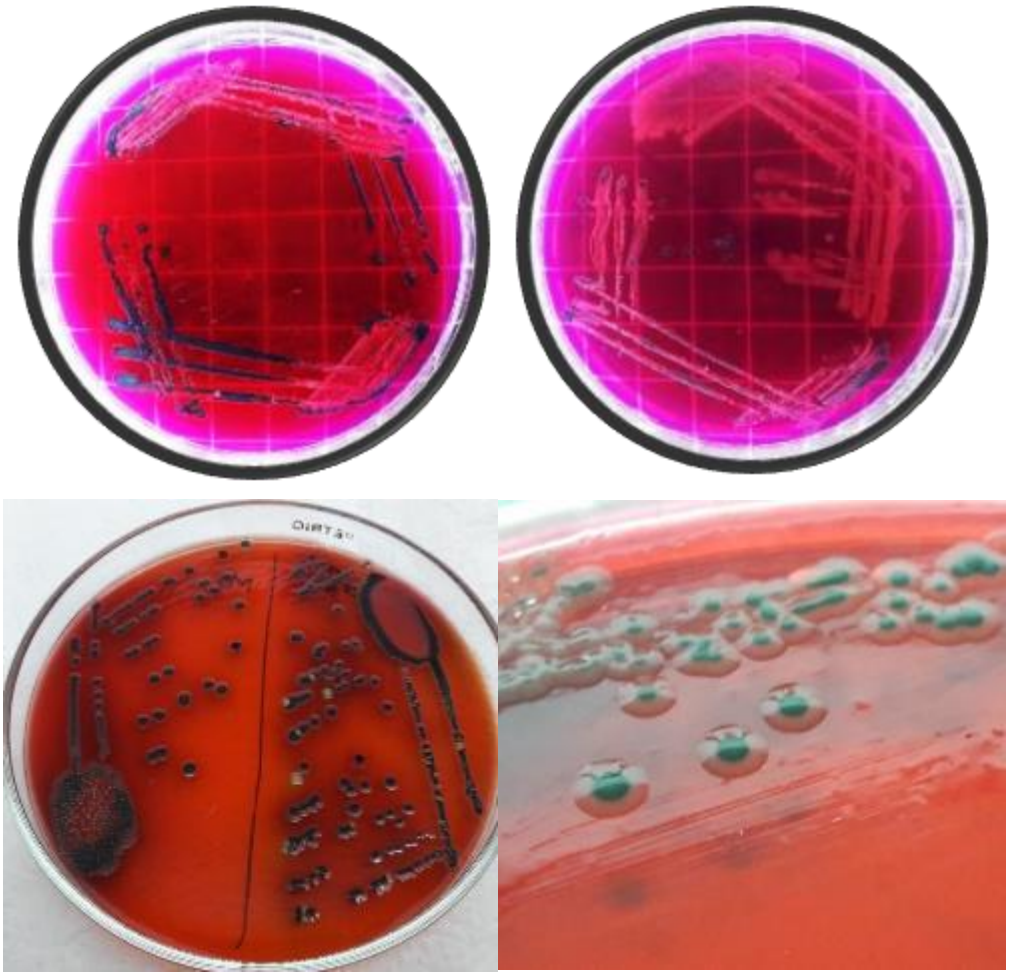


Figure 21: Isolation and colony characteristics of Salmonella in XLD-Agar.

4.4 Identification of *Staphylococcus*

Twenty-seven samples showed characteristics in mannitol salt agar as pink, yellow and white colony.

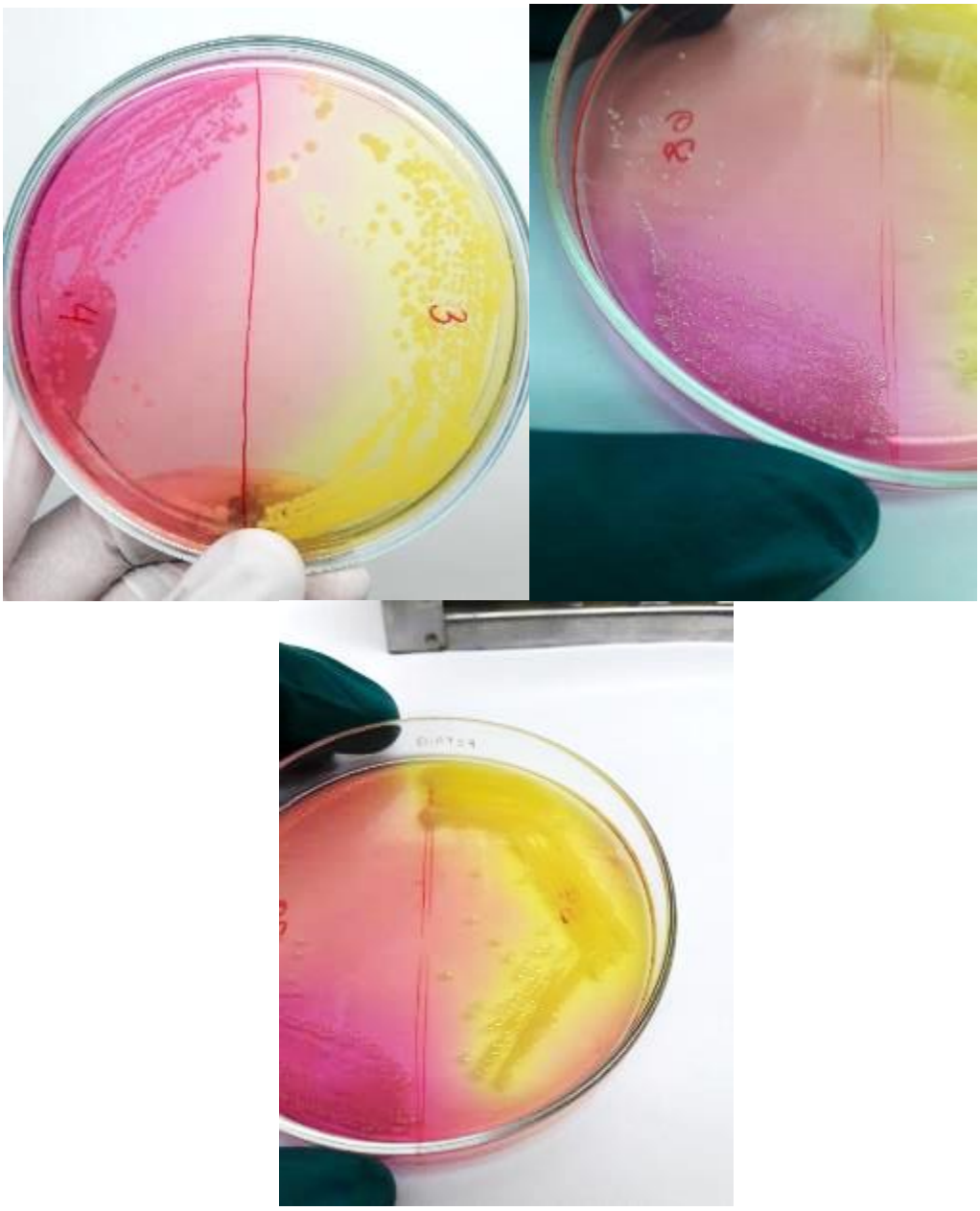


Figure 22: Isolation of Staphylococcus morphological characteristics in Mannitol Salt Agar.

The same sample that was given in mannitol salt agar was inoculated in Baird parker agar (BPA) and 27 isolates that grew on mannitol salt agar were also inoculated on BPA. Total 27 showed the characteristic of a Black colony and gray colony with an opaque zone in BPA.

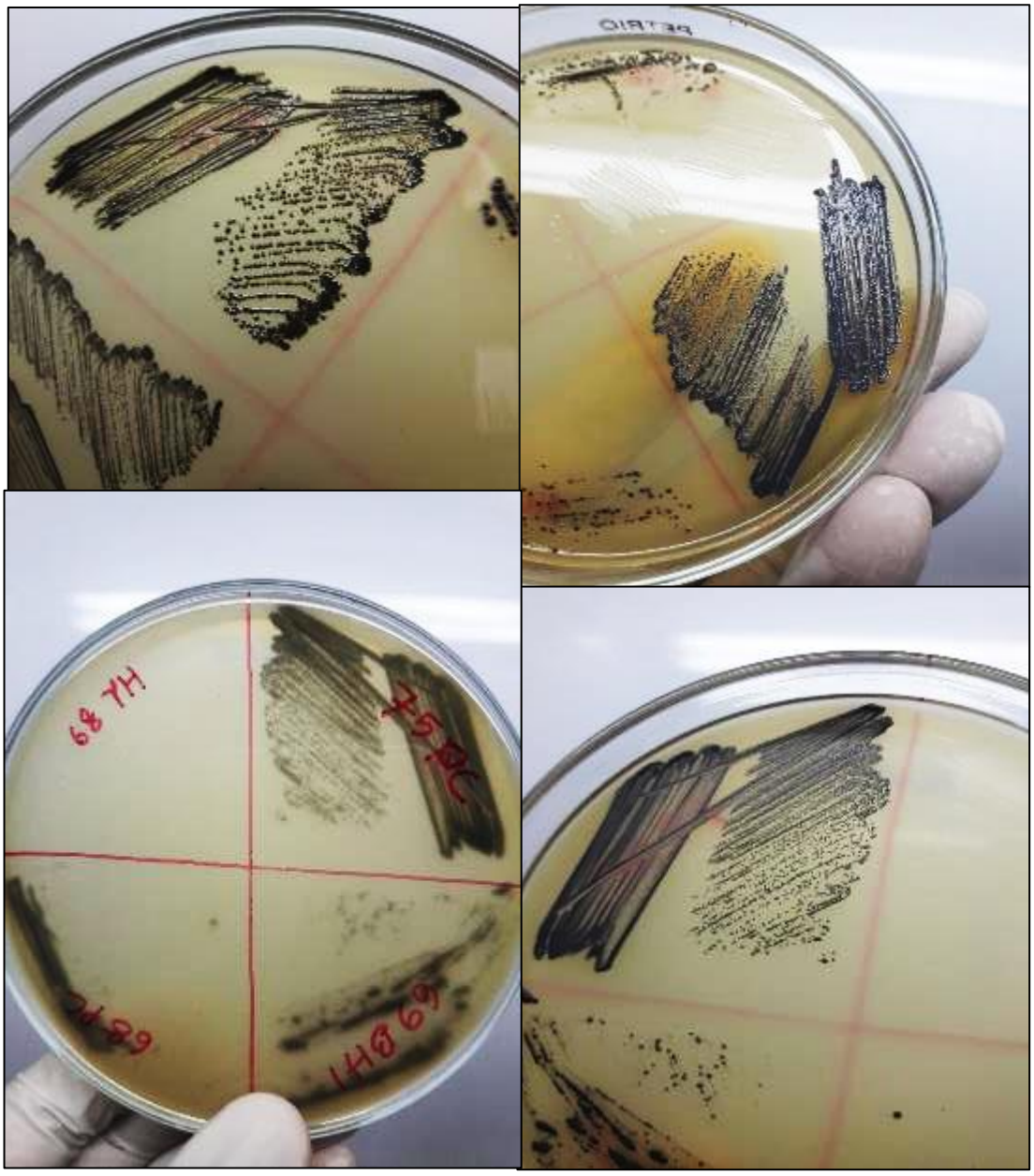


Figure 23: Isolation of Staphylococcus in Baird parker agar.

The samples were sub cultured 3 times to get pure colonies and all of the 27-sample showed growth in BPA.

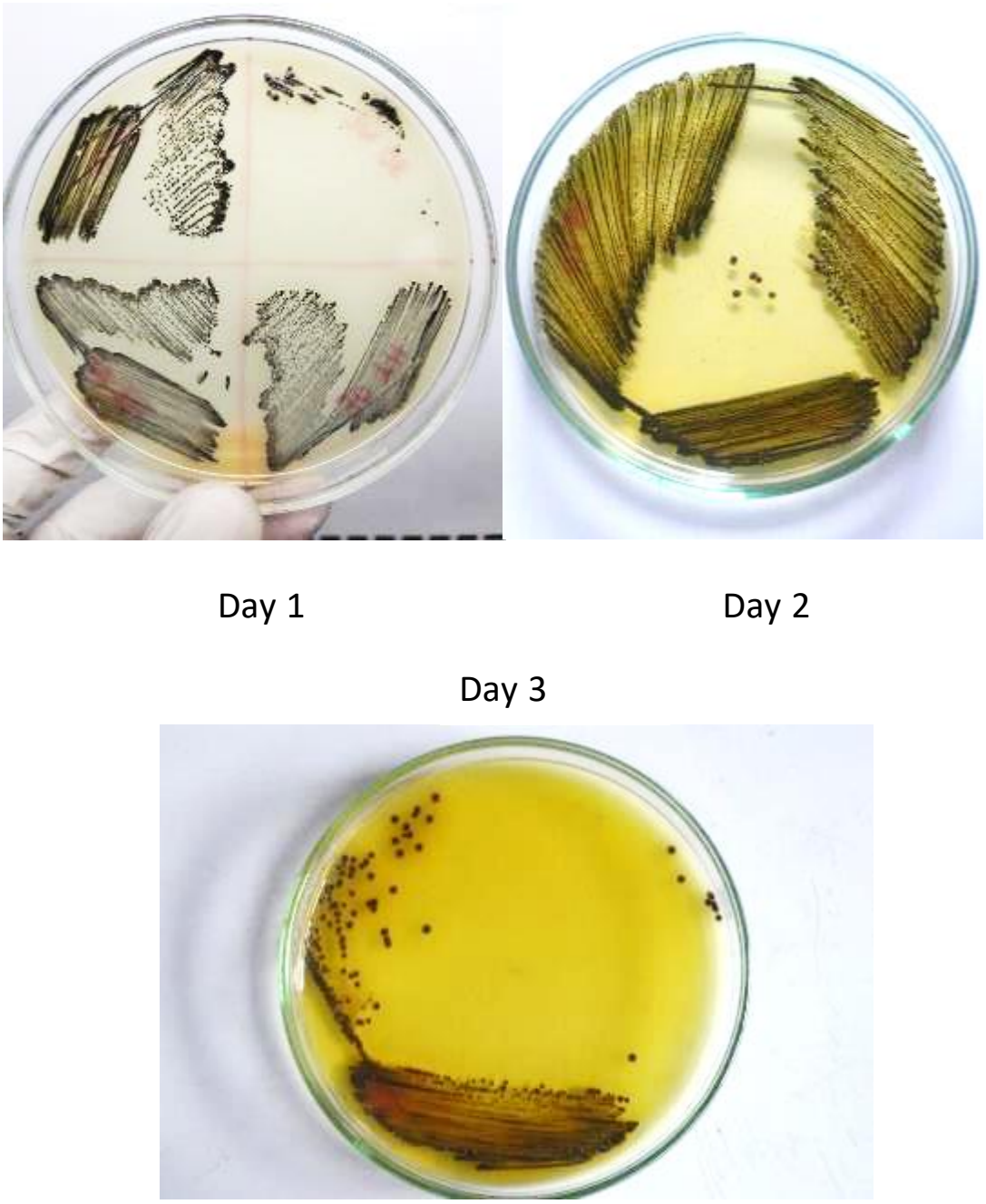


Figure 24: Subculturing of Staphylococcus in Baird parker agar.

4.5 Gram Staining

A total of 113 isolates were isolated from 61 milk samples. Different types of colonies were observed and according to gram staining, gram-positive and gram-negative isolates were found among the samples. Raw milk showed the highest number of gram-positive isolates with 41, pasteurized milk showed 24-gram positive isolates and UHT milk showed 8-gram positive isolates. Gram-negative isolates were a little less than gram-positive isolates. Raw showed 28-gram negative organism, pasteurized milk showed 7 isolates and UHT milk showed 5 isolates.

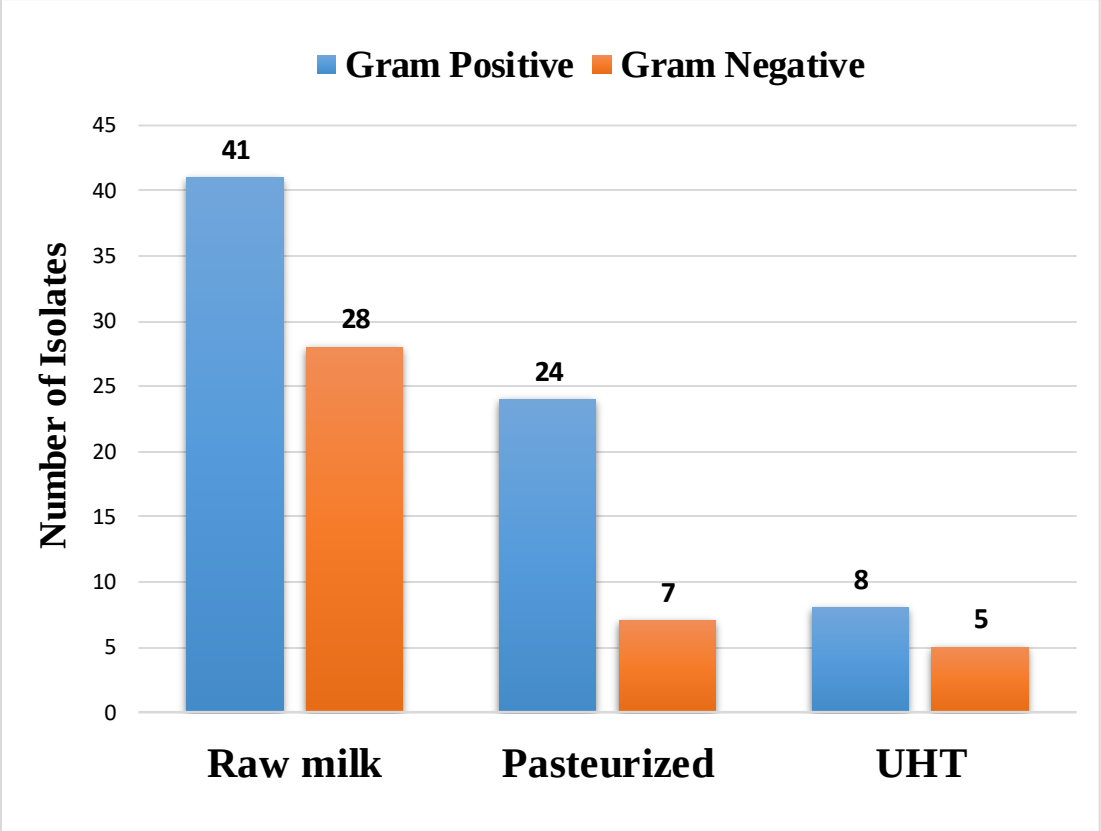


Figure 25: Gram-positive & gram-negative organism found in different types of sample

Depending on the morphological characteristics of round shape and round shape the analysis was done. Raw milk showed the highest number of round shape gram-positive

isolates (28), 10 in pasteurized milk, 8 in UHT milk. Round gram-negative isolates were found 3 in raw milk, 1 in pasteurized milk, 1 in UHT treated milk.

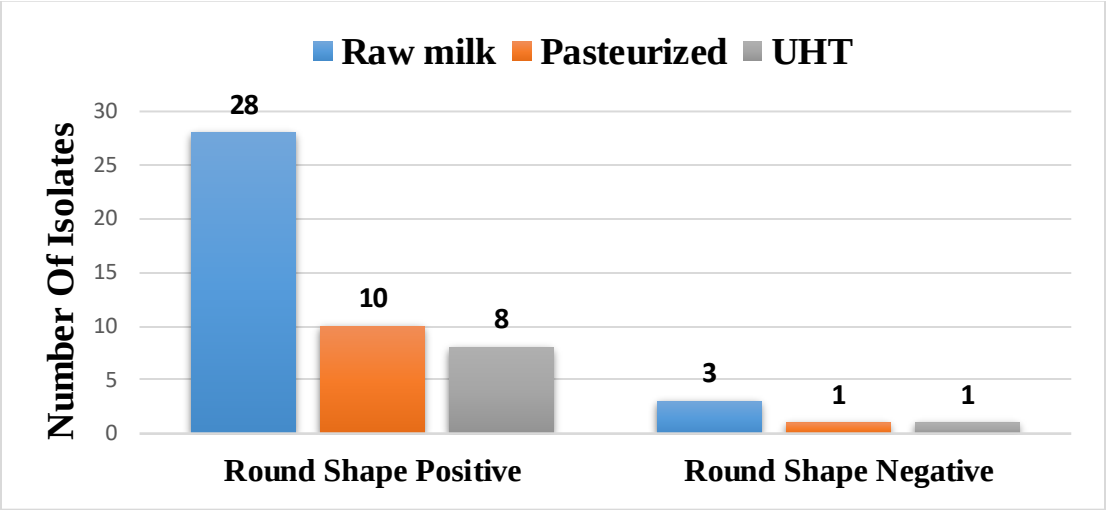


Figure 26: Morphologically round shape organism found in different types of sample

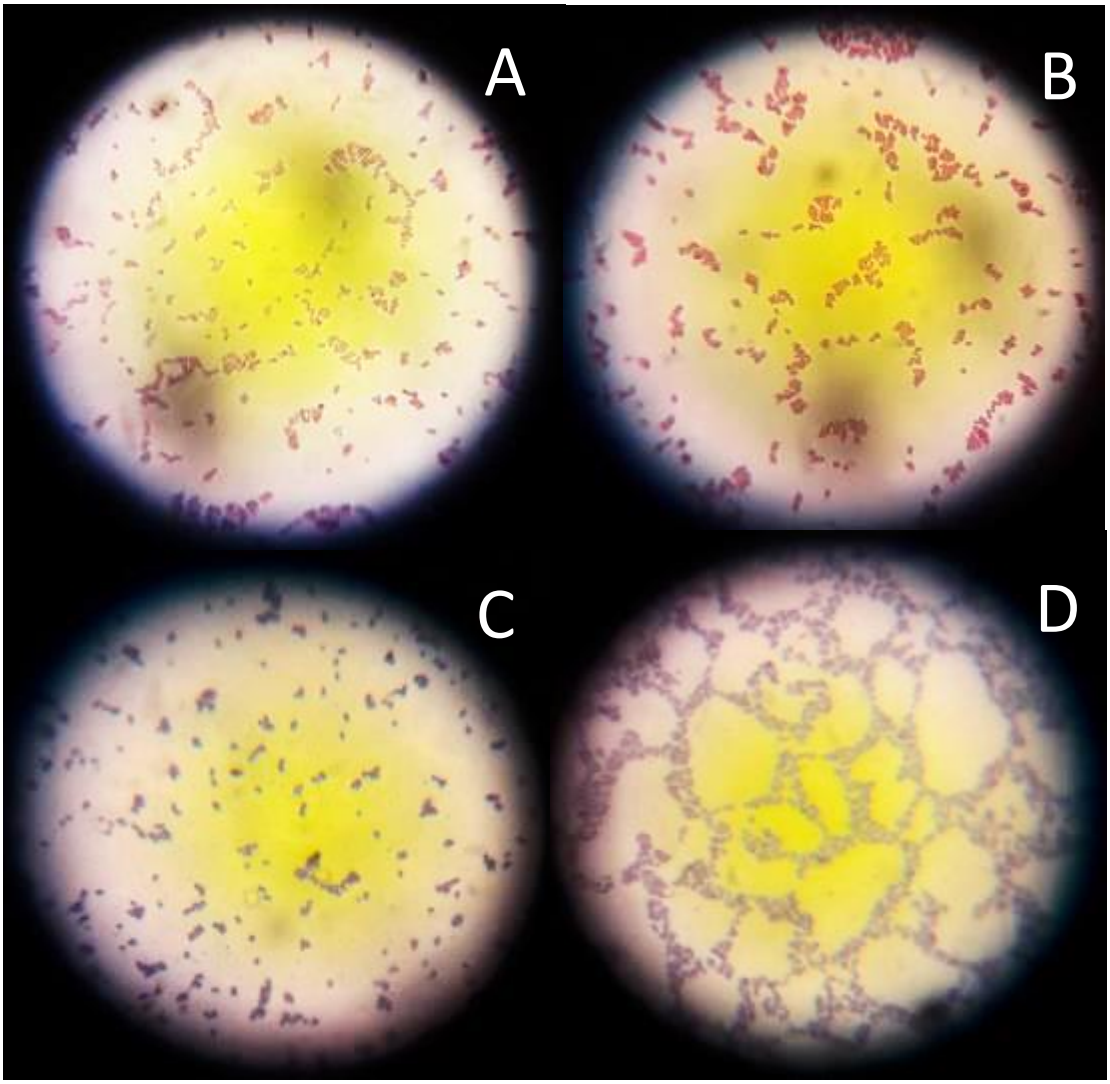


Figure 27: Gram-positive and negative organism of the round shape. Round shape gram-positive organism (A), (B). round shape negative organism (C), (D).

Raw milk showed 13-rod shape gram-positive isolates, 14 in pasteurized milk, and none in UHT milk. Rod gram-negative isolates were found highest in raw milk (25), 6 in pasteurized milk, 4 in UHT treated milk.

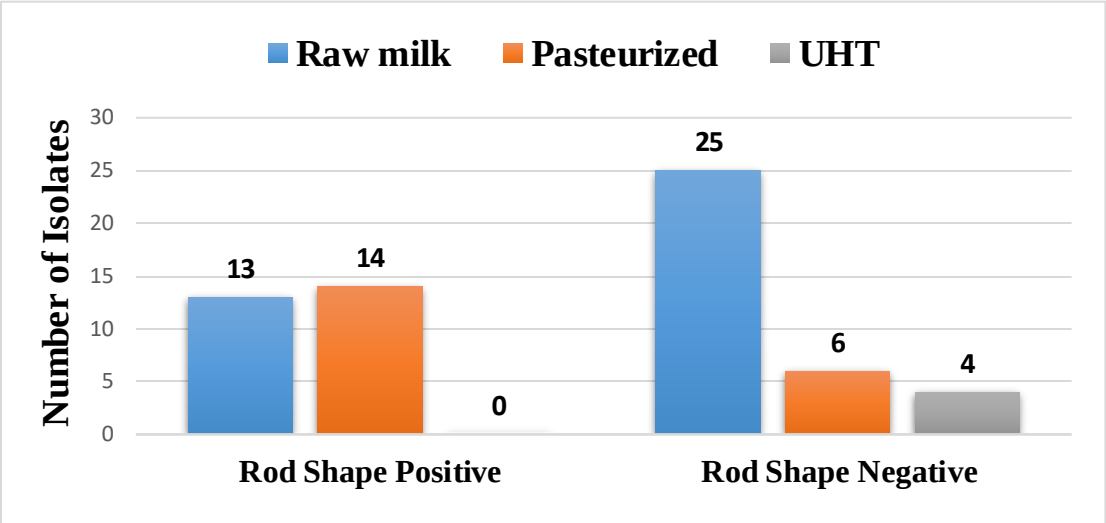


Figure 28: Morphologically round shape organism found in different types of sample

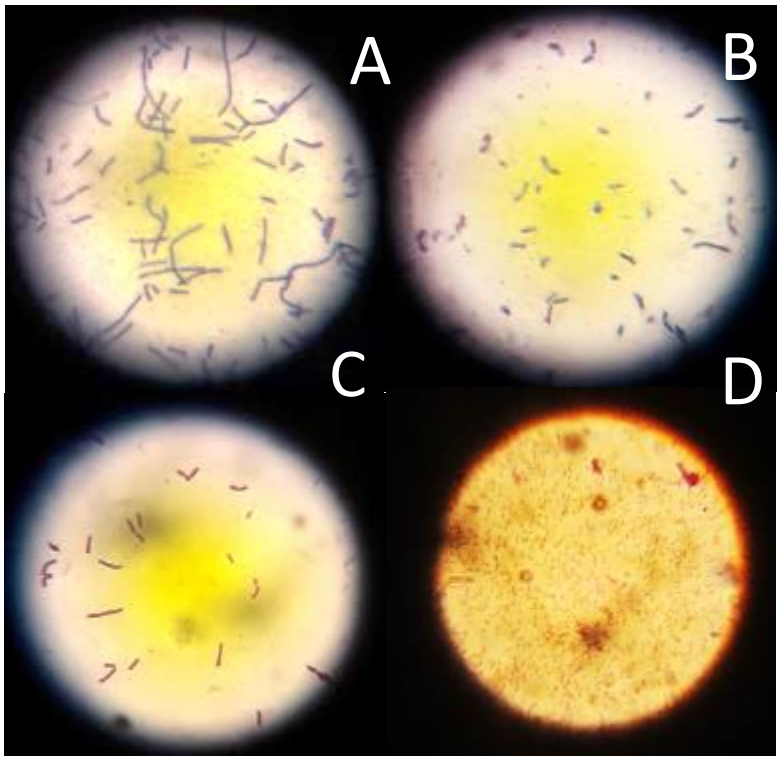


Figure 29: Gram-positive and negative organism of rod shape. Rod shape gram-positive organism (A), (B). rod shape negative organism (C), (D).

4.6 Biochemical Identification

Biochemical identification was for 34 *Salmonella* isolates. 22 was positive in case of methyl red test among them 14 sample was from raw milk and 8 sample was from pasteurized commercial milk and none from UHT milk.

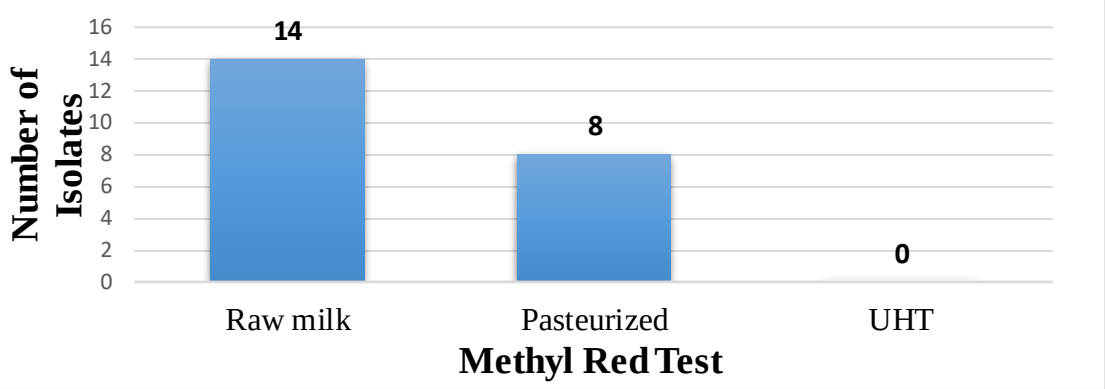


Figure 30: Methyl red test positive sample among different type of samples

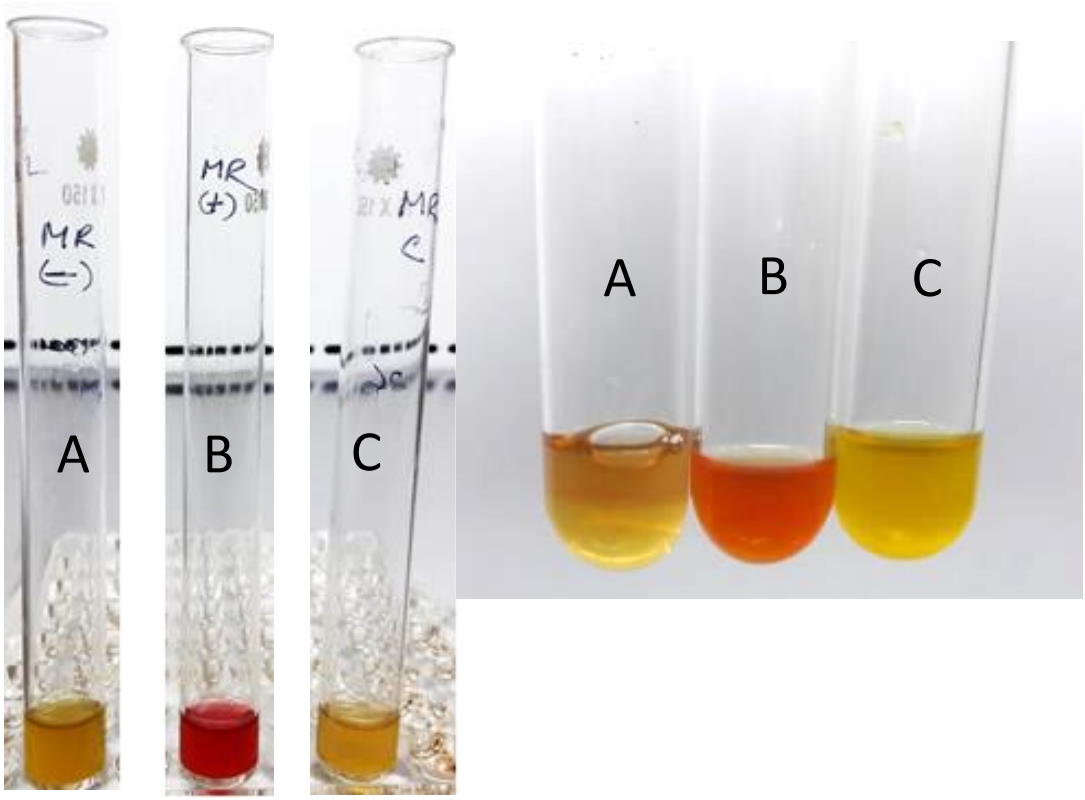


Figure 31: Methyl red test. Methyl red test negative (A), Methyl red test positive (B), Methyl red test control negative (C).

Total 23 isolates showed Voges-Proskauer test negative among which 15 were from raw milk, 7 from pasteurized milk, and 1 from UHT milk.

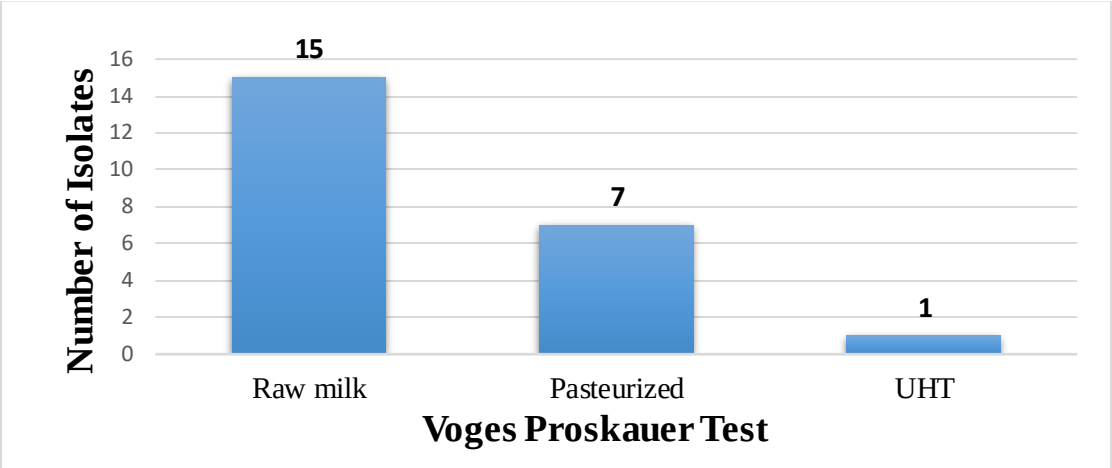


Figure 32: Voges-Proskauer test positive sample among different type of sample

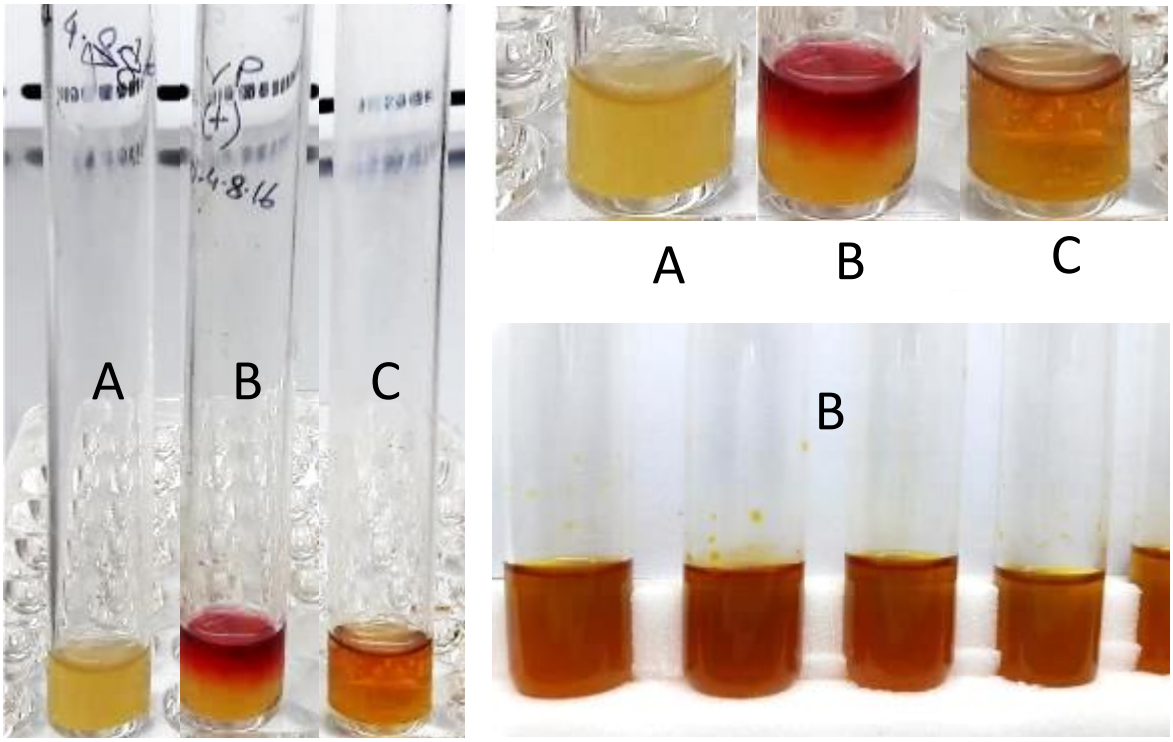


Figure 33: Voges Proskauer test. Voges Proskauer test control negative (A), Voges Proskauer test positive (B), Voges Proskauer test negative (C).

9 samples showed positive in indole test among them 6 from raw milk, 3 from pasteurized milk, and none from UHT milk.

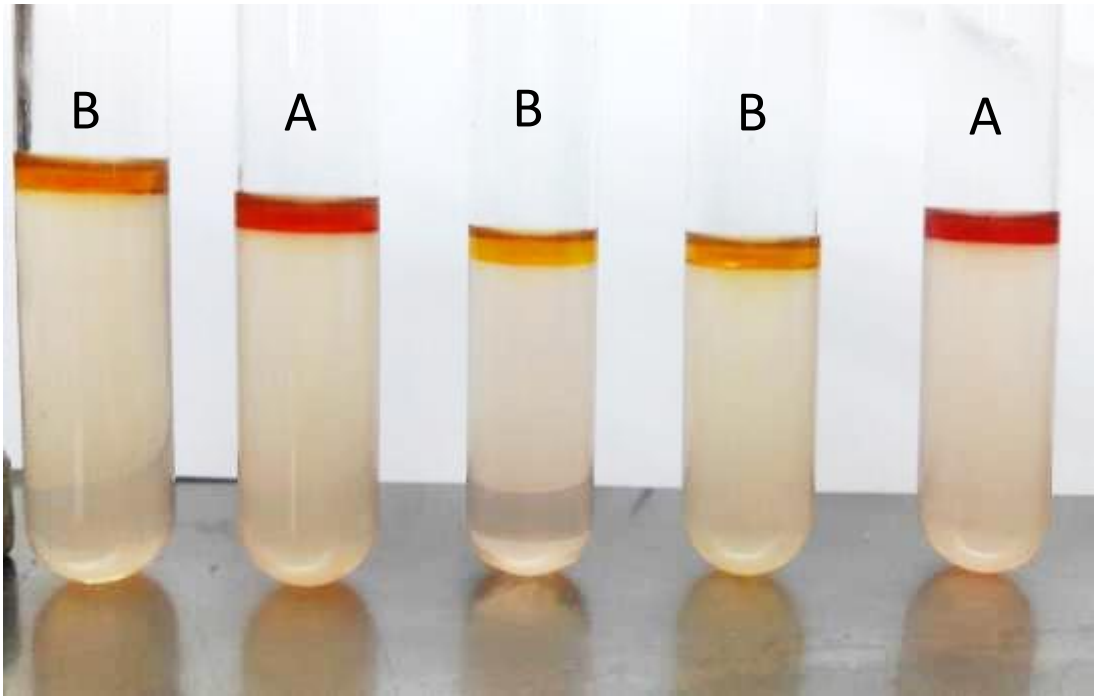


Figure 34: Indole test. Indole test positive (A), Indole test negative (B).

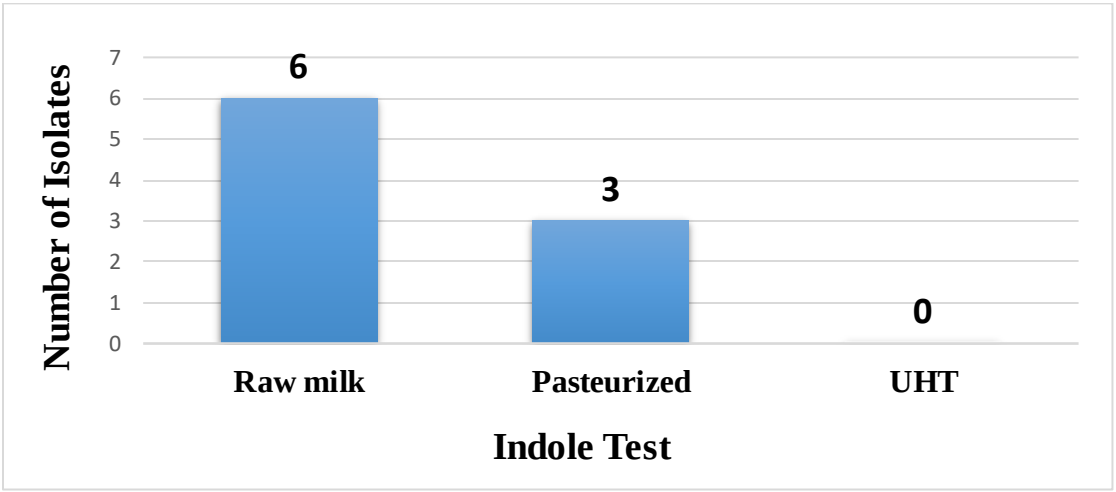


Figure 35: Indole test positive sample among different types of samples

4.7 Triple Sugar Iron Test

Five samples showed characteristics of reaction, which showed positive result in the case of *Salmonella*.

Table 3: Triple sugar iron test and interpretation

Isolate number	Slant Color	The butt of Tube's Color	Gas production	H_2S production
Isolate 1	Yellow	Black + Red	✓	✓
Isolate 2	Yellow	Black + Red	✓	✓
Isolate 3	Yellow	Black + Red	✓	✓
Isolate 4	Red	Black	×	✓
Isolate 5	Yellow	Black + Red	✓	✓
Isolate 6	Yellow	Black + Red	✓	✓
Positive control	Yellow	Black + Red	✓	✓

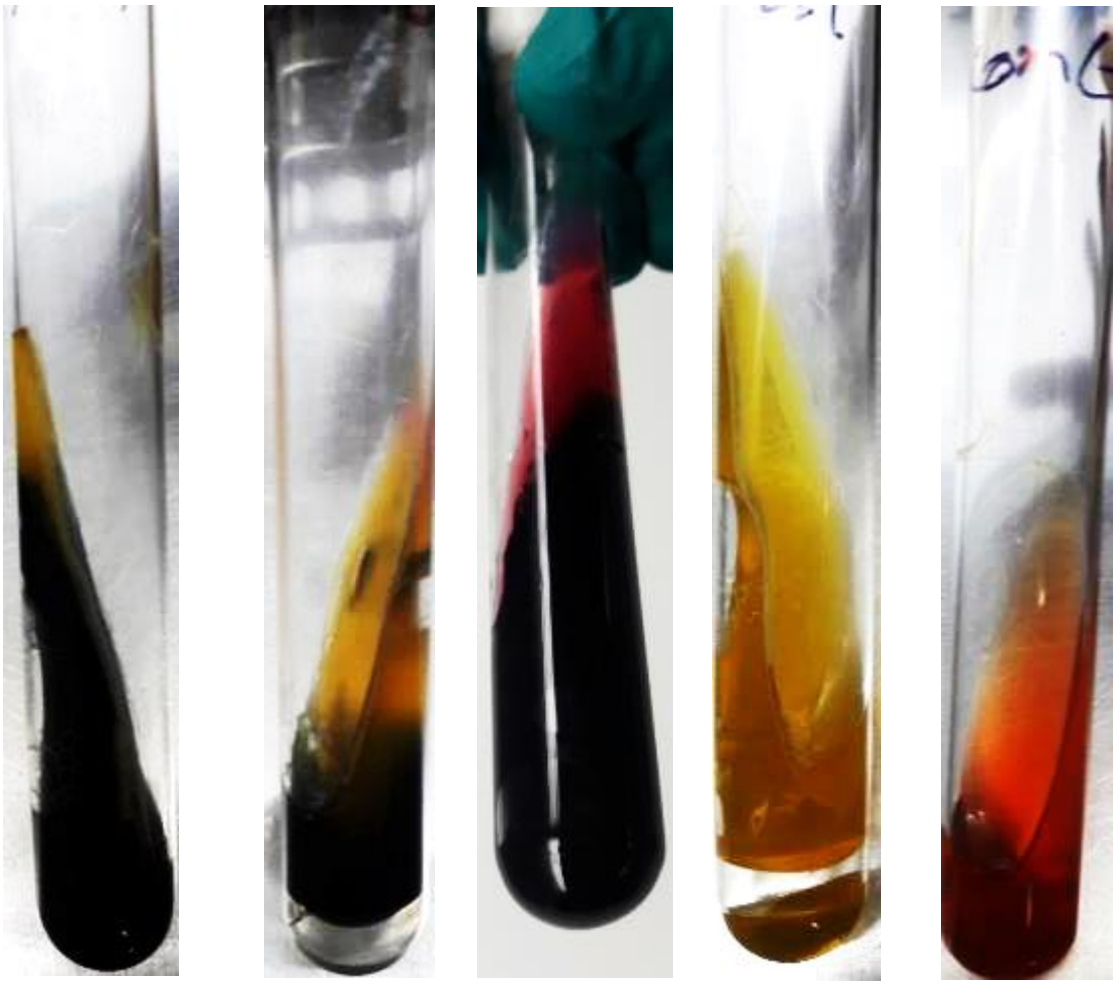


Figure 36: Formation of hydrogen sulfide indicative by black precipitation at the butt of the test tube, yellow at the slant indicates alkaline, bottom light red indicates acid production, and gas formation is indicative of carbohydrate fermentation (A) and (B).

4.8 Antimicrobial Susceptibility

Table 4: shows the twenty-three (23) antimicrobial agents used for the susceptibility testing of the 20 *Salmonella* isolates. All 20 (100%) isolates were susceptible to imipenem (100%) while 18 (90%) isolates were susceptible to amoxicillin/clavulanic acid. Fifteen (75%) isolates were susceptible to Chloramphenicol, fourteen (70%)

isolates were susceptible to Cefotaxime, twelve (60%) isolates were susceptible to Ciprofloxacin. Eleven (55%) isolates were susceptible to Cefixime, ten (50%) isolates were susceptible to gentamicin, levofloxacin, norfloxacin. Eight (40%) isolates were susceptible to ampicillin, streptomycin. Six (30%) isolates were susceptible to kanamycin and 3 (15%) isolates were susceptible to Co-trimoxazole.

Based on the antibiotic susceptibility test, all 20 (100%) isolates were resistant to tetracycline, pefloxacin, oxytetracycline, nalidixic acid, erythromycin, doxycycline followed by enrofloxacin, cefepime (85%) then azithromycin (75%), and gentamycin (50%).

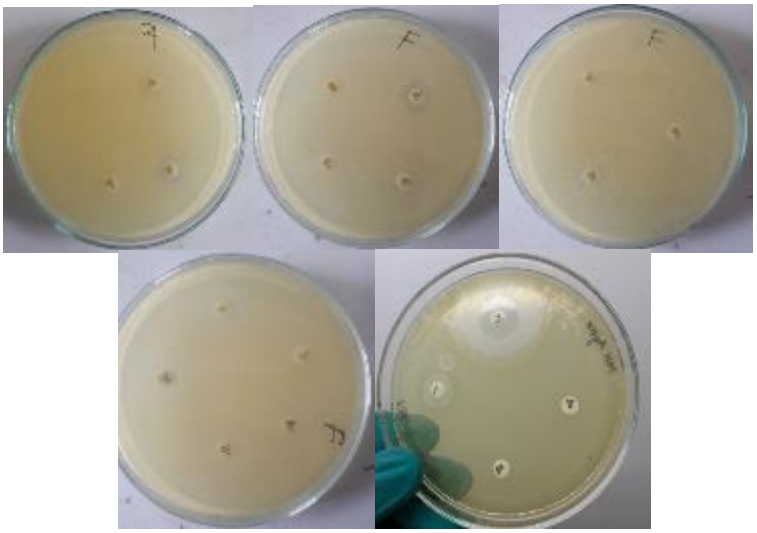


Figure 37: Antibiogram test plates for interpretation of the result

Sl. No.	Antibiotic Name	Concentration	No. of *Salmonella* isolates susceptible	% of *Salmonella* isolates susceptible	No. of *Salmonella* isolates resistant	% of *Salmonella* isolates resistant
1	Amoxicillin/Clavulanic acid	AMC 30µg	18	90	1	5
2	Ampicillin	AMP 10µg	8	40	6	30
3	Azithromycin	AZM 15µg	-	-	15	75
4	Cefixime	CFM 5µg	11	55	4	20
5	Chloramphenicol	C 30µg	15	75	4	20
6	Ciprofloxacin	CIP 5µg	12	60	6	30
7	Ceftriaxone	CTR 30µg	11	55	5	25
8	Cefotaxime	CTX 30µg	14	70	6	30
9	Doxycycline	DO 30µg	-	-	20	100
10	Enrofloxacin	ENR 5µg	-	-	17	85
11	Erythromycin	E 15µg	-	-	20	100
12	Cefepime	FEP 30µg	-	-	17	85
13	Gentamicin	CN 10µg	10	50	10	50
14	Imipenem	IPM 10µg	20	100	-	-
15	Kanamycin	K 30µg	6	30	7	35
16	Levofloxacin	LEV 5µg	10	50	7	35
17	Nalidixic acid	NA 30µg	-	-	20	100
18	Norfloxacin	NOR 10µg	10	50	3	15
19	Oxytetracycline	OT 30µg	-	-	20	100
20	Pefloxacin	PEF 5µg	-	-	20	100
21	Streptomycin	S 10µg	8	40	7	35
22	Co-trimoxazole	SXT 25µg	3	15	17	85
23	Tetracycline	TE 10µg	-	-	20	100

Table 4: Salmonella antibiogram result of antibiotic susceptibility and resistance

Key

Amoxicillin/Clavulanic acid (AMC 30µg), Ampicillin (AMP 10µg), Azithromycin (AZM 15µg), Cefixime (CFM 5µg), Chloramphenicol (C 30µg), Ciprofloxacin (CIP 5µg), Ceftriaxone (CTR 30µg), Cefotaxime (CTX 30µg), Doxycycline (DO 30µg), Enrofloxacin (ENR 5µg), Erythromycin (E 15µg), Cefepime (FEP 30µg), Gentamicin (CN 10µg), Imipenem (IPM 10µg), Kanamycin (K 30µg), Levofloxacin (LEV 5µg), Nalidixic acid (NA 30µg), Norfloxacin (NOR 10µg), Oxytetracycline (OT 30µg), Pefloxacin (PEF 5µg), Streptomycin (S 10µg), Co-trimoxazole (SXT 25µg), Tetracycline (TE 10µg).

Here is a resistant pattern of 20 isolates in which tetracycline, pefloxacin, oxytetracycline, nalidixic acid, erythromycin, doxycycline is not included because of their 100% resistance in all the sample.

Sl. No.	**Isolates**	**Resistant Pattern**	**Frequency**
1	M1	ENR, FEP, K, SXT	1
2	MMO3	AZM, ENR, FEP, SXT	1
3	MMO1	AMP, ENR, FEP, K, SXT	1
4	MMO6	AZM, ENR, FEP, K, SXT	1
5	MH3	CTX, ENR, FEP, CN, S	1
6	MH1	AZM, CTX, FEP, CN, S	1
7	MS1	AMP, ENR, FEP, CN, LEV, SXT	1
8	MV4	AZM, CFM, CTR, ENR, CN, SXT	1
9	MS1	AZM, CTR, ENR, FEP, LEV, SXT	1
10	MMO2	AZM, CIP, ENR, CN, LEV, SXT	1
11	MH9	AZM, C, ENR, CN, S, SXT	1
12	MH12	AMP, AZM, CIP, ENR, FEP, IPM, SXT	1
13	MMO4	AZM, ENR, FEP, LEV, NOR, S, SXT	1
14	MM2	CTX, ENR, FEP, CN, K, S, SXT	1
15	M2	AZM, C, CTX, FEP, CN, LEV, SXT	1
16	M3	AMP, AZM, CIP, CTR, CTX, ENR, FEP, SXT	1
17	MS3	AMP, AZM, C, CTR, ENR, FEP, CN, K	1

18	MMO5	AMP, AZM, CFM, CIP, CTR, CTX, FEP, K, SXT	1
19	MS2	AZM, CFM, CIP, ENR, FEP, K, LEV, NOR, S, SXT	1
20	MM1	AMC, AZM, CFM, C, CIP, ENR, FEP, CN, LEV, NOR, S, SXT	1

Table 5: Multidrug resistance and frequency of Salmonella isolates

Key

Amoxicillin/Clavulanic acid (AMC 30µg), Ampicillin (AMP 10µg), Azithromycin (AZM 15µg), Cefixime (CFM 5µg), Chloramphenicol (C 30µg), Ciprofloxacin (CIP 5µg), Ceftriaxone (CTR 30µg), Cefotaxime (CTX 30µg), Doxycycline (DO 30µg), Enrofloxacin (ENR 5µg), Cefepime (FEP 30µg), Gentamicin (CN 10µg), Imipenem (IPM 10µg), Kanamycin (K 30µg), Levofloxacin (LEV 5µg), Norfloxacin (NOR 10µg), Streptomycin (S 10µg), Co-trimoxazole (SXT 25µg), MMO (Isolate from Mohakhali farm), MM (Isolate from Mirpur farm), M (Brand 1), MS (Isolate from Savar farm), MH (Isolate from Hemayetpur farm)

Table 6 shows ten (10) antimicrobial agents used for the susceptibility testing of the 27 Staphylococcal isolates. 26 (96.3%) isolates were susceptible to chloramphenicol while 25 (92.6%) isolates were susceptible to ciprofloxacin and gentamicin. 24 (88.9%) isolates were susceptible to nitrofurantoin, twenty-three (85.2%) isolates were susceptible to linezolid, eighteen (66.7%) isolates were susceptible to Trimethoprim/Sulphamethaxazole. Twelve (44.4%) isolates were susceptible to erythromycin and one (3.7%) isolate was susceptible to ampicillin.

Based on the antibiotic susceptibility test, all 27 (100%) isolates were resistant to penicillin followed by 26 (96.3%) isolates resistant to ampicillin, 5 (18.5%) isolates resistant to tetracycline, 4 (14.8%) isolates resistant to linezolid and 3 (11.1%) isolates resistant to Trimethoprim/sulphamethoxazole.

Sl. No.	Antibiotic Name	Concentration	No. of Staphylococcal isolates susceptible	% of Staphylococcal isolates susceptible	No. of Staphyloccal isolates resistant	% of Staphylococcal isolates resistant
1	Ampicillin	AMP 10µg	1	3.7	26	96.3
2	Chloramphenicol	C 30 µg	26	96.3	-	-
3	Ciprofloxacin	CIP 5 µg	25	92.6	-	-
4	Erythromycin	E 15 µg	12	44.4	1	3.7
5	Gentamicin	CN 10 µg	25	92.6	-	-
6	Linezolid	LZD 30 µg	23	85.2	4	14.8
7	Nitrofurantoin	F 300 µg	24	88.9	1	3.7
8	Penicillin	P 10 µg	-	-	27	100
9	Tetracycline	TE 30 µg	22	81.2	5	18.5
10	Trimethoprim/ Sulphamethaxazole	SXT 25 µg	18	66.7	3	11.1

Table 6: Staphylococcus antibiogram result of antibiotic susceptibility and resistance

Key

Ampicillin (AMP 10µg), Chloramphenicol (C 30µg), Ciprofloxacin (CIP 5µg), Erythromycin (E 15µg), Gentamicin (CN 10µg), Linezolid (LZD 30 µg), Nitrofurantoin (F 300 µg), Penicillin (P 10 µg), Tetracycline (TE 10µg), Trimethoprim/Sulphamethaxazole (SXT 25 µg).

Sl.no.	Resistant Pattern	Frequency
1	AMP, P	14
2	AMP, P, TE	4
3	AMP, P, LZD	4
4	AMP, P, F, TE	1

Table 7: Multidrug resistance and frequency of Staphylococcus isolates

Key

Ampicillin (AMP 10µg), Penicillin (P 10µg), Tetracycline (TE 15µg), Linezolid (LZD 30 µg), Nitrofurantoin (F 300 µg).

4.9 PCR Result of Salmonella identification

Among 20 isolates that were tested for antibiotic susceptibility, 5 (25%) of the sample showed a positive result in the conventional PCR method. In-gel electrophoresis, the band appeared at 284 bp. These five samples were for 2 from Mohakhali farm, 2 from Hemayetpur farm, 1 from the pasteurized milk of Brand 1. Four isolates were from raw milk and one of them was from commercial milk.

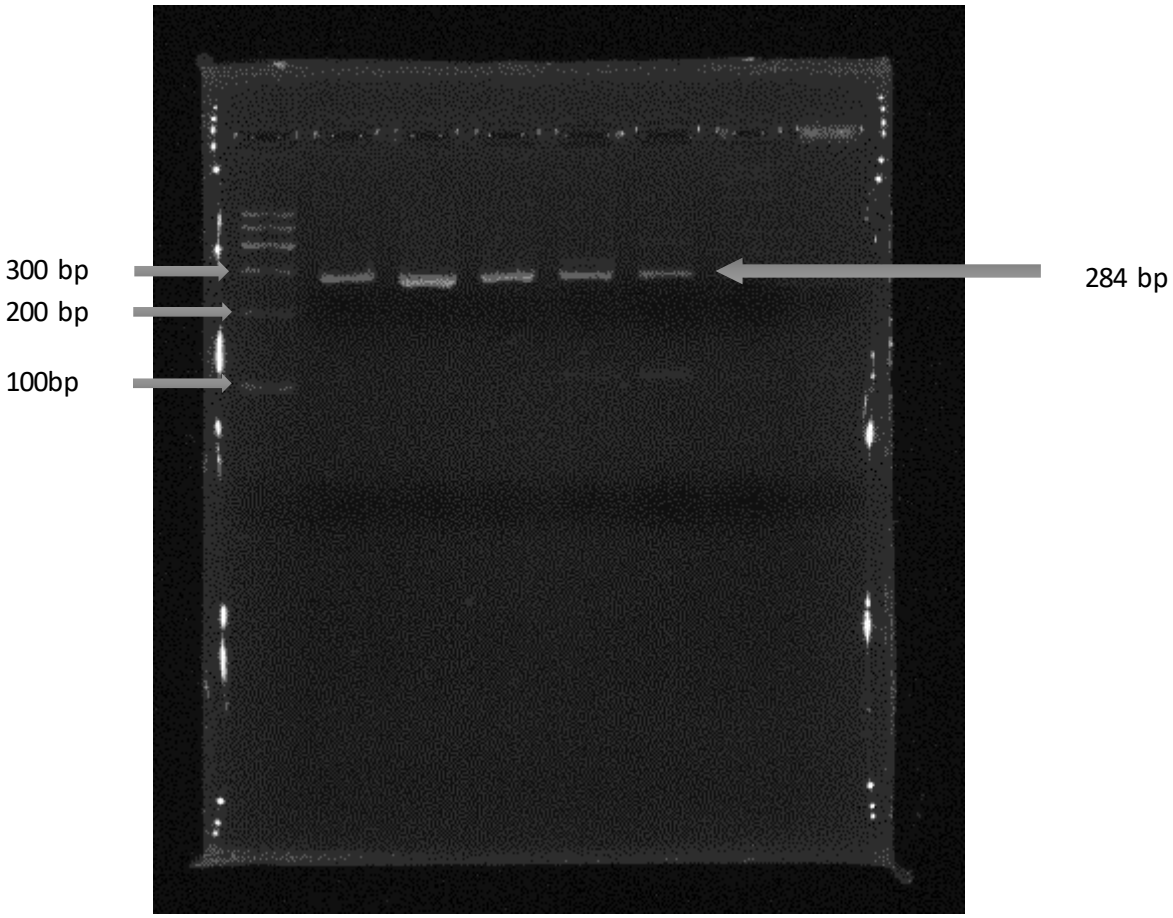


Figure 38: PCR positive Salmonella isolates by inv A gene amplification.

Chapter 5: Discussion

The investigation on microbial analysis of raw and different brands of pasteurized milk was conducted to evaluate milk samples obtained from three important sources viz., raw, pasteurized, and UHT milk. The samples passed many categories of various standards. Fourteen of the raw milk samples (31%) had Total viable bacterial counts (TVBC) higher than the permissible level recommended by World Health Organization (W. H. O.), while eight (32%) of the commercial milk samples had TVBC higher than the W.H.O. standard. The raw milk samples had values from 3-8.2 $\log_{10}$ CFU/mL and commercial milk samples had values that ranged between 0 - 8.6 $\log_{10}$ CFU/mL. This could be due to poor hygiene and a lack of pasteurization. It is in agreement with (Lawan et al., 2012) who reported lower total viable bacterial counts range before pasteurization between 5.7 - 6.04 $\log_{10}$ CFU/mL and after pasteurization (3.7-4.20 $\log_{10}$ CFU/mL). Furthermore, the WHO maximum permissible value for TVBC in milk is 6.0 log10 CFU/ml (Ajogi et al., 2005). The total viable bacterial count of raw milk ranged between 7.11-7.71 log10 CFU/mL which corresponds with the results reported in the present work. The most frequent cause of high bacterial load is normally a result of poor cleaning of the milking system. The bacterial count may be high due to milking with dirty udders, maintaining an unclean milking and housing environment, and failing to rapidly cool milk to less than 40 °F (Banik et al., 2015). (Aaku et al., 2004) and (Arenas et al., 2004) have found (6.74-7.74 $\log_{10}$ CFU/mL) of the total number of microorganisms in pooled raw milk, respectively, which were comparatively lower than that of this experiment. Hossain et al., 2011 experimented in India and found that the bacterial count in raw milk ranged from 6.24-8.07 $\log_{10}$ CFU/mL.

There are several reasons for the occurrence of bacterial contamination in the pasteurized milk samples such as faulty pasteurization machinery, the capacity of organisms to survive even after pasteurization, and contamination in the post-pasteurized process due to poor processing and handling and storage conditions and/or maintenance of substandard hygienic practices by working personnel. The TVBC (total viable bacterial count) of the pasteurized milk samples in this study was ranged (0 - 8.6 $\log_{10}$ CFU/mL), higher than that recommended by BSTI and USPHS (not exceeding 20,000 CFU/ml) (Institution, 2002), (Jay, 2003). (Hossain et al., 2011) found the

bacterial count in pasteurized milk samples were in between 7.9-8.1 $\log_{10}$ CFU/mL. interestingly, three milk samples (M2, A2, A4) showed no growth at all.

According to the definition of the UHT process, the presence of bacteria in UHT milk should be minimal or not at all (Ammara et al., 2009). In the present study, UHT milk ranged from 0-5.95 $\log_{10}$ CFU/mL This was an indication that there might be a problem in the UHT process. The reasons for the presence of bacteria in UHT milk may be due to milk quality, sanitation of process plant, the status of packaging material, and also the process of handling (Tekinsen et al., 2007).

The presence of *Escherichia coli* (one of the members of coliform bacteria) in milk is a common indicator of fecal contamination. Coliform count in the raw milk ranged from 0 – 7.73 $\log_{10}$ CFU/mL which was higher than that obtained by Saitanu, 1996, who found TCC (total coliform count) of <1000 CFU/ml. However, TCC obtained in the study of (Sraïri et al., 2006) varied from less than 1.5-7.3 $\log_{10}$ CFU/mL in raw milk. In another study by Uddin et al., 2011, the range of total coliform count was from 4-6.9 $\log_{10}$ CFU/mL. Reasons for higher coliforms counts in raw milk may be as a result of poor hygiene, contaminated water, unsanitary milking practices, and improperly washed and maintained equipment.

Coliform count of pasteurized milk ranged 0 – 6.7 $\log_{10}$ CFU/mL from 4 (40%) samples and 0 – 5.9 $\log_{10}$ CFU/mL from 2 (25%) sample in case of UHT milk. Coliform bacteria are supposed to be absent in pasteurized milk as they can't survive the pasteurization temperature. Because of the faulty machinery in the pasteurization process or post pasteurization contamination which includes contamination in packaging materials, defects in pipelines; TCC may be detected in the pasteurized milk samples (Dey and Karim, 2013), (Agriculture., 2008). These results of detecting the coliform bacteria test indicate that processed milk available in Bangladesh is of not good quality and will cause a health risk to consumers.

The prevalence of *Salmonella* in raw milk was 10% while commercial milk had 4% prevalence. The overall prevalence of 8.2 % (Raw milk 10 % while commercial milk 4 %) in this study is higher than that is obtained by (Karshima et al., 2013) who found a prevalence of 6.4% from raw milk and 0.8% from fermented milk. (Mhone et al., 2012) who carried out a study in Zimbabwe from selected farms on raw and processed cow

milk and showed no *Salmonella spp*. However, Karshima et al., 2013 and Mhone et al., 2012 used the same method of isolation (Hendriksen, 2003).

(Munsi et al., 2016) found 35.71% of vendor milk sample positive for *Salmonella* which higher than the result in this study but the presence of *Salmonella* in brand milk sample was not found but in this study, 4% was observed. (Rahman et al., 2018) found 1.85% prevalence in the milk sample, collected from various small dairy farms. This study shows a higher prevalence than 1.85%. Tamba et al., 2016 found prevalence of 4% *Salmonella* in raw milk samples.

The 8.2% prevalence established in this work is of public health importance since the presence of one *Salmonella* species can lead to the recall of food items from the market following the WHO standard (Codex Alimentarius Commission). The prevalence found in this study is probably due to the poor sanitary conditions of the milkman's hands, clothing's and the environment. The presence of flies in the environment where raw milk was sold and also the addition of polluted water could be potential sources of the *Salmonella* organism. The higher prevalence of *Salmonella spp*. in milk could be due to the poor sanitary environment where the milk was sold. The antibiotic susceptibility of *Salmonella* isolates showed that *Salmonella* isolates from raw and commercial milk were resistant to commonly used antibiotics. There was also resistance to multiple antimicrobials, including tetracycline, pefloxacin, oxytetracycline, nalidixic acid, erythromycin, doxycycline but all the isolates were sensitive to imipenem.

This study also revealed 20 antibiotic patterns from the 23 antimicrobial agents used for the antibiotic susceptibility testing on 20 *Salmonella* isolates, which is very alarming. This shows multiple drug resistance, and 20 (100%) of the isolates showed resistance to at least 4 antibiotics (Table 4).

The resistance of (100%) *Salmonella* isolates for tetracycline groups recorded in this study. *Salmonella* isolates showed Pefloxacin, nalidixic acid, and erythromycin (100%) resistant. Azithromycin 5 (25%), enrofloxacin 3 (15%), cefepime 3 (15%) samples were intermediate according to CLSI 2016 guideline. All (100%) of the isolate were resistant to multidrug (Table 5).

The probable reason for multidrug resistance with 100% resistance on including tetracycline, pefloxacin, oxytetracycline, nalidixic acid, erythromycin, doxycycline may be due to the inappropriate use of antibiotics by farmers and animal feed producers

in preventing or treating certain diseases of their animals (FVE, 2012). This could lead to mutations from susceptible bacteria to new resistant bacteria through gene transfer (that is emergence of antimicrobial resistance). It could also lead to prolonged treatment and additional cost of diagnostic testing on animals and calls for concern (Acar, 1997) and (Addis, 2015).

The prevalence of *Staphylococcus* in raw milk was 69.2% while in commercial milk *Staphylococcus* was not found. The overall prevalence of this study is lower than (Ateba et al., 2010) who found a prevalence of 100% from 28 raw milk samples.

This study also revealed 4 antibiotic patterns from the 10 antimicrobial agents used for the antibiotic susceptibility testing on 27 *Salmonella* isolates. This shows multiple drug resistance, and 14 (51.6%) of the isolates showed resistance to ampicillin and penicillin. Chloramphenicol 1 (3.7%), ciprofloxacin 2 (7.4%), erythromycin 14 (51.9%), gentamicin 2 (7.4%), Nitrofurantoin 2 (7.4%), Trimethoprim/Sulphamethaxazole 6 (2.2%) sample were intermediate according to CLSI 2016 guideline. This is indicative of β lactamase gene resistance (Table 6) and (Table 7).

β-lactams play a major role in the treatment of humans and animals. Many of the β-lactams like Amoxicillin, Penicillin, Cloxacillin, Ampicillin, and their combinations with βlactamase inhibitors are being used in human and animal treatment. Therefore, antimicrobial discs were selected depending on their use in animal treatment. Ampicillin is active against many Gram-positive and Gram-negative bacteria. It was the first 'broad spectrum' Penicillin with activity against Gram-positive bacteria including *Streptococcus pneumonia, Streptococcus pyogenes*, and some isolates of *S. aureus* (but not Penicillin-resistant or Methicillin-resistant strains). Its spectrum of activity is enhanced by co-administration of Sulbactam, a drug that inhibits βlactamase, an enzyme produced by bacteria to inactivate ampicillin and related antibiotics.

Conclusion

In conclusion, this study has established high values of aerobic plate count in milk samples which is above the permissible values of the WHO standard. This indicates poor hygienic levels among the nomads and therefore calls for corrective measures through public health enlightenment.

This research has established the presence of *Salmonella* species with an overall prevalence of 8.2% in the milk samples, with raw milk recording 10% prevalence and commercial milk recording 4% prevalence. Also, in the presence of *Staphylococcus* the prevalence of *Staphylococcus* in raw milk was 69.2%. Codex Alimentarius Commission of the World Health Organization (WHO) states that milk for human consumption must be free of *Salmonella*. Thus, the observed presence of *Salmonella* species in the sampled milk is contrary to the standard of the Codex Alimentarius Commission. that milk for human consumption must be free of *Salmonella*.

Also, the work has established that there were *Salmonella spp.* and *Staphylococcus spp.* resistant to commonly used antibiotics and pose considerable health hazards to the consumers unless prudent control measures are instituted. This could be due to indiscriminate use of antibiotics in those areas or the use of substandard antibiotics or improper storage of antibiotics (as this could affect the potency of the drugs by the farm owners.

Recommendations

Based on the finding in the study, the following recommendations are provided:

1. The milkman should practice personal hygiene by wearing clean clothing and washing their hands regularly with soap and water before milking the animals.
2. There should be active surveillance of salmonellosis in cattle herds to screen and treat those with the infection to reduce the prevalence of salmonellosis in the environment.
3. There should be public health education on the need to keep the environment where the animals are kept clean and also to wipe the udder of the cow before milking with a clean cloth with warm water and edible disinfectant.
4. Government agencies like the National Agency for The Directorate General of Drug Administration (DGDA) should assess and prevent the indiscriminate use of antibiotics by animal owners.
5. The minimum inhibitory concentration evaluation should be carried out on the resistant isolates of *Salmonella* and *Staphylococcus* to determine the breaking point of the resistant organisms isolated.
6. Identification of β-lactamase producing gene by PCR needs to be done for confirmation.
7. Further studies should be carried out to identify the *Staphylococcus spp.* in foods using more sensitive and faster techniques to reveal the true prevalence of food-borne diseases.

Reference

AAKU, E. N., COLLISON, E. K., GASHE, B. A. & MPUCHANE, S. 2004. Microbiological quality of milk from two processing plants in Gaborone Botswana. *Food control,* 15**,** 181-186.

ACAR, J. F. 1997. Consequences of bacterial resistance to antibiotics in medical practice. *Clinical infectious diseases,* 24**,** S17-S18.

ADDIS, M. 2015. A Review on Antibiotic Resistance and Implication on food chain. *Food Science and Quality Management,* 42**,** 2225-2247.

AGRICULTURE., C. D. O. F. A. 2008. New Coliform standard for milk sold to consumers. *California Department of Food and Agriculture*.

AJOGI, I., KABIR, J., OKOLOCHA, E., LUGA, I., DZIKWI, A., EGEGE, S., UMOH, J., ADESIYUN, A., AGANGA, A. & DUSAI, D. 2005. Manual for Clinics in Veterinary Public Health and Preventive Medicine. *Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.(3rd edition). Published by Asekome & Co, Zaria Nigeria***,** 17-23.

ALAM, M., AKTHER, S. & SARWAR, N. 2017. An Investigation on Risk Factors Related to the Occurrence of Escherichia coli in Fluid Milk Marketed in Chittagong, Bangladesh. *Journal of food quality and hazards control,* 4**,** 9-13.

ALTHOUSE, C., PATTERSON, S., FEDORKA-CRAY, P. & ISAACSON, R. E. 2003. Type 1 fimbriae of Salmonella enterica serovar Typhimurium bind to enterocytes and contribute to colonization of swine in vivo. *Infection immunity,* 71**,** 6446-6452.

AMMARA, H., IMRAN, A. & SHAHID, M. 2009. Microbiological and physicochemical analysis of different UHT milk available in a local market. *Asian Journal of Food and Agro-Industry,* 2**,** 434-447.

ANDERSON, R. C., BUCKLEY, S. A., KUBENA, L. F., STANKER, L. H., HARVEY, R. B. & NISBET, D. J. J. J. O. F. P. 2000. Bactericidal effect of sodium chlorate on Escherichia coli O157: H7 and Salmonella typhimurium DT104 in rumen contents in vitro. 63**,** 1038-1042.

ANDREWS, W. H., T.S. HAMMACK 2000. Salmonella. *Bacteriological Analytical Manual*.

ANONYMOUS 2002. Microbiology of food and animal feeding stuffs horizontal method for the detection of Salmonella (ISO 6579:2002). *International Organization for Standardization. Geneva, Switzerland*.

ANTUNES, P. C., RÉU, C., SOUSA, J. C., PEIXE, L. S. & PESTANA, N. 2003. Incidence of Salmonella from poultry products and their susceptibility to antimicrobial agents. *International Journal of Food Microbiology,* 82**,** 97-103.

APLIN., J. 2014. Presence of coagulase-negative Staphylococcus in dairy products. *MSc. Thesis (Environmental science and research)*.

ARENAS, R., GONZALEZ, L., BERNARDO, A., FRESNO, J. & TORNADIJO, M. 2004. Microbiological and physico-chemical changes in Genestoso cheese, a Spanish acid curd variety, throughout ripening. *Food Control,* 15**,** 271-279.

ARGUDIN, M. A., MENDOZA, M.C. AND RODICIO, MR. 2010. Food poisoning and Staphylococcus aureus enterotoxins. *Toxins***.,** 1751- 1773.

ARUMUGASWAMY, R., RUSUL, G., HAMID, S. A. & CHEAH, C. 1995. Prevalence of Salmonella in raw and cooked foods in Malaysia. *Food Microbiology,* 12**,** 3-8.

ASAO, T., KUMEDA. Y . KAWAI, T.. SHIBATA, T., ODA, H., HARUKI, K.N. AND KOZAKI, S. 2003. An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan:estimation of enterotoxin A in the incriminated milk and powdered skim milk. *Epidemiology Infection,* 130**,** 33-40.

ATEBA, C., MBEWE, M., MONEOANG, M. & BEZUIDENHOUT, C. 2010. Antibiotic-resistant Staphylococcus aureus isolated from milk in the Mafikeng Area, North West province, South Africa. *South African Journal of Science,* 106**,** 1-6.

BANIK, S. K., DAS, K. K. & UDDIN, M. A. 2015. Microbiological quality analysis of raw, pasteurized, UHT milk samples collected from different locations in Bangladesh. *2015,* 4**,** 4.

BARBARA, M., TONY, C. & GRAHAME, W. 2000. The microbiological safety and quality of food. Maryland: Aspen Publishers.

BAUER, A. W., KIRBY, W. M., SHERRIS, J. C. AND TURCK, M. 1966. .Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology,* 45**,** 493-496.

BÄUMLER, A. J., TSOLIS, R. M. & HEFFRON, F. 2000. *Virulence mechanisms of Salmonella and their genetic basis,* CABI Publishing Wallingford, Oxon, UK.

BEARSON, B. L., WILSON, L. & FOSTER, J. W. 1998. A low pH-inducible, PhoPQ-dependent acid tolerance response protects Salmonella typhimurium against inorganic acid stress. *Journal of bacteriology,* 180**,** 2409-2417.

BEARSON, S. M., BEARSON, B. L. & RASMUSSEN, M. A. 2006. Identification of Salmonella enterica serovar Typhimurium genes important for survival in the swine gastric environment. *Applied environmental microbiology*

72**,** 2829-2836.

BEKELE, B. & ASHENAFI, M. 2010. Distribution of drug resistance among enterococci and Salmonella from poultry and cattle in Ethiopia. *Tropical animal health and production,* 42**,** 857-864.

BHARATHY, S., GUNASEELAN, L. PORTEEN, K. AND BOJIRAJ, M. 2015. Prevalence of Staphylcoccus aureus in raw milk: can it be a potential public health threat. *International Journal of Advanced Research,* 3**,** 801-806.

BOQVIST, S. & VÅGSHOLM, I. 2005. Risk factors for hazard of release from Salmonella-control restriction on Swedish cattle farms from 1993 to 2002. *Preventive veterinary medicine,* 71**,** 35-44.

BRANDS, D. A., INMAN, A. E., GERBA, C. P., MARÉ, C. J., BILLINGTON, S. J., SAIF, L. A., LEVINE, J. F. & JOENS, L. A. 2005. Prevalence of Salmonella *spp.* in oysters in the United States. *Applied and environmental microbiology,* 71**,** 893-897.

BRENNER, F., VILLAR, R., ANGULO, F., TAUXE, R. & SWAMINATHAN, B. 2000. Salmonella nomenclature. *Journal of clinical microbiology,* 38**,** 2465-2467.

BUTT, T., AHMAD, R. N., MAHMOOD, A. & ZAIDI, S. 2003. Ciprofloxacin treatment failure in typhoid fever case, Pakistan. *Emerging infectious diseases,* 9**,** 1621.

BYARUGABA, D. 2004. Antimicrobial resistance in developing countries and responsible risk factors. *International journal of antimicrobial agents,* 24**,** 105-110.

CABRERA, R., RUIZ, J., MARCO, F., OLIVEIRA, I., ARROYO, M., ALADUENA, A., USERA, M. A., DE ANTA, M. T. J., GASCÓN, J. & VILA, J. 2004. Mechanism of resistance to several antimicrobial agents in Salmonella clinical isolates causing traveler's diarrhea. *Antimicrobial agents and chemotherapy,* 48**,** 3934-3939.

CARRAMINANA, J. J., ROTA, C., AGUSTIN, I. & HERRERA, A. 2004. High prevalence of multiple resistance to antibiotics in Salmonella serovars isolated from a poultry slaughterhouse in Spain. *Veterinary Microbiology,* 104**,** 133-139.

CARTER. G. R. AND CHENGEPPA, M. M. 1991. Enterobacteriaceae In: Essentials of Veterinary bacteriology and mycology. *Lea and Febiger, Philadelphia,* 150-164.

CHANDA, G. C., UDDIN, G. M. N., DEB, A., BILKIS, T., CHOWDHURY, S. & UDDIN, M. B. 2008. Microbiological profile of the traditionally collected industrial raw milk

from the milk pocket zones of Bangladesh. *Bangladesh Journal of Microbiology,* 25**,** 17-20.
CHAO, G., ZHOU, X., JIAO, X., QIAN, X. & XU, L. 2007. Prevalence and antimicrobial resistance of foodborne pathogens isolated from food products in China. *Foodborne pathogens and disease,* 4**,** 277-284.
CHEESBROUGH, M. 2002. Medical laboratory manual for tropical countries. *Cambridge University Press.*
CHEN, S., ZHAO, S., WHITE, D. G., SCHROEDER, C. M., LU, R., YANG, H., MCDERMOTT, P. F., AYERS, S. & MENG, J. 2004. Characterization of multiple-antimicrobial-resistant Salmonella serovars isolated from retail meats. *Applied and Environmental Microbiology,* 70**,** 1-7.
CHIN, J. 2000. Control of communicable diseases manual.
CHIU, C.-H., SU, L.-H. & CHU, C. 2004. Salmonella enterica serotype Choleraesuis: epidemiology, pathogenesis, clinical disease, and treatment. *Clinical microbiology reviews,* 17**,** 311-322.
COHEN, J. I., BARTLETT, J. A., AND G. R. COREY. 1987. Extra-intestinal manifestations of Salmonella infections. *Medicine (Baltimore),* 66**,** 349-388.
COLEMAN, M. E., SANDBERG, S. & ANDERSON, S. A. 2003. Impact of microbial ecology of meat and poultry products on predictions from exposure assessment scenarios for refrigerated storage. *Risk Analysis: AN INTERNATIONAL JOURNAL,* 23**,** 215-228.
COTTER, P. A. & DIRITA, V. J. 2000. Bacterial virulence gene regulation: an evolutionary perspective. *Annual Reviews in Microbiology,* 54**,** 519-565.
COWAN, S. T. 2003. *Cowan and Steel's manual for the identification of medical bacteria*, Cambridge university press.
DALLAL, M. M. S., DOYLE, M. P., REZADEHBASHI, M., DABIRI, H., SANAEI, M., MODARRESI, S., BAKHTIARI, R., SHARIFIY, K., TAREMI, M. & ZALI, M. R. 2010. Prevalence and antimicrobial resistance profiles of Salmonella serotypes, Campylobacter and Yersinia *spp.* isolated from retail chicken and beef, Tehran, Iran. *Food Control,* 21**,** 388-392.
DARWIN, K. H. & MILLER, V. L. 1999. Molecular Basis of the Interaction of Salmonella with the Intestinal Mucosa. *Clinical microbiology reviews,* 12**,** 405-428.
DAWSON, P. 1992. Control of Salmonella in poultry in Great Britain. *International journal of food microbiology,* 15**,** 215.
DE JONG, B. & EKDAHL, K. 2006. The comparative burden of salmonellosis in the European Union member states, associated and candidate countries. *BMC Public Health,* 6**,** 4.
DE OLIVEIRA, L. P., BARROS. L.S.S., SILVA, V.C. AND CIRQUEIRA, M.G. 2011. Study of Staphylococcus aureus in raw and pasteurized milk consumed in the Reconcavo area of the State of Bahia, Brazil. *Journal Food Process Technolgy***,** 2-6.
DEB, M. & KAPOOR, L. 2005. Salmonella nomenclature seen in the literature. *Indian journal of medical microbiology,* 23**,** 204.
DEY, S. & KARIM, M. 2013. Study on physicochemical and microbial quality of available raw, pasteurized and UHT milk during preservation. *Int. J. Sci. Invent. Today,* 2**,** 150-157.
DOYLE, M. P., M.C. ERICKSON 2006. Emerging microbiological food safety issues related to meat. *Meat Science. ,* 74**,** 98-112.
DUNCAN, M. J., MANN, E. L., COHEN, M. S., OFEK, I., SHARON, N. & ABRAHAM, S. N. 2005. The distinct binding specificities exhibited by enterobacterial type 1 fimbriae are determined by their fimbrial shafts. *Journal of Biological Chemistry,* 280**,** 37707-37716.

EVEN, S., LEROY, S., CHARIER. C . ZAKOUR, N.B., CHACOMAC, J.P., LEBERT, I., JAMET, E., DESMONT. M.H., COTON. E. POCHET, S., DORMK), P.Y., GAULIER. M., TALON. R. AND LE LOIR, Y. 2010. Low occurrence of safety hazards in coagulase-negative Staphylococci isolated from fermented food stuffs. *International Journal of Food Microbiology,* 139.

FARKAS, J. 1998. Irradiation as a method for decontaminating food. . *A review. International Journal of Food Microbiology,* 44**,** 189-204.

FDA 2012. Bad Bug Book Foodbome Pathogenic Microorganisms and Natural Toxins Handbook. 2 Edn.**,** 87-92.

FEDORKA-CRAY, P. J., KELLEY, L. C., STABEL, T. J., GRAY, J. T. & LAUFER, J. A. 1995. Alternate routes of invasion may affect pathogenesis of Salmonella typhimurium in swine. *Infection Immunity,* 63**,** 2658-2664.

FISCHER, A. R. H., L.J. FREWER AND M.J. NAUTA 2006. Toward improving food safety in the domestic environment: A multi-item Rasch scale for the measurement of the safety efficacy of domestic food-handling practices Risk Analysis. 26**,** 1323-1338.

FLUIT, A. C. 2005. Towards more virulent and antibiotic-resistant Salmonella? *FEMS Immunology & Medical Microbiology,* 43**,** 1-11.

FORSHELL, L. P. & WIERUP, M. 2006. Salmonella contamination: a significant challenge to the global marketing of animal food products. *Rev. sci. tech. Off. int. Epiz,* 25**,** 541-554.

FORSYTHE, S. J. 2008. *The microbiological risk assessment of food,* John Wiley & Sons.

FOSTER, J., W. 1991. Salmonella acid shock proteins are required for the adaptive acid tolerance response. *Journal of Bacteriology,* 173**,** 6896-6902.

FRENZEN, P. D., R.T. LYNN, J.C. BUZBY, T. BREUER, T. ROBERTS, D.VOETSCH AND S. REDDY 1999. Salmonella cost estimate updated using Food Net data. Food Review. 22(2)**,** 10-15.

FRYE, J. G. & FEDORKA-CRAY, P. J. 2007. Prevalence, distribution and characterisation of ceftiofur resistance in Salmonella enterica isolated from animals in the USA from 1999 to 2003. *International journal of antimicrobial agents,* 30**,** 134-142.

FVE, F. O. V. O. E. 2012. Antibiotic resistance and prudent use of antibiotics in veterinary medicine.

GALLAND, J. C., TROUTT, H. F., BREWER, R. L., OSBURN, B. I., BRAUN, R. K., SEARS, P., SCHMITZ, J. A., CHILDERS, A. B., RICHEY, E. & MURTHY, K. 2001. Diversity of Salmonella serotypes in cull (market) dairy cows at slaughter. *Journal of the American Veterinary Medical Association,* 219**,** 1216-1220.

GANDRA, E., FERNANDEZ, MA, SILVA, JA, AND SILVA. W.P.D. 2011. Standardization of a multiplex PCR for the identification of coagulase-positive. *Staphylococcus Clencia Tecnologia Alimentaria,* 31**,** 310-312.

GEBREYES, W. A. & THAKUR, S. 2005. Multidrug-resistant Salmonella enterica serovar Muenchen from pigs and humans and potential interserovar transfer of antimicrobial resistance. *Antimicrobial agents and chemotherapy,* 49**,** 503-511.

GHARIB, A., ADEL ATTIA, MA AND BENDARY, M M. 2013. Detection of the CoA gene in Staphylococcus aureus from different sources by polymerase chain reaction. *International Journal of Microbiological Research,* 4**,** 37-42.

GŁOŚNICKA, R., PIEŃKOWSKA, D., DERA-TOMASZEWSKA, B. & ILNICKA, M. 1987. Characteristics of lactose-fermenting Salmonella strains from Poland. *Bulletin of the Institute of Maritime and Tropical Medicine in Gdynia,* 38**,** 69-75.

GOMEZ, H. F. A. T. G., CLEARLY 1997. Principles of pediatric infectious diseases. *New York: Churchill Livingstone***,** 923-929.

GRAY, V. L., O'REILLY, M., MÜLLER, C. T., WATKINS, I. D. & LLOYD, D. 2006. Low tyrosine content of growth media yields aflagellate Salmonella enterica serovar Typhimurium. *Microbiology,* 152**,** 23-28.

GUNDOGAN, N., CITEK, S. AND TURAN, E. 2005. Slime production, DNase activity and antibiotic resistance ofStaphylococcus aureus isolated from raw milk, pasteurised milk and ice cream samples. *Food Control,* 17**,** 389-392.

GWIDA, M. M., EL-GOHARY, FA 2013. Zoonotic Bacterial Pathogens Isolated from Raw Milk with Special Reference to Escherichia coli and Staphylococcus aureus in Dakahlia Govemorat. Egypt,. 2**,** 705.

HAFEZ, H. 2005. Governmental regulations and concept behind eradication and control of some important poultry diseases. *World's Poultry Science Journal,* 61**,** 569-582.

HASAN, T. & RAKIB, R. 2017. Physiochemical and microbiological quality assessment and detection of adulterants of raw milk available in Nakla Upazila, Sherpur, Bangladesh. *2017,* 14**,** 8.

HENDRIKSEN, R. 2003. A global Salmonella surveillance and laboratory support project of the World Health Organization: Laboratory Protocols Level 1 Training Course Identification of Salmonella 4th Ed. April 2003.

HENNEKINNE, J. A., DE BUYSER. M.L. AND DRAGACCI, S. 2012. Staphylococcus aureus and its food poisoning toxins: characterization and outbreak investigation. *Microbiology Review Journal,* 36**,** 815-836.

HENSEL, M. 2004. Evolution of pathogenicity islands of Salmonella enterica. *International Journal of Medical Microbiology,* 294**,** 95-102.

HENSON, S. 2003. The economics of food safety in developing countries. 12**,** 3-19.

HEYNDRICKX, M., PASMANS, F., DUCATELLE, R., DECOSTERE, A. & HAESEBROUCK, F. 2005. Recent changes in Salmonella nomenclature: the need for clarification.

HOERTLE, J. L. A. B., A 2009. Antimicrobial resistance of Staphylococcus aureus isolated from the intensive care unit of a general hospital in southern Brazil. *Journal of Infection in Developing Countries.,* 3**,** 504-510.

HOSSAIN, T., ALAM, M. & SIKDAR, D. 2011. Chemical and microbiological quality assessment of raw and processed liquid market milks of Bangladesh.

ICMSF 1996. Staphylococcus aureus. In: Microorganisms in food Microbiological specifications of food pathogens.**,** 299-333.

INSTITUTION, B. S. A. T. 2002. Bangladesh standard: Specification for pasteurized milk. Bangladesh Standards and Testing Institution, Tejgaon Industrial Area, Dhaka.

IOVINE, N. M. & BLASER, M. J. 2004. Antibiotics in animal feed and spread of resistant Campylobacter from poultry to humans. *Emerging Infectious Diseases,* 10**,** 1158.

IRLINGER, F. 2008. Safety assessment of dairy microorganism: coagulase-negative Staphylococci. *International Journal of Food Microbiology,* 126**,** 302-310.

JABER, N. N. 2011. Isolation and biotyping of Stapnylococcus aureus from white cheese in Basrah local markets. *Journal of Veterinary Research.,* 10**,** 2-4.

JAHAN. M . RAHMAN. M.. SHAFIULLAH. M.. PARVEJ. S.. MD. CHOWDHURY, M. Z. H. H., M.E., TALUKDER, MAX AND AHMED, S. 2015. Isolation and characterization of Stapnylococcus aureus from raw cow milk in Bangladesh. *Journal of advance Veterinary and Animal Research.,* 2**,** 49-55.

JAMALI, H., PAYDAR, M., RADMEHR, B., ISMAIL, S. AND DADRASNIA, A. 2015. Prevalence and antimicrobial resistance of Staphylococcus aureus isolated from raw milk and dairy products. *Food Control,* 54**,** 383-388.

JANDA, J., ABBOTT, SL 2006. The Genera Klebsiella and Raoultella. *The Enterobacteria***,** 115-129.

JAY, J. M. 2003. Modem Food Microbiology.

JAY, J. M., MARTIN J.L. AND DAVID AG. 2005. Modern Food Microbiology. 241-244.
JJ, J. J. 2012. Post harvest contamination of milk obtained from various sources. *Food microbiology,* 2**,** 50-57.
JOHNSON, J. M., RAJIC, A. & MCMULLEN, L. M. 2005. Antimicrobial resistance of selected Salmonella isolates from food animals and food in Alberta. *The Canadian Veterinary Journal,* 46**,** 141.
JONES, B. D. 2005. Salmonella invasion gene regulation: a story of environmental awareness. *J Microbiol,* 43**,** 110-117.
JONES, L. 2000. Molecular methods in food analysis: principles and examples. Key topics in Food Science and Technology, No. 1. Campden & Chorleywood Food Research Association, Chipping Campden, UK.
JORGENSEN, H. J., MODE TV, HOGASENAND, H.R. AND RORVIK, M. 2005. Enterotoxigenic Staphylococcus aureus in bulk milk in Norway. *Journal of Applied Microbiology,* 99**,** 158-166.
JOSHI, S., RAY, P., MANCHANDA, V.. BAJAJ, J., CHITNTS, D.S., GAUTAM, V., GOSWAMI, P., GUPTA, V.. HARISH. B.N., KAGAL, A., KAPIL, A., RAO, R., RODRIGUES, N.C., SARDANA. R., DEVI, S., SHARMA, A. AND BALAJI, V. 2013. Methicillin resistant Staphylococcus aureus (MRSA) in India: Prevalence & susceptibility pattern. *Indian Journal of Medical Research,* 137**,** 363-369.
JUN, W., ZENGREN, Z. & JINGYU, W. 2007. Risk assessment of Salmonella in animal derived food. *Chinese journal of animal quarantine,* 24**,** 23.
KADARIYA, J., SMITH. T.C. AND THAPALIYA, D. 2014. Staphylococcus aureus and staphylococcal food-borne disease: An ongoing challenge in public health. *Bio Medical Research International.*
KARSHIMA, N., PAM, V., BATA, S., DUNG, P. & PAMAN, N. 2013. Isolation of Salmonella species from milk and locally processed milk products traded for human consumption and associated risk factors in Kanam, Plateau State, Nigeria. *Journal of Animal Production Advances,* 3**,** 69-74.
KAUSHIK, S., SINGH, R.T., TIWARI, U., SARASWAT, A. AND SHRIVASTAV, A. 2014. Antimicrobial investigation of different medications against nosocomial infections causing Staphylococcus aureus. *International Journal of Development Research,* 4**,** 215-217.
KHANAL, B., SHARMA, S. K., BHATTACHARYA, S. K., BHATTARAI, N. R., DEB, M. & KANUNGO, R. 2007. Antimicrobial susceptibility patterns of Salmonella enterica serotype typhi in eastern Nepal. *Journal of health, population, nutrition,* 25**,** 82.
KORSAK, N., DEGEYE, J.-N., ETIENNE, G., BEDUIN, J.-M., CHINA, B., GHAFIR, Y. & DAUBE, G. 2006. Use of a serological approach for prediction of Salmonella status in an integrated pig production system. *International journal of food microbiology,* 108**,** 246-254.
KUMAR, R., AND AMIT PRASAD 2010. Detection of Ecoli and Staphylococcus in milk and milk products in and around Pantnagar. *Veterinary World,* 3**,** 495-496.
LAN, X., ZHAO, S., ZHENG, N., LI, S., ZHANG, Y., LIU, H., MCKILLIP, J. & WANG, J. 2017. Microbiological quality of raw cow milk and its association with herd management practices in Northern China. *Journal of dairy science,* 100**,** 4294-4299.
LAUDERDALE, T.-L., AARESTRUP, F. M., CHEN, P.-C., LAI, J.-F., WANG, H.-Y., SHIAU, Y.-R., HUANG, I.-W. & HUNG, C.-L. 2006. Multidrug resistance among different serotypes of clinical Salmonella isolates in Taiwan. *Diagnostic microbiology infectious disease,* 55**,** 149-155.

LAWAN, M., ABDULSALAWU, F., SULEIMAN, A., ALUWONG, T. & YAQUB, L. 2012. Microbial assessments of bulk milk before and after pasteurization in two different dairy farms in Zaria, Nigeria. *International Journal of Dairy Science,* 7**,** 103-108.

LAXMINARAYAN, R. 2010. *Battling resistance to antibiotics and pesticides: An economic approach*, Routledge.

LEVIN, R. E. 2009. *Rapid detection and characterization of foodborne pathogens by molecular techniques*, CRC Press.

LIU, D. 2010. *Molecular detection of foodborne pathogens*, CRC Press.

LIU, X. M., Y. CHEN, Y.C. GUO AND Z.T. WANG 2008. Foodborne diseases outbreaks in 2005. Report of National Foodborne Diseases Surveillance Network in China. *ChinesemJournal of Food Hygiene.,* 20**,** 506-509.

LOWY, F. D. 2003. Antimicrobial resistance: the example of Staphyiococus aureus. *Journal of Clinical lnvestlgattion,* 111**,** 1265-1273.

LUKÁŠOVÁ, J. & ŠUSTÁČKOVÁ, A. 2003. Enterococci and antibiotic resistance. *Acta Veterinaria Brno,* 72**,** 315-323.

LYNCH, M., PAINTER, J., WOODRUFF, R. & BRADEN, C. 2006. Surveillance for Foodborne-disease Outbreaks: United States, 1998--2002.

MALEK, M., AKTER, J., AHMED, T. & UDDIN, M. A. 2016. Isolation and quantification of microorganisms from some common milk products within Dhaka city, Bangladesh. *Stamford Journal of Microbiology,* 5**,** 13-17.

MATHEWS, S., NGOMA. L. GASHE. B. AND MPUCHANE. S. 2013. Assessment of pathogenic bacteria from ice cream and ice pop sold in Gaborone. *Botswana Ethno Medicine,* 7**,** 195-203.

MEAD, P. S., L.SLUTSKER, V.DIETZ, L.F. MCCAIG, J.S. BRESEE, C. SHAPIRO, P.M. GRIFFIN AND & TAUXE, R. V. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.,* 5**,** 607-625.

MEHMETI, I., BYTYQI, H., MUJI, S., NES, I. F. & DIEP, D. B. 2017. The prevalence of Listeria monocytogenes and Staphylococcus aureus and their virulence genes in bulk tank milk in Kosovo. *The Journal of Infection in Developing Countries,* 11**,** 247-254.

MHONE, T. A., MATOPE, G. & SAIDI, P. T. 2012. Detection of Salmonella *spp.,* Candida albicans, Aspergillus spp., and antimicrobial residues in raw and processed cow milk from selected smallholder farms of Zimbabwe. *Veterinary medicine international,* 2012.

MIHAIU, L. 2013. Antimicrobial Resistance Evaluation of Pathogen Salmonella Strains Isolated in Pork and Poultry Meat MIHAIU Liora, Marian MIHAIU 2, Alexandra TĂBĂRAN 2, Sorin Daniel DAN 2, Ionut Vlad CORDIŞ 2, Bogdan PIVARIU 2, Darius CORDEA 2. *Bulletin UASVM,* 70**,** 2.

MILLER, S. I., HOHMANN, E.L., D.A. PEGUES, 1995. Salmonella (including Salmonella typhi). *Mandell, G.L., Bennett, J.E. and Dollin, R. editors. Mandell and Bennett's principles and practice of infectious diseases*.

MONTVILLE, T. J., MATTHEWS. K.R. 2008. Food Microbiology An Introduction. 2 Edn.

MORITA, M., MORI, K., TOMINAGA, K., TERAJIMA, J., HIROSE, K., WATANABE, H. & IZUMIYA, H. 2006. Characterization of lysine decarboxylase-negative strains of Salmonella enterica serovar Enteritidis disseminated in Japan. *FEMS Immunology & Medical Microbiology,* 46**,** 381-385.

MUEHLHERR. J.E., Z. C., CORTI, S., BLANCO, J.E., STEPHAN, R. 2003. Microbiological Quality of Raw Goat's and Ewe's Bulk Tank Milk in Switzerland. *Journal of Dairy Science,* 86.

MUNSI, M. N., SARKER, N. R., KHATUN, R. & ALAM, M. K. 2016. Identification and antibiogram study of bacterial species isolated from milk samples of different

locations in Bangladesh. *Asian Journal of Medical and Biological Research,* 1**,** 457-462.
NAYAK, R., STEWART, T., WANG, R.-F., LIN, J., CERNIGLIA, C. & KENNEY, P. 2004. Genetic diversity and virulence gene determinants of antibiotic-resistant Salmonella isolated from preharvest turkey production sources. *International Journal of Food Microbiology,* 91**,** 51-62.
NEMA, V., AGRAWAL, R., KAMBOJ, D.V., GOEL, A.K. AND SINGH, L. 2007. Isolation and characterization of heat resistant entertoxigenic Staphylococcus aureus from a food poisoning outbreak in Indian subcontinent. *International Journal of Food Microbiology,* 117**,** 29-35.
NORMANNO, G., SALANDRA, G.L, DAMBROSIAO, A, QUAGLIA. N.C., CRIESETTI, E. AND CELANO, G.V. 2006. Occurrence, characterization and antimicrobial resistance of enterotoxlgenic Staphylococcus aureus isolated from meat and dairy products. *Journal of Food Microbiology,* 10**,** 48-49.
NØRRUNG, B. & BUNCIC, S. 2008. Microbial safety of meat in the European Union. *Meat Science,* 78**,** 14-24.
NOWAK, B., SAMMET, K., KLEIN, G. & MUEFFLING, T. 2006. Trends in the production and storage of fresh meat–the holistic approach to bacteriological meat quality. *International journal of food science technology,* 41**,** 303-310.
OHL, M. E. & MILLER, S. I. 2001. Salmonella: a model for bacterial pathogenesis. *Annual review of medicine,* 52**,** 259-274.
OIE 2005. Salmonellosis. *Center for Food Security and Public Health,* College of Veterinary Medicine Iowa State University.
OKEKE, I. N., LAXMINARAYAN, R., BHUTTA, Z. A., DUSE, A. G., JENKINS, P., O'BRIEN, T. F., PABLOS-MENDEZ, A. & KLUGMAN, K. P. 2005. Antimicrobial resistance in developing countries. Part I: recent trends and current status. *The Lancet infectious diseases,* 5**,** 481-493.
OKOLOCHA, E., EGWU, P., UMOH, J. & LUGA, I. 2008. Hazards and critical control points of yoghurt produced in a research institute farm in Zaria, Nigeria. *Nigerian Veterinary Journal,* 27**,** 15-24.
OLIVER, S. P. A. G. B. E. 2005. Simultaneous detection of mastitis pathogens. Staphylococcus aureus, Streptococcus uberis and Streptococcus agalactiae by multiplex real time polymerase chain reaction. *Journal of Dairy Science,* 88**,** 3510-3518.
ORMAN, B. E., PINEIRO, S. A., ARDUINO, S., GALAS, M., MELANO, R., CAFFER, M. I., SORDELLI, D. O. & CENTRÓN, D. 2002. Evolution of multiresistance in nontyphoid Salmonella serovars from 1984 to 1998 in Argentina. *Antimicrobial agents and chemotherapy,* 46**,** 3963-3970.
PAPAGRIGORAKIS, M. J., SYNODINOS, P. N. & YAPIJAKIS, C. 2007. Ancient typhoid epidemic reveals possible ancestral strain of Salmonella enterica serovar Typhi. *Infection, Genetics and Evolution,* 7**,** 126-127.
PARISI, A., CARUSO, M., NORMANNO, G., LATORRE, L., SOTTILI, R., MICCOLUPO, A., FRACCALVIERI, R. & SANTAGADA, G. 2016. Prevalence, antimicrobial susceptibility and molecular typing of methicillin-resistant Staphylococcus aureus (MRSA) in bulk tank milk from southern Italy. *Food microbiology,* 58**,** 36-42.
PATERSON, D. L. 2006. Resistance in gram-negative bacteria: Enterobacteriaceae. *American journal of infection control,* 34**,** S20-S28.
PEGUES, D. 2005. Salmonella species, including Salmonella typhi. *Principles practice of infectious diseases***,** 2636-2654.
PEKANA, A., NWODO, U. U., OKOH, A. I. & GREEN, E. 2017. Distribution and Antibiotic Susceptibility Profiles of Staphylococcus spp Isolated from Unpasteurized

Cow Milk Locally Consumed in Nkonkobe Local Municipality, South Africa. *International Journal of Applied Research in Veterinary Medicine,* 15.
PINTO, CLAUDIA, L., SOUZA, L. V., MELONI, V. A., BATISTA, C. S., SILVA, R., MARTINS, E. M., CRUZ, A. G. & MARTINS, M. L. 2018. Microbiological quality of Brazilian UHT milk: Identification and spoilage potential of spore-forming bacteria. *International Journal of Dairy Technology,* 71**,** 20-26.
POPOFF & MICHEL, Y. 1997. Antigenic formulas of the Salmonella serovars. *WHO Collaborating center for reference and research on Salmonella*.
POPOFF, Y, M., BOCKEMÜHL, JOCHEN, BRENNER, W, F. & GHEESLING, L. L. 2001. Supplement 2000 (no. 44) to the Kauffmann-White scheme. *Research in microbiology,* 152**,** 907-909.
POPOFF, Y, M., BOCKEMÜHL, JOCHEN, GHEESLING & L, L. 2004. Supplement 2002 (no. 46) to the Kauffmann–White scheme. *Research in microbiology,* 155**,** 568-570.
PORWOLLIK, S., SANTIVIAGO, C., CHENG, P., FLOREA, L. & MCCLELLAND, M. 2005. Differences in gene content between Salmonella enterica serovar Enteritidis isolates and comparison to closely related serovars Gallinarum and Dublin. *Journal of bacteriology,* 187**,** 6545-6555.
PRODHAN, U. K., ALAM, M. J., SADIA, M. K., SULTANA, S., HYE, M. A. & AHAMED, M. S. 2016. STUDY ON THE QUALITY OF COMMERCIAL MARKET MILK AVAILABLE IN BANGLADESH.
QUINN, P. J., MARKEY, B. K., CARTER, M. E., DONNELLY, W. J. C., LEONARD, F. C. 2002. Veterinary Microbiology and Microbial disease. *Oxford: Blackwell Science*.
QUINTAVALLA, S., & VICINI, L. 2002. Antimicrobial food packaging in meat industry. *Meat science,* 62**,** 373-380.
RAHIMI, E. 2013. Enterotoxigenicity of Staphylococcus aureus isolated from traditional and commercial dairy products marketed in Iran. *Brazilian Journal of Microbiology,* 44**,** 393-399.
RAHMAN, M. A., RAHMAN, A. K. M. A., ISLAM, M. A. & ALAM, M. M. 2018. DETECTION OF MULTI–DRUG RESISTANT SALMONELLA FROM MILK AND MEAT IN BANGLADESH. *2018,* 16**,** 6 %J Bangladesh Journal of Veterinary Medicine.
RAHN, K., DE GRANDIS, S., CLARKE, R., MCEWEN, S., GALAN, J., GINOCCHIO, C., CURTISS III, R. & GYLES, C. 1992. Amplification of an invA gene sequence of Salmonella typhimurium by polymerase chain reaction as a specific method of detection of Salmonella. *Molecular and cellular probes,* 6**,** 271-279.
RANSOM, J., BELK, K., BACON, R., SOFOS, J., SCANGA, J. & SMITH, G. 2002. Comparison of sampling methods for microbiological testing of beef animal rectal/colonal feces, hides, and carcasses. *Journal of food protection,* 65**,** 621-626.
RAO, P. R. 2004. *Essentials of microbiology*, CBS Publishers.
REID, R., PORTER, R. & BALL, H. 1993. The isolation of sucrose-fermenting Salmonella Mbandaka. *Veterinary microbiology,* 37**,** 181-185.
RETA, M. A., BEREDA, T. W. & ALEMU, A. N. 2016. Bacterial contaminations of raw cow's milk consumed at Jigjiga City of Somali Regional State, Eastern Ethiopia. *International Journal of Food Contamination,* 3**,** 4.
ROBERT KOCH INSTITUTE, B.
SAFETY, E. F. 2009. The community summary report on trends and sources of zoonoses and zoonotic agents in the European Union in 2007. 7**,** 223.
SAITANU, I. A., ET AL. 1996. Microbiological quality of raw cow milk. *Thai J. Vet. Med.,* 26**,** 193-214.
SANTOS, E., DOS, C, GENIGEORGIS, M., FARVER, C. AND THOMAS. B. 1981. Prevalence of Staphylococcus aureus in raw and pasteurized milk used for

commercial manufacturing of Brazilian Minas cheese. *Journal of Food Protection,* 5**,** 172-240.
SANTOS, M., BARROS, L., SILVA, I., ANDRADE, M. & LIMA, D. 2016. Escherichia coli O157, Staphylococcus aureus and coliforms in crude and processed bovine milk marketed. *African Journal of Microbiology Research,* 10**,** 1888-1893.
SANTOS, R., TSOLIS, R., BÄUMLER, A. & ADAMS, L. 2003. Pathogenesis of Salmonella-induced enteritis. *Brazilian Journal of Medical and Biological Research,* 36**,** 03-12.
SASIDHARAN, S., PREMA. B. AND YOGA. LL 2011. Antimicrobial drug resistance of Staphylococcus aureus in dairy products. *Asian Pacific Journal of Biomedicine„* 1**,** 130-132.
SCOTT, F., THRELFALL, J. & ARNOLD, C. 2002. Genetic structure of Salmonella revealed by fragment analysis. *International journal of systematic and evolutionary microbiology,* 52**,** 1701-1713.
SHAH, T., SHAH, Q. A., SHAH, J. M., ARAIN, M. A., SAEED, M., SIYAL, F. A., SOOMRO, R. N. & BROHI, S. A. 2016. Microbiological Quality of Raw Milk and Associated Health Risk in the Hyderabad Region of Pakistan. *Int. J. Food Nutr. Saf,* 7**,** 61-77.
SHARMA, D., SHARMA. P.K. AND MALIK. A. 2011. Prevalence and Antimicrobial Susceptibility of Drug Resistant Staphylococcus aureus in Raw Milk of Dairy Cattle. *International Research Journal of Microbiology,* 2**,** 466-470.
SHIFERAW, S. & AHMAD, M. 2016. Prevalence and antibiotic susceptibility of Staphylococcus aureus from lactating cows milk in Bahir Dar dairy farms. *African Journal of Microbiology Research,* 10**,** 1444-1454.
SINELL, H.-J. 1995. Control of food-borne infections and intoxications. *International journal of food microbiology,* 25**,** 209-217.
SINGH, P. A. P. A. 2010. Prevalence of coagulase postive pathogenic Staphylococcus aureus in milk and milk products collected from unorganized sector of Agra. *Acta argiculturae Slovenica,* 96**,** 37-41.
SMITH, J. L., PALUMBO, S. A. & WALLS, I. 1993. Relationship between foodborne bacterial pathogens and the reactive arthritides. *Journal of food safety,* 13**,** 209-236.
SNIJDERS, J. & VAN KNAPEN, F. 2002. Prevention of human diseases by an integrated quality control system. *Livestock Production Science,* 76**,** 203-206.
SOFOS, J., N. 2008. Challenges to meat safety in the 21st century. *Meat science,* 78**,** 3-13.
SOLARI, C., MANDARINO, J., PANIZZUTTI, M. & FARIAS, R. 2003. A new serovar and a new serological variant belonging to Salmonella enterica subspecies diarizonae. *Memórias do Instituto Oswaldo Cruz,* 98**,** 501-502.
SPANU, V. S. C., VIRDIS; S.. COSSU, F., SCARANO, C, PIETRO, E. AND SANTIS, L.D. 2012. Virulence factors and genetic variability of Staphylococcus aureus strains isolated from raw sheep's milk cheese. *Journal of Food Microbiology,* 53**,** 53-57.
SRAÏRI, M., MOUDNIB, J., RAHHO, L. & HAMAMA, A. 2006. How do milking conditions affect the hygienic quality of raw milk? Case study from Moroccan dairy farms. *Livestock research for rural development,* 18**,** 1-9.
STEVENS, A., KABORÉ, Y., PERRIER-GROS-CLAUDE, J.-D., MILLEMANN, Y., BRISABOIS, A., CATTEAU, M., CAVIN, J.-F. & DUFOUR, B. 2006. Prevalence and antibiotic-resistance of Salmonella isolated from beef sampled from the slaughterhouse and from retailers in Dakar (Senegal). *International journal of food microbiology,* 110**,** 178-186.
STEWART, C., COLE, M B. AND SCHAFFNER, D.W. 2003. Managing the Risk of staphylococcal Food Poisoning from Cream-Filled Baked Goods to Meet a Food Safety Objective. *Journal of Food Protection,* 7**,** 1310-1325.

SUAREZ. M.J., A. M. L., GAMBOA. M. 2008. Staphylococcus aureus enterotoxin A detection using the polymerase chain reaction (PCR) and its correlation with coagulase and thermonuclease tests. *Archivos Latinoamericanos de Nutrition,* 58**,** 59-63.

SUELAM, I. I., AMAL, R., RASLAN, A. AND MOHAMED, EM. 2012. Isolation of Staphylococcus aureus from milk and human with reference to its survival on surfaces. *World Journal of Dairy and Food Sciences.,* 7**,** 142-145.

SUKUPOLVI, S., LORENZ, R. G., GORDON, J. I., BIAN, Z., PFEIFER, J. D., NORMARK, S. J. & RHEN, M. 1997. Expression of thin aggregative fimbriae promotes interaction of Salmonella typhimurium SR-11 with mouse small intestinal epithelial cells. *Infection Immunity,* 65**,** 5320-5325.

SUMNER, J., RAVEN, G. & GIVNEY, R. 2004. Have changes to meat and poultry food safety regulation in Australia affected the prevalence of Salmonella or of salmonellosis? *International Journal of Food Microbiology,* 92**,** 199-205.

TAMBA, Z., BELLO, M. & RAJI, M. 2016. OCCURENCE AND ANTIBIOGRAM OF SALMONELLA SPP. IN RAW AND FERMENTED MILK IN ZARIA AND ENVIRONS. *Bangladesh Journal of Veterinary Medicine,* 14**,** 103-107.

TANZIN, T., KHMNH, N., ZAHAN, M. N., PARVEJ, M. S., ZESMIN, K. & RAHMAN, M. T. 2016. Antibiotic resistance profile of bacteria isolated from raw milk samples of cattle and buffaloes. *Journal of Advanced Veterinary and Animal Research,* 3**,** 62-67.

TASCI, F., SAHINDOKUYUCU, F., OZTURK, D. 2011. Detection of Staphylococcus species and staphylococcal enterotoxins by ELISA in ice cream and cheese consumed in Burdur Province. *African Journal of Agricultural Research,* 6**,** 937-942.

TEGEGNE, B. & TESFAYE, S. 2017. Bacteriological milk quality: possible hygienic factors and the role of Staphylococcus aureus in raw bovine milk in and around Gondar, Ethiopia. *International Journal of Food Contamination,* 4**,** 1.

TEKINSEN, K. K., ELMALI, M. & ULUKANLI, Z. 2007. Microbiological quality of UHT milk consumed in Turkey. *Internet Journal of Food Safety,* 7**,** 45-48.

THAKER, H. C., BRAHMBHATT., M.N, NAYAK, J.B. 2013. Isolation and identification of Staphylococcus aureus from milk and milk products and their drug resistance patterns in Anand. *Gujarat Veterinary World*., 6**,** 10-13.

THRELFALL, E. J., WARD, L. R., FROST, J. A. & WILLSHAW, G. A. 2000. The emergence and spread of antibiotic resistance in food-borne bacteria. *International journal of food microbiology,* 62**,** 1-5.

TRAVERS, K. & MICHAEL, B. 2002a. Morbidity of infections caused by antimicrobial-resistant bacteria. *Clinical Infectious Diseases,* 34**,** S131-S134.

TRAVERS, K. & MICHAEL, B. J. C. I. D. 2002b. Morbidity of infections caused by antimicrobial-resistant bacteria. 34**,** S131-S134.

UDDIN, M. A., MOTAZZIM-UL-HAQUE, H. M. & NOOR, R. 2011. Isolation and identification of pathogenic Escherichia coli, Klebsiella spp. and Staphylococcus spp. in raw milk samples collected from different areas of Dhaka city, Bangladesh. *Stamford Journal of Microbiology,* 1**,** 19-23.

VAN ASTEN, A. J. & VAN DIJK, J. E. 2005. Distribution of "classic" virulence factors among Salmonella spp. *FEMS Immunology & Medical Microbiology,* 44**,** 251-259.

VAN DEN BOGAARD, A. E. & STOBBERINGH, E. E. 2000. Epidemiology of resistance to antibiotics: links between animals and humans. *International journal of antimicrobial agents,* 14**,** 327-335.

VAN DUIJKEREN, E., WANNET, W., HOUWERS, D. & VAN PELT, W. 2003. Antimicrobial susceptibilities of Salmonella strains isolated from humans, cattle, pigs, and chickens in the Netherlands from 1984 to 2001. *Journal of Clinical Microbiology,* 41**,** 3574-3578.

VELGE, P., CLOECKAERT, A. & BARROW, P. 2005. Emergence of Salmonella epidemics: The problems related to Salmonella enterica serotyp Enteritidis and multiple antibiotic resistance in other major serotypes. *Veterinary research,* 36**,** 267-288.

WALIA, K. Emerging problem of antimicrobial resistance in developing countries: Intertwining socioeconomic issues. ISSN 1020 4237© World Health Organization 2002 Publications of the World Health Organization enjoy copyright protection in accordance with the provisions of Protocol 2 of the Universal Copyright Convention. For rights of reproduction or translation, in part or in toto, of publications issued by the WHO Regional Office for South, 2003. 1.

WANJALA, G., MATHOOKO, F., KUTIMA, P. & MATHARA, J. 2017. Microbiological quality and safety of raw and pasteurized milk marketed in and around Nairobi region. *African Journal of Food, Agriculture, Nutrition and Development,* 17**,** 11518-11532.

WEILL, F.-X., GUESNIER, F., GUIBERT, V., TIMINOUNI, M., DEMARTIN, M., POLOMACK, L. & GRIMONT, P. A. 2006. Multidrug resistance in Salmonella enterica serotype Typhimurium from humans in France (1993 to 2003). *Journal of clinical microbiology,* 44**,** 700-708.

WOTEKI, C. E., S.L. FACINOLI AND D. SCHOR 2001. Keep food safe to eat: Healthy food must be safe as well as nutritious. In: The dietary guidelines: Surveillance issues and research needs. . *Journal of Nutrition,* 131**,** 502s-509s.

WRAY, C. & WRAY, A. 2001. Salmonella in domestic animals, book review. *Veterinary Microbiology,* 81**,** 281-282.

YAN, H., LI, L., ALAM, M. J., SHINODA, S., MIYOSHI, S.-I. & SHI, L. 2010. Prevalence and antimicrobial resistance of Salmonella in retail foods in northern China. *International journal of food microbiology,* 143**,** 230-234.

YAN, S. S., PENDRAK, M. L., ABELA-RIDDER, B., PUNDERSON, J. W., FEDORKO, D. P. & FOLEY, S. L. 2004. An overview of Salmonella typing: public health perspectives. *Clinical and Applied Immunology Reviews,* 4**,** 189-204.

YANG, S. J., PARK, K. Y., SEO, K. S., BESSER, T. E., YOO, H. S., NOH, K. M., KIM, S. H., KIM, S. H., LEE, B. K. & KOOK, Y. H. 2001. Multidrug-resistant Salmonella typhimurium and Salmonella enteritidis identified by multiplex PCR from animals. *Journal of veterinary science,* 2**,** 181-188.

ZAMAN, M., ARA, A. & HAQUE, M. 2017. Effect of season on production and quality of milk of crossbred dairy cows at Sylhet district government dairy farm in Bangladesh. *2017,* 45**,** 6.

ZESSIN, K. H. 2006. Emerging diseases: a global and biological perspective. *Journal of Veterinary Medicine, Series B,* 53**,** 7-10.

ZHAO, S., WHITE, D., FRIEDMAN, S., GLENN, A., BLICKENSTAFF, K., AYERS, S., ABBOTT, J., HALL-ROBINSON, E. & MCDERMOTT, P. 2008. Antimicrobial resistance in Salmonella enterica serovar Heidelberg isolates from retail meats, including poultry, from 2002 to 2006. *Applied and environmental microbiology,* 74**,** 6656-6662.

ZOUHAROVA, M., AND D. RYSANEK. 2008. Multiplex PCR and RPLA identification of Stapnylococcus aureus enterotoxigenic strains from bulk tank milk. *Zoonoses Public Health,* 55.