HYGIENE PRACTICES OF DAIRY FARMERS AND MILK BULKING CENTRES AND THEIR INFLUENCE ON DEVELOPMENT OF BIOFILM; A CASE OF LILONGWE, MALAWI

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A thesis submitted to the Graduate School in Partial Fulfilment for the Requirements of Master of Science Degree in Food Science of Egerton University

EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

I declare that this is my original work and has not been submitted in this or any other University for an award of any degree.

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Approval

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DEDICATION

This thesis is dedicated to my family;

My father Mr. Fredrick Dickson Banda,

My late mum Mrs. Martha Makina Banda,

My brothers Christopher, Precious,

My sisters Linly, Ellina, Eunice,

My wife Lucy Jamali Banda and

Our daughter Promise.

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ABSTRACT

Hygiene practices from milk production to processing are significant on the quality of milk and milk products. In Malawi, hygiene practices in small and medium enterprises (SME) milk processing plants and their influence on development of biofilm and subsequent microbial safety of milk had little attention. A cross-section study design was used where a survey on hygiene practices of handling raw cow milk from the farm to the bulking centre in Lilongwe district was carried out to assess the influence on biofilm formation in the handling equipment. A Semi-structured questionnaire was administered to 256 respondents. A complete randomized design (CRD) was used to sample 90 samples (30 each of milk, water and swabs) from farmers' households and at bulking centres clustered in six bulking groups. The samples were analysed for total viable count (TVC), coliform counts (CC), Lactic acid Bacteria (LAB) and Yeasts and Moulds. Biofilm indicator isolates of *Pseudomonas, Bacillus,* and *Salmonella,* which were isolated from the samples were tested for biofilm forming capacity using tube method. Results indicate that up to 100% of the dairy farmers from the six milk bulking group sourced water from boreholes and up to 80% use without treating. Plastic containers were the major handling (up to 66.7%) and storage containers (up to 91.7%). Evening milk was stored at a room temperature overnight (up to 100%) and mixed with morning milk to be taken to bulking centres. The mean TVC was 7.57±0.45 $\log_{10}$ cfu/ml, CC was 6.06±0.36 $\log_{10}$ cfu/ml, Y/M was 5.33±0.38 $\log_{10}$ cfu/ml and LAB was 5.73±0.36 $\log_{10}$ cfu/ml in all the 30 milk samples taken. Gram negative rods were the dominant group of bacteria accounting for 85.9% at the farm as compared to bulking centres which was70.6%. The Gram-positive cocci and rods were less than 14.1% and 14.1% respectively at farm level while at the bulking centres they were 5.9% and 52.9%. Biofilm indicator microorganisms *Salmonella, Pseudomonas* and *Bacillus* were detected at farm level at 20%, 12% and 10.7% respectively while at bulking centres they were *Salmonella* (15.8%) and *Bacillus* (42.1%). The swab had the highest biofilm indicator microorganisms (17.5%). Using the tube method, 17.95% of the isolates formed strong biofilms and 43.59% formed moderate biofilm. Farmers and workers at bulking centres need knowledge on proper hygiene practices and sanitation in handling milk for further processing by large scale processors to produce quality and safe milk products

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS/ACRONYMS

^{0}C	Degree Celsius
ANOVA	Analysis of Variance
CAC	Codex Alimentarius Commission
CC	Coliform Counts
cfu/ml	Colony forming unit per millilitre
CIP	Clean –in-place
CRAs	Chlorine releasing agents
DAHLD	Department of Animal Health and Livestock Development
ELISA	Enzyme-Linked Immune-sorbent Assay
EO	Electrolyzed-Oxidizing
EPS	Extracellular polymeric substances
ESCOM	Electricity Supply Cooperation of Malawi
FAO	Food and Agriculture Organisation of the United Nations
GDP	Gross Domestic Production
LAB	Lactic Acid Bacteria
LSD	Least Significant Difference
MBGs	Milk Bulking Groups
MBS	Malawi Bureau of Standards
MMM	Malawi Milk Marketing
PHL	Post-Harvest Loss
QACs	Quaternary Ammonium Compounds
Stderr	Standard Error
SME	Small and Medium enterprise
SPSS	Statistical Package for Social Science
SSOP	Sanitation Standard Operating Procedures
TVC	Total Viable Counts
UV	Ultra-violet
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Milk processing is one of the pre-requisite for safety of the consumer. Milk is processed from the farm through handling, cooling and transportation to the processing plant where it is transformed into other products. The products quality depends on good manufacturing practices; basic hygiene to minimize microbial contamination. Cross-contamination can occur from other foods, processing environment, personnel, water and food contact surfaces which include holding and mixing tanks, knives, pipes and conveyor belts (López-carballo *et al.,* 2008 Niemira *et al.,* 2014). Microorganisms can also be inherent in the milk if the milk comes from a sick cow, for example mastitis.

The practices of clean milk production, transportation and storage are key to milk quality. Clean milk production composes the healthy cow, healthy personnel, potable water, food grade equipment and milking technique. For transportation, time taken to reach processor, means of transportation and type of container will have direct effects to the quality of the milk. For storage, if the temperature is not controlled then the microbial multiplication will be highly encouraged (Kashongwe, *et al*., 2017).

The type and design of equipment coming into contact with food is one of critical elements in ensuring consistent safety of food (Hasting, 2012). Equipment made of materials which are not of food grade with poor hygienic designs result in ineffective cleaning. This will lead to retention of the food residues (soil), which promotes survival, multiplication and attachment of microorganisms (Hauser *et al.,* 2004). The type and design of food-contact surfaces influences the level of attachment of microorganisms and effectiveness of cleaning once the attachment occurs (Silva, *et al*., 2010).

The undesirable presence of microorganisms in food has significant effect to both food processors and consumers. Spoilage microorganisms make food unpalatable while pathogenic microorganisms cause foodborne diseases. Pathogenic microorganisms are eliminated through pasteurization and sterilization during processing, while spoilage microorganisms are managed by time-temperature controls (Purnell *et al.,* 2012).

Microorganisms such as bacteria can adhere to and colonize food contact surfaces forming layered complex structures known as biofilms, which gives them the ability to respond to and

protect themselves against exposure to environmental stresses. The cells in biofilms are embedded in extracellular polymeric substance (EPS) composed of Exopolysaccharides, protein and nucleic acid, exhibiting altered growth, gene transcription and increased resistance to most antimicrobial agents as compared to unattached cells (Bridier *et al.,* 2011). Biofilms are difficult to remove and act as permanent source of contamination if not removed. Some of the microorganisms associated with surface attachment include *Pseudomonas, Bacillus*, and *Salmonella*.

An ideal food hygiene programme should cover elements of processing environment, equipment, personnel, water, and food contact surfaces throughout the supply chain. This will include primary production where environmental hygiene, hygienic production, handling storage and transport, cleaning and personnel hygiene are key elements. Cleaning and food hygiene procedures for equipment should always be checked using visual, analytical and microbiological methods (Silva *et al.,* 2010).

Cleaning is the process of removing "soil", (food residues), 'dirt', grease or other objectionable matter from surfaces that come in contact with food, using specified detergents under specified condition such as temperature and time of contact (Schmidt, 2012). Cleaning and sanitizing procedure for food contact surfaces involves rinsing, cleaning, rinsing again and finally sanitizing. Cleaning using detergents only reduces surface microorganisms, but it is not adequate enough to reduce their populations to the acceptable levels. However there are other detergents formulated with disinfectants. But these are expensive and cannot be accessed by the farmers. Resultantly, disinfection must be done to reduce 99.9% of surface microorganisms. Disinfectants used are thermal, radiation and chemical based. Chemical sanitization is done by the use of chlorine based, chlorine dioxide based, iodine based, Quaternary ammonium compounds, acid-anionic, fatty acid peroxide and hydrogen peroxide based sanitizers (CAC, 2009; Schmidt, 2012). The surface microbial load on the food contact surface varies from on processor to another depending on the microbial quality of the food being processed, the cleaning regimes used, and the disinfection post-cleaning (Evans *et al.,* 2004).

Malawi produces 64,747 tons of milk per annum and post-harvest loss is estimated at 17% due to microbial contamination causing spoilage (Sindani, 2012; FAOSTAT, 2014). Economically this post-harvest loss to the dairy actors accounts for approximately US$ 606 194. 57 per annum. The dairy industry in Malawi is composed of formal and informal sector, with the formal sector selling milk to processors through Milk Bulking Groups (MBGs). The

sole purpose of the MBGs is to collect milk, check for quality before acceptance, store the milk in cooling tanks and sell to processors (Sindani, 2012). A major limitation to the quality of the milk at MBGs is lack of sustainable supply of energy. Persistence rationing of electricity supply in Malawi has made MBGs to fail to effectively cool milk and hence spoilage. However, Few MBGs have diesel or petrol generators as power backups, however, some do not have enough power rating to effectively cool the milk in the cooling tanks (Sindani, 2012, Wiggins, 2016). At times, large scale milk processors fail to collect milk from the MBGs on the scheduled collection day and this has led to loss due to spoilage.

Previous studies indicate that the spoilage is largely due to poor hygiene at milking stage, use of unsterile containers to collect and transport milk to MBGs. The indicators for poor hygiene pointed out included; lack of treated water, not using sanitizers to wash milk handling equipment, no observation of personal hygiene for example not washing hand before milking among others. At the MBGs, there are no routine screening tests for microbial quality. Additionally, udder health is not practiced, there are no prior tests for udder infection, the udder and teats are not washed before milking, and the calf is let to suckle before milking. Occasionally, re-used pieces of cloth are used to dry the udder and the teats. The farmers use sand and ash as a scourer to scrub the milk handling containers. Transportation of milk takes long time to reach bulking centres. The time also varies with distance from the farmers' premises to the bulking centres and with farmers' practices, as some keep the afternoon's milk and deliver it together with the morning milk (Sindani 2012, Wiggins, 2016).

These practices are likely to lead to microbial contamination, hence the high loss of 17% of Malawi's milk production. The farmer's awareness of hygienic production of milk is scanty, the personnel working in bulking centres lack handling skills and the equipment used for milk transportation is difficult to clean. The strength of cleaning agents used is not routinely assessed. The water used for cleaning the equipment and processing premises is not treated.

These gaps are likely causative factors for microbial contamination and biofilm formation. This study aimed at assessing the hygiene practices from farm level to milk processing plants and their influence on biofilm formation.

1.2 Statement of the Problem

Malawi produces 64,747 tons of milk per annum and post-harvest loss is estimated at 17% due to microbial contamination causing spoilage. Economically, this accounts for approximately US$ 606 194. 57 per annum. This suggests cross-contamination occurs from processing environment, personnel, equipment, water and equipment contact surfaces. There has not been a clear system of monitoring and evaluation of hygiene practices in the milk supply chain in Malawi and this may be contributing to the loss of milk. The danger of biofilm development in transportation equipment and storage tanks is likely to be enhanced. This will exacerbate the milk loss. This study aimed at analysing hygiene practices of dairy farmers and milk bulking centres and their influence on biofilm development.

1.3 Objectives of the Study

1.3.1 Overall Objective

The study aimed at contributing to food and nutrition security by reducing milk post-harvest loss at farm and bulking centres through improved sanitation practices of dairy actors and reduction of biofilm development.

1.3.2 Specific Objectives

i. To determine sanitation practices of dairy farmers, and milk bulking centres that lead to the development of biofilms.
ii. To determine microbial load and type at farm and bulking centres
iii. To determine biofilm indicator bacteria and their biofilm capacity at farm and bulking centres

1.4 Research Question

The study aimed at responding to the following question for specific objective one;

i. What are the hygiene practices of the farmers, small and medium enterprise milk processing plants in Lilongwe Malawi

1.5 Hypotheses

The study aimed at testing the following hypotheses for specific objective 2 and 3 respectively;

i. There is no difference in microbial load and type of microorganisms on the milk handling containers.
ii. Microorganisms on the milk handling containers are not biofilm forming microorganisms.

1.6 Justification of the Study

In Malawi, the dairy industry is one of the enterprises which are being explored by the Malawian government and donor partners to improve the economic livelihood of smallholder farmers. However, though the industry has the capacity to develop the industry is faced with postharvest loss due to spillage as well as microbial contamination (spoilage). The cleaning processes that the small scale enterprise practice is one of the sources of the microorganisms. The hygiene practices encourage microorganisms to attach to the surface of the milk handling containers forming biofilms. Biofilms are a chronic source of milk microbial contamination, resulting to persistent postharvest losses. The postharvest losses of milk results to significant economic losses to the farmers and processors and hence affect the national economy, food security and consumer safety. This research is, therefore, justified since the results obtained will help to develop ways to enhance dairy industry productivity; increase profitability, and improve the farmers' livelihood by reducing milk post-harvest loss as well as protect consumers. This can be achieved by assessing the sanitation practices at dairy farm and milk bulking centre, how they contribute to the biofilm formation and hence suggest the mitigation practices.

1.7 Scope and Limitations of the Study

The study was undertaken in Lilongwe district, central region milk shed, in Malawi, targeting dairy farmers and milk bulking centres handling milk. The study assessed the sanitation practices at dairy farm and milk bulking centre that may lead to biofilm formation on milk handling containers, determined microorganisms on the milk handling containers, and determined biofilm forming microorganisms on the milk handling container and thereby reducing milk post-harvest losses. Data for the empirical study was collected from dairy farm and milk bulking centre using a survey questionnaire, sampling procedures and (microbial) experimental analyses. Relevant authorities such as agricultural extension workers were consulted to help in collecting data.

CHAPTER TWO

LITERATURE REVIEW

2.1 Milk Production in Malawi

The dairy industry in Malawi is divided into formal and informal sector, with 65, 027 heads of dairy animals (Revoredo-Giha *et al.*, 2013; FAOSTAT, 2014). The informal sector produce around 27, 000 tons of milk for consumption in a year (Imani Development Consultants, 2004). Milk from the informal sector is mainly produced from the Malawi Zebu cattle (*Bos indicus*), a breed kept by Malawians for a long time for subsistence, prestige and insurance for drought. The formal sector, emerged due to urbanization and industrialization, has about 4,000 dairy farmers with a dairy herd of approximately 15, 000 Friesians and crossbreeds of Malawi Zebu producing about 64, 747 tons of milk per annum (Sindani, 2012; FAOSTAT, 2014). The sector is reliant on smallholder farmers, with only five large scale farms. The informal sector sells raw milk directly to consumers, while the formal sector sale the milk to milk processors through MBGs as portrayed in Figure 1; the dairy value chain mapping in Malawi.

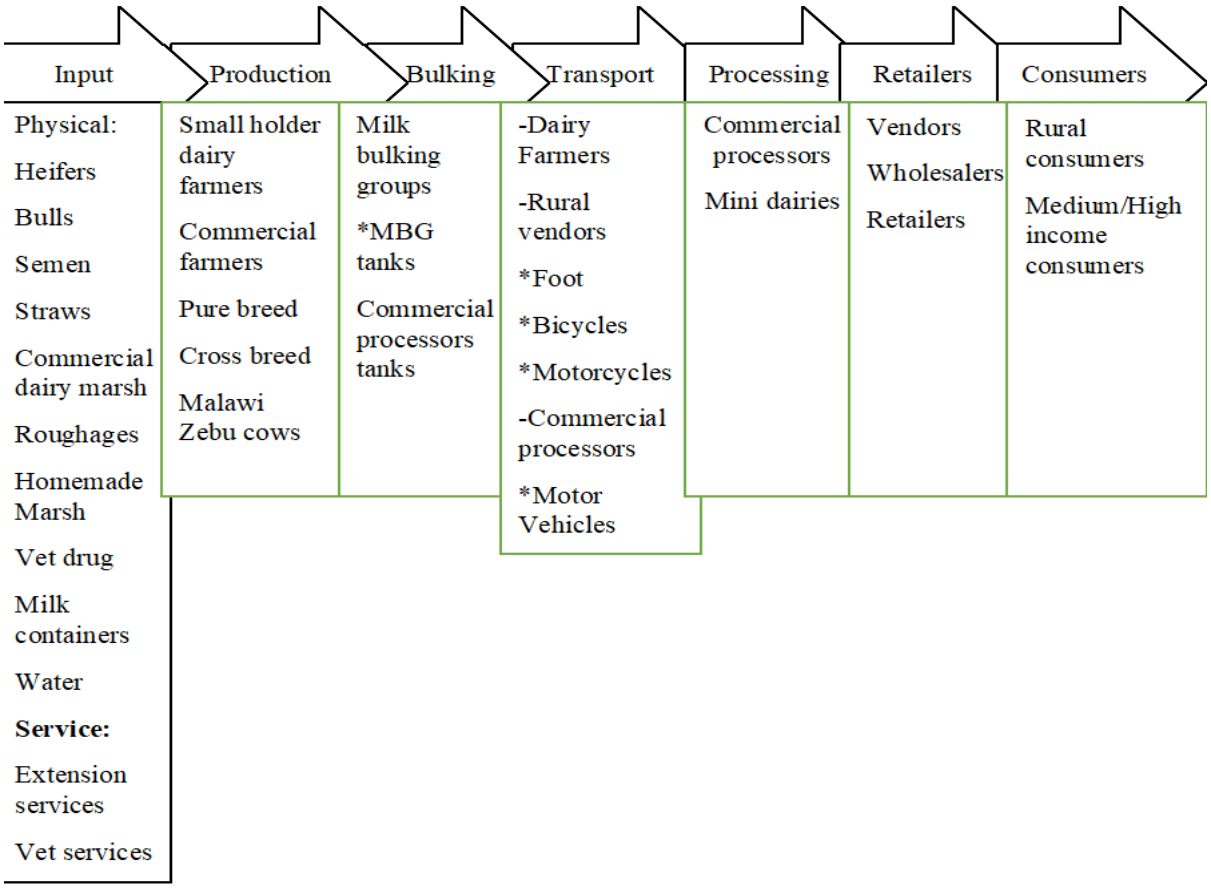

Figure 1. Dairy Value chain mapping in Malawi**.**

Source: M-Livestock Consultants, (2013)

Milk bulking groups (MBGs) are registered under Department of Animal Health and Livestock Development (DAHLD) under ministry of Agriculture, which operates by Malawi Milk and Milk Products Act of 1972, under chapter 67:05 of the laws of Malawi (Sindani, 2012). The act provides regulations to improve and control production, processing and marketing of milk and milk products. DAHLD inspects premises where milk is produced, stored or processed. Furthermore, milk and milk products, are subjected to quality tests by Malawi Bureau of Standards (MBS) under the MBS Act, 1972 (chapter 51:02), which routinely inspect premises quarterly in a year. MBS provides regulation on hygiene at production and processing; Food and food processing units–Code of hygienic conditions (MS 21:2002) and Dairy Farming-Code of hygienic conditions for milking (MS 111:1988) (Sindani, 2012).

The dairy industry in Malawi has the potential to grow. It is promoted by the government of Malawi and non-governmental organizations through research funding, dissemination of technologies to farmers, capacity building and development, (Sindani, 2012). However, its development is hindered by milk post-harvest loss.

2.2 Milk Post-harvest Loss

Post-harvest loss (PHL) is the measured loss both qualitatively and quantitatively along the value chain starting from the time of harvest up to its end use; is caused by forced consumption, spillage and microbial contamination (Kiaya, 2014). In Malawi, PHL of milk due to microbial spoilage is mostly detected at reception of the milk at processing plant, now stands at 17 % (Wiggins, 2016). Milk spoilage causes economic loss both to the dairy farmer and the government, and foodborne diseases to the consumer. Most cases of foodborne illness are a result of consumption of pathogens with food. According to WHO (2015), the effects of food borne diseases is significant and affects entire populations in the world. Thermal treatments applied during food processing such as pasteurization and sterilization eliminates most foodborne pathogens (Purnell & James, 2012). However, cross-contamination can occur from other food, processing environment, personnel and most importantly food contact surfaces (López-carballo *et al.*, 2008). Contamination of food may occur from direct contact with surfaces such as holding and mixing tanks, knives, pipes and conveyor belts among others (Niemira *et al*., 2014).

The type and design of equipment coming into contact with food is one of critical elements in ensuring consistent safety of food (Hasting, 2012). Equipment made of materials which are not food grade with poor hygienic designs result in ineffective cleaning causing retention of

soil, thereby promoting survival, multiplication and attachment of microorganisms (Hauser *et al*., 2004). The type and design of food-contact surfaces influences the level of attachment of microorganisms and effectiveness of cleaning once the attachment occurs (Silva et al., 2010), resulting in the formation of biofilms.

The microbial contaminations of the milk occur at the farm level, transportation channels, and at selling points in the dairy value chain. At farm level, contamination of milk has been attributed to failure to sufficiently clean milk handling equipment, resulting from use of poor design and type of the equipment, use of poor quality water for cleaning, use of ineffective sanitation agents or inappropriate use of sanitation agents and cleaning without disinfecting (Yilma, 2012; Wafula *et al.*, 2016).

2.3 Dairy Actors Hygiene Practices in Malawi

In Malawi, the dairy value chain actors' hygiene practices are poor. According to Imani Development Consultants (2004) and Sindani (2012), the dairy farmer fails to thoroughly clean the udder and teats before milking, they let the calf to suckle the milk before milking instead of washing, a dirty towel is used to dry the udder and the teats. The farmers use sand and ash as a scourer to scrub the milk handling containers. The water used in the cleaning processes is of poor quality and it is not treated before use. Therefore, containers with high microbial load on the surface are used to transport the milk to Milk Bulking Centres. In addition the milk is brought to the MBGs either by a pushbike or by foot (Sindani, 2012). Thus, it takes long time to transport milk to milk bulking centres. The time also varies with distance from the farmers' premises to the bulking centres and with farmers' practices, as some keep the afternoon's milk and deliver it together with the morning milk (Sindani, 2012).

2.4 Process of Cleaning

Cleaning is the process of removing soil, food residues, dirt, grease or other objectionable matter from surfaces that come in contact with food, using specified detergents under specified condition such as temperature and time of contact (Schmidt, 2012). The detergents are compounded specifically for certain jobs such as Cleaning-in-place (CIP), and/or high pressure washer. They are also made to clean specific "soil". For instance, acid-cleaning compound is appropriate for removal of inorganic compound and an alkaline cleaner is more effective in removing organic deposits. There are several factors affecting cleaning performance such as time, action, concentration, temperature, water, individual, nature of the soil, and surface (Marriott and Gravani, 2006). The cleaning time needs to be long enough to allow sufficient chemical contact with surfaces, but not so long that it becomes cost

ineffective. The temperature of the cleaning agent needs to be hot enough to effectively clean, but not so hot that it bakes on proteins. The concentration and chemistry of the cleaning agents is dependent on the type and nature of soil present. The action/manual scrubbing and velocity of the cleaning solution is very important in the cleaning process, because it is used to lift soil from the surface and pipelines. Finally, the surface being treated must be cleanable, with a smooth, corrosion-resistant finish (Stewart and Seiberling, 1996).

The cleaning chemicals play an important role in cleaning process; they must first be transported to the solid–liquid interface, then contact and penetrate the deposit. The cleaning solution reacts with the deposit, which is then able to be removed, due in part to the manual scrubbing and velocity of the solution through the pipes (Changani *et al.*, 1997). However, under conditions of lowered temperature, and detergent and sanitizer concentrations, those cells that are present after CIP are viable and show attachment fibrils (Stone and Zottola, 1985; Bremer *et al*., 2009). Therefore, it is essential to maintain and control the CIP to the soil conditions so optimum cleaning is always achieved.

The major functions of water as a cleaning medium include: pre-rinse for the removal of large soil particles; wetting (or softening) of soils on the surface where removal is essential; transport of the cleaning compound to the area to be cleaned; suspension of soil to be removed; transport of suspended soil from the surface being cleaned; rinsing of the cleaning compound from the area being cleaned; transport of a sanitizer to the cleaned area. Satisfactory water is required to complement the cleaners. The water should be, free of microorganisms, clear, colourless, noncorrosive, and free of minerals (known as *soft water*). Hard water, which contains minerals, may interfere with the action of some cleaning compounds, thereby limiting their ability to perform effectively (although some cleaning compounds can counteract the adverse effects of hard water). The hardness of water affects cleaning compound consumption and may cause the formation of films, scale, or precipitates on equipment surfaces (Marriott and Gravani, 2006).

The major functions of a cleaning compound are to lower the surface tension of water so that soils may be dislodged and loosened, and to suspend soil particles for subsequent flushing away. To complete the cleaning process, a sanitizer is applied to destroy residual microorganisms that are exposed through cleaning. Sanitation means to adequately treat food-contact surfaces by a process that is effective in destroying vegetative cells of microorganisms of public health significance, and in substantially reducing the numbers of

other undesirable microorganisms, but without adversely affecting the product or its safety for the consumer (CAC, 2009).

2.4.1 Sanitation

Cleaning process is not complete if sanitation does not follow soon after cleaning. Sanitation is the application of science to provide wholesome food, processed, prepared, merchandised and sold in a clean environment by health workers; to prevent contamination with microorganisms that cause foodborne illness and to minimize the proliferation of food spoilage microorganisms (Marriott and Gravani, 2006). Sanitation attained through physical involves use of moist heat (steam, hot water, and autoclave), hot air, dry heat, freezing, filtration, smoking and dry ice. Other physical methods include use of radiations and sound waves. Heat which denatures enzymatic protein and removes water in microbial cells is the most applied mechanism in the control of microorganisms in sanitation practices. Freezing and sound waves methods only slow microbial growth but do not kill. Filtration method is used to take out suspended soil particles and microorganisms that attach onto these surfaces. Radiations that are used in sanitation include gamma, X-rays and ultra-violet (UV). Sun drying of milk handling equipment where the sun rays contain UV light of 260 nm wavelength that has bactericidal properties is a widely used method in sanitation by dairy farms. Smoke is the aerosol product as a result of wood pyrolysis by fire and low oxygen supply. The smoke contains several acids and alcohols that have antimicrobial properties. Dry ice process is whereby solid CO_2 is passed over a surface to be cleaned at a very high velocity in a stream. This cause's local undercooling thus inhibiting growth (Yilma, 2012).

Microbiological sanitation involves the use of enzyme-based agents of protease, lipases that break down "soils" such as blood, body fluids, secretions and excretions from surfaces and equipment. Enzyme-based agents are used on surfaces that contain biofilms. The enzymes digest the extracellular polymeric substances loosening and dissolving the organic substances prior to cleaning. Most enzymatic cleaning agents also contain a detergent (Wasserman and Plescia, 1989).

Chemical sanitation in the dairy industry uses chemical agents grouped in four main groups; chlorine releasing agents (CRAs), quaternary ammonium compounds (QACs), acidic agents and iodophors. Ozonized water is another chemical gaining popularity in the dairy industry. CRAs are used in the form of gaseous chlorine, hypochlorite and chloride dioxide (ClO_2). The chlorine gas and hypochlorite are potent oxidizing agents that react with reducing agents in bacteria in the bacterial cell membrane affecting transport of materials across the

membrane. Resultantly, cellular enzymes involved in glucose metabolism and oxidize cellular proteins are inhibited (Schmidt, 2012).

Iodophors are iodine releasing compounds. In aqueous solutions, iodine exists in four form; elemental iodine (I_2), hypoiodus acid (HIO), periodide (I_3) and iodate ion (IO_3). The elemental iodine and hypoiodus acid possess bactericidal properties, which is attained by diminishing the oxygen supplies to the aerobic microorganisms. This is through interfering with microorganisms' respiratory chain by blocking the transport of electrons through electrophilic reactions with the enzymes of the respiratory chain (Wasserman and Plescia, 1989).

Quaternary ammonium compound (QAC) is a positively charged polyatomic ion of the structure NR_4^+, R being an alkyl or aryl group. QAC's being positively charged (cationic), is therefore attracted to the negative charge of the bacterial cytoplasmic membrane i.e. phospholipids. There is thus a loss of structural organization and integrity of the cytoplasmic membrane in bacteria, together with other damaging effects to the bacterial cell (Wasserman and Plescia, 1989; Schmidt, 2012)

Ozone is a triatomic form of oxygen (O_3). Oxygen atom (O_2) splits into singlet oxygen and the single atom (O) rapidly combines with oxygen (O_2) to form ozone. It is a very strong oxidant that reacts either directly with compounds in the bacterial membrane or form compounds that are highly reactive, causing death of a bacterial cell. There is also, electrolyzed oxidizing (EO) water. It has strong bactericidal effects on most microorganisms that are important to milk spoilage. It is produced by passing a diluted salt solution through an electrolytic cell, within which the anode and cathode are separated by a membrane (Hsu, 2005). Lastly, the other most common disinfecting chemical agents are acids. They include inorganic acids such as hydrochloric, nitric, sulphuric, sulphamic and phosphoric 4 acids. Also organic acids that are used include lactic, citric, mallic, formic and propionic acids. Acids dissociate in water to form hydrogen ions (H^+) and hydroxyl ions (OH^-) (McDonnell and Russell, 1999).

2.5 Biofilms

According to Shunmugaperumal (2010), a biofilm is a microbial derived sessile community which is characterized by cells that are irreversibly attached to a substratum, interface, and/or each other. Korber *et al.* (2009) stipulates that biofilms usually consist of assemblages of different species of microorganisms living together in a complex community, but not always,

rarely the biofilm is composed of a single strain of microorganism. The cells in biofilms are embedded in extracellular polymeric substance (EPS) composed of exopolysaccharides, protein and nucleic acid, exhibiting altered growth, gene transcription and increased resistance to UV light as compared to unattached cells (Bridier *et al.*, 2011).

2.6 Biofilm Formation

2.6.1 The Formation of a Conditioning Film

Organic and inorganic compounds (proteins, lipids and nucleic acids) from the milk accumulate on the food contact surface, increasing the nutrient content and changing physicochemical properties of the surface forming a conditioning film (Bryers, 1987). This stage is however not a requirement for some microorganisms as some will attach to the milk handling equipment by the pili and flagellum and some secrete and attach by small amount of Exopolysaccharide (Mogha *et al.*, 2014)..

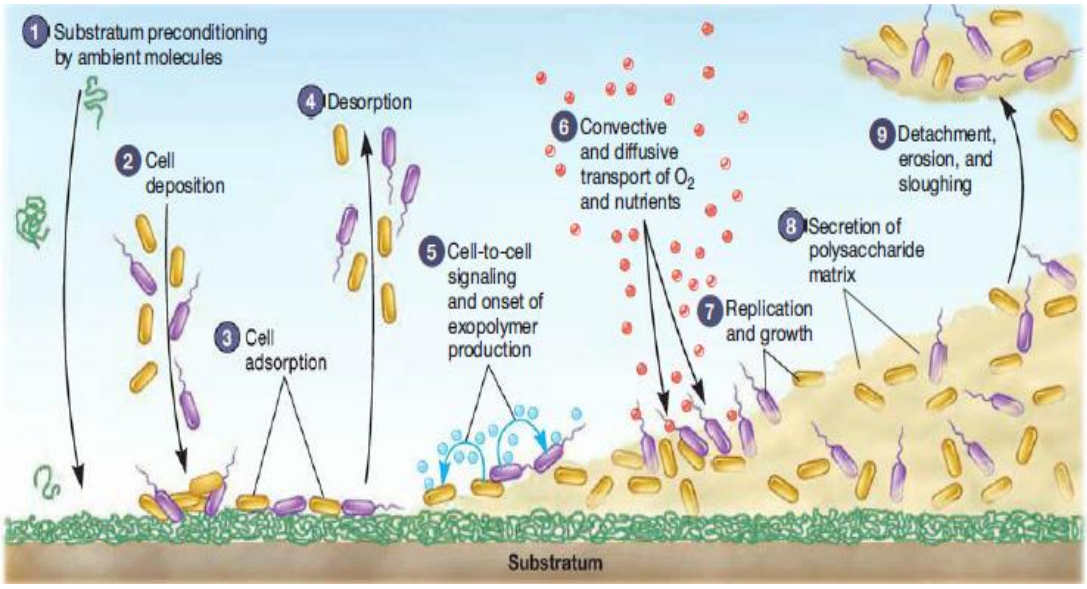

Figure 2. Stages of biofilm formation

Source: Breyers and Ratner (2004).

2.6.2 Attachment of Cells

The bacteria attach to the surface either through a reversible or irreversible adhesion; active or passive depending on cell motility. Passive attachment is driven by gravity, diffusion and fluid dynamics while active adhesion the attachment is driven by the bacterial cell surface properties such as flagella, pili, adhesin protein, capsules, and surface charge, as shown at stage 2 in Figure 2 (Kumar and Anand, 1998). The cellular physiological surface proteins, such as pili and adhesins, and synthesis of polysaccharides help in the attachment by altering

the diameter-dependent repulsion experienced by microbial cells as they approach a surface (Bos *et al.*, 1999; Davey and O'Toole, 2000). The attachment (reversible followed by irreversible adhesion) occurs within 5 to 30 s (Mittelman, 1998).

In reversible cell attachment, cells are attached to the surface by long-range forces such as van der Waals, electrostatic, and hydrophobic. The cells can still be removed by cleaning, rinsing and sanitization. However, if the surface is not cleaned and sanitized at stage 3 in figure 2, the biofilm grow, developing micro-colonies and water channels, becoming difficult to remove otherwise the cells are desorbed (Chmielewski and Frank, 2003)

Irreversible attachment of cell involves interactions such as dipole–dipole interactions, hydrogen, ionic and covalent bonding, as well as hydrophobic interactions. Production and secretion of bacterial EPS and receptor-based attachment (Lawrence *et al*., 1996), together with specific sets of genes that express biofilm-specific proteins, also influence the long-term adhesion (Wingender *et al.,* 1999; Böckelmann *et al.*, 2006). EPS fibrils form bridges between the microbial cells and the surface, facilitating irreversible attachment. Bacterial EPS not only enhances bacterial adhesion and biofilm stability, but also plays putative roles in nutrient storage, resistance to antimicrobial agents and desiccation, maintenance of the biofilm microenvironment, and predator avoidance (Wolfaardt *et al*., 1999).

2.6.3 Micro-colony Formation and Biofilm Development

Bacterial cells irreversibly attached to the surface, grow and multiply into micro colonies. The micro-colonies enlarge and merge to form a layer of cells covering the surface that further develop into fully formed biofilms. The bacteria biofilm mode of growth includes changes in the expression of genes and proteins (Pringent-Combaret, *et al.,* 1999; Ren *et al.*, 2004; Beloin and Ghigo, 2010). Proteins that have shown to be up-regulated in the biofilm cells relative to planktonic cells include biosynthesis of EPS components, membrane proteins, Quorum-sensing proteins, adaptation and protection proteins, and proteins associated with metabolic cycles (Korber and Lawrence, 2004; Mangalappalli-Illathu *et al.,* 2008a). Enzymes associated with stress response, especially oxidative stress, are found to be up-regulated in the biofilm cells as a result of nutrient and O_2 limitation in the depth of the biofilms (Ren *et al.*, 2004). Genes associated with the flagella biosynthesis and other motility structures are repressed in biofilm cells (Pringent-Combaret *et al.,* 1999; Mangalappalli-Illathu, *et al.,* 2008a). The cells are different in biofilm from "planktonic", but the ability of the cells to have adaptive and programmed responses to changing conditions has predisposed many bacteria for reproductive success on surfaces (Mangalappalli-Illathu, *et al*., 2008a, 2008b).

2.6.4 Biofilm Redistribution (Biofilm Dispersion and Re-colonization)

Once the biofilm has fully matured, clumps of cells or individual daughter cells are detached and transported to new locations with sufficient nutrients and space, where they recolonize and initiate a new biofilm formation (Korber *et al.*, 1989). Nutrients and O_2 concentration, and accumulation of waste products, facilitates dispersion of bacteria where the bacteria in the biofilm produce lytic enzymes that hydrolyse the extra polymeric substances (EPS) (Allison *et al.,* 1998; Decho, 2000). Daughter cells that are ready to be released from biofilms adopt planktonic cell phenotype, with the up-regulation of motility appendages such as flagella (Korber and Lawrence, 2004).

2.7 Methods to Determine Biofilms

Several instrumentation as well as methods have been used to detect and study the structure of biofilms. According to Chmielewski and Frank, (2003) light, fluorescence, differential interference contrast (DIC), transmission electron (TEM) scanning electron (SEM), atomic force (AFM), and confocal laser scanning microscopy (CLSM) are used to analyse biofilm structure. Microelectrodes can detect the presence of O_2 and observe molecular diffusion within the biofilm. Molecular biological methods, including 16-23S rRNA hybridization and fluorescent in-situ hybridization (FISH) with CLSM (Davey and O'Toole, 2000), have been used to observe microstructure and metabolism of biofilms (Wimpenny *et al.,* 2000). The FISH method was used to confirm the decrease in viability of cells as the biofilm ages. Viable cells were detect in the biofilm, and young biofilm were determine to have about 80% viable cells and about 50% in old biofilm (Wimpenny *et al.*, 2000). Phenotypic identification of microorganisms involved in biofilm formation is done by Tube Method, Congo red agar method and Tissue culture plate method. Tube method has been reported to detect approximately 63% of the biofilm forming isolates while Congo red agar detects approximately 53% of the isolates and Tissue culture method detects 78% of the biofilm forming isolates.

2.8 Implications of Biofilm Formation

Biofilms formed in food-processing environments are a persistent source of microbial contamination that may lead to food spoilage and/or transmission of diseases. Biofilm are more resistant-tolerant to biocides/sanitizers than their planktonic counterparts. For instance, the antimicrobial efficacy of various aqueous sanitizers is lower for biofilm-associated than for planktonic *Pseudomonas spp.* and *Salmonella spp.* (Van Houdt and Michiels, 2009).

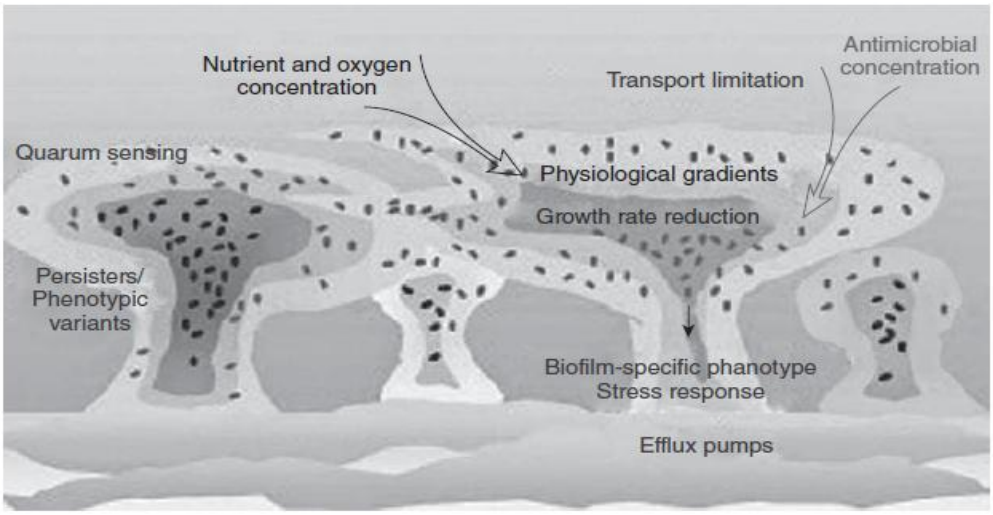

Figure 3. Schematic representation of mechanisms proposed to be involved in *P. aeruginosa* biofilm resistant antimicrobial agents.

Source: Shunmugaperumal (2010).

The resistance-tolerance mechanisms to biocides/sanitizers of biofilm are related to the morphology of the biofilm, and the gene expression in biofilm. The changes in gene expression induced by surface attachment lead to emergence of biofilm specific phenotype. The biofilm provides a shelter for the bacteria to thrive; the production of exopolysaccharides matrix contributes to increasing cell survival by delaying antimicrobial penetration. This is shown by the narrowing arrows in the Figure 3. As the biofilm matures, the increase in cell density creates gradients of nutrients of oxygen availability leading to a reduction in metabolic activity and growth rate, and the cell density induces activation of quorum sensing. The nutrient starvation and oxygen limitation induces stress response and up-regulation of efflux pumps. In the biofilm, there is exchange of genetic material through conjugation process at rates up to 1000-fold higher than those in planktonic populations, and hyper-mutation of bacteria; a bacterial mutation at high rates to evolve under stressful conditions. Thus development of resistant mechanism can quickly be selected and propagated throughout the community. The environmental conditions in the biofilm induce and select for phenotypic/persister variants resistant to antimicrobials/sanitizers (Shunmugaperumal, 2010)

2.9 Control of Biofilms in Dairy Manufacturing Plants

Prevention of the formation of biofilms is the key step to control biofilms. The prevention of cell attachment can be achieved through altering the surface chemistry; treating the surface with antimicrobial agents; optimizing process and equipment design, and the use of cleaning regimes (Bower *et al.,* 1996). The simplest way to control biofilms in Dairy Manufacturing

Plant (DMP) is to clean the production line using chemicals. The cleaning process revolves around the clean-in-place (CIP) system; having a variability in eliminating surface adherent bacteria (Bremer *et al.*, 2009).

Novel methods that scientist are working on include; use of enzymes, biofilm degrading enzyme that has the ability to attack the bacterial cells in the biofilm and the biofilm matrix, substantially reducing the biofilm, application of enzymatic cleaning product known as "green chemicals". Phages and bio-regulation are also exploited to control biofilms. Microbial molecules, commonly used as bio-preservatives, such as nisin, lauricidin, reuterin and pediocin control potential against microorganisms commonly found in dairy processing facilities, including *L. monocytogenes*. Molecule have been developed having capacity to interfere quorum sensing (Bridier *et al.*, 2011).

2.10 Foodborne Pathogenic and Spoilage Microorganisms that Develop Biofilms.

Pathogenic and spoilage microorganism in the dairy industry that form biofilms include *Pseudomonads, Bacillus*, and *Salmonella.* According to Chmielewski and Frank (2003), *Pseudomonads* have been isolated from drains and floors, on fruits, vegetables, meat surfaces and in low acid dairy products. The species of *Pseudomonas* produce large amounts of EPS. Pseudomonads are said to attach and form biofilms on stainless steel surfaces and survive in multispecies biofilms (Barnes *et al.*, 1999; Chmielewski and Frank, 2003). For instance, *Pseudomonads* are reported to coexist within biofilms with *Listeria, Salmonella* and other pathogens (Fatemi, and Frank, 1999; Bagge *et al*., 2001)

Bacillus is ubiquitous, heat stable microorganism, and has been reported to accumulate on pipelines and joints in the processing environment (Jeong and Frank, 1994; Chmielewski and Frank, 2003). The microorganism and other thermoduric bacteria form biofilm when hot fluid continuously flows over a surface for over 16 hours (Frank, 2000; Chmielewski and Frank, 2003).

Salmonella is a microorganism that causes salmonellosis. It has been associated with poultry, mainly the processing equipment especially in the slaughter and evisceration area (Joseph *et al.*, 2001; Helke and Wong, 1994). The wet environment in poultry processing operation is ideal for biofilm formation (Chmielewski and Frank, 2003). *Salmonella* can attach and form biofilms on surfaces found in food processing plants, including plastic, cement, and stainless steel (Joseph *et al.*, 2001; Chmielewski and Frank, 2003).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area.

The study was conducted in Lilongwe district located in the Central Region of Malawi. Lilongwe is located at the approximate latitude of 13°58′S and 33°47′E. Lilongwe lies at an altitude of between 1,300 to 1,700 meters above the sea level. The mean annual temperature varies between 22 degrees Celsius in low altitude areas and 18 degrees Celsius in high altitude areas. Annual rainfall ranges from 900mm to 1500 mm (Saka, Rao, & Sakala, 2003)

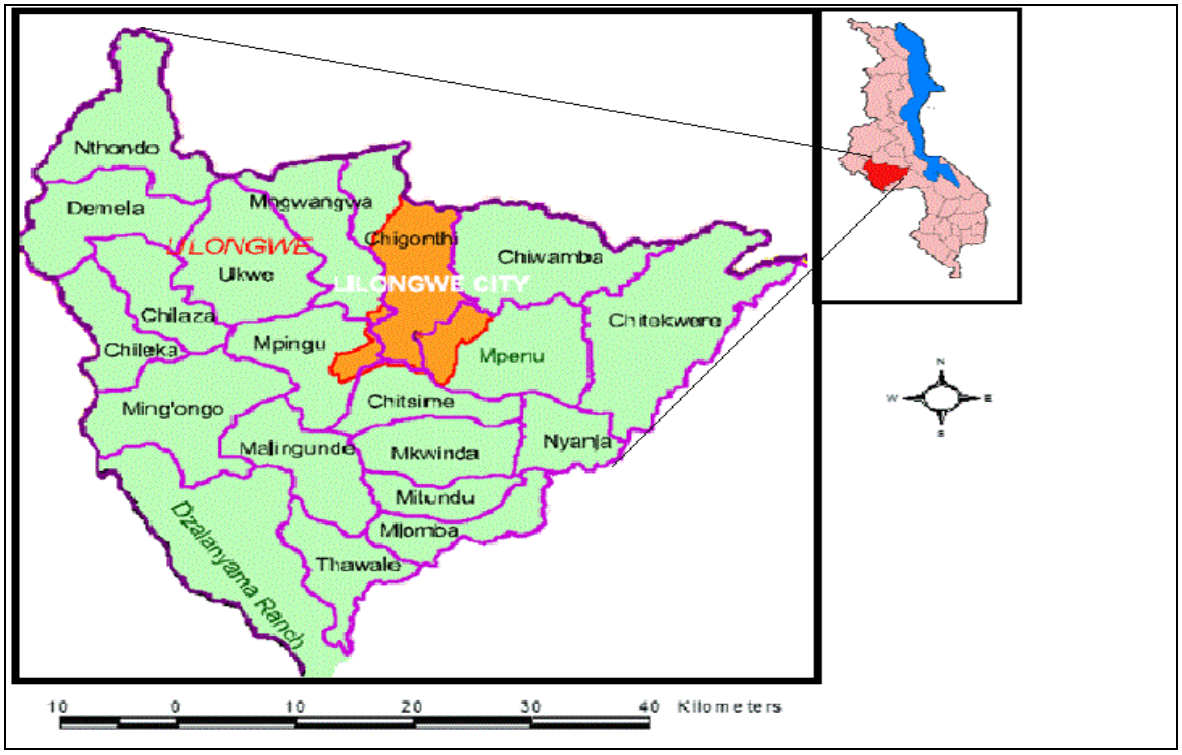

Figure 4. Map of Malawi showing the study site; Lilongwe

Source: Kundhlande *et al.* (2015)

3.2 Sample Size and Experimental Design

3.2.1 Determine sanitation practices of dairy farmers

Across-sectional study design was used along the dairy value chain in Lilongwe to determine sanitation practices. The number of respondents in all dairy systems was two hundred and sixty three. This was determined by the following formula:

$$n = 2\left[\frac{ZC}{d}\right]^2$$

(Barllet *et al.*, 2001)…… (Equation 1)

Where: n is the sample size, Z= 1.96 for 95% confidence interval, **C** is coefficient of variation = 30% and **d** is level of difference set at 5%.

The formula yields a sample size of 280 respondents. The value for Z is found in statistical tables which contain the area under the normal curve. However, the 262 respondents were reached after a hundred percent sampling of dairy farmers (Appendix A. 256) in six milk bulking groups; who had an experience of milk handling, and at the six bulking centres (Appendix B).

The questionnaires were administered in local language to small holder dairy farmers, milk bulking centres to obtain qualitative data. The data comprise of cleanliness of animals (udder), milking environment, milking person and milk harvesting and storage containers; the type of container farmers used to handle milk, type of detergents and/or sanitizers used in cleaning and sanitization, the cleaning method and procedure, the source of their water, availability and accessibility in terms of time to milk cooling facilities. In addition, it determined if the dairy actors have water treatment programme in place, how many times milk is delivered to the cooling facility and how is milk kept waiting delivery to the cooling facility at a bulking centre.

3.2.2 Determine Microbial Load and Type.

A complete randomized design CRD in nested arrangement was used for analysis of (milk, water, swab and rinse sample) microbial characteristics. There were three factors with different levels; Site (MBG1, MBG2, MBG3, MBG4, MBG5, and MBG6), Dairy actor (Farm and bulking centre) and Microbiological load (TVC, CC, LABs, and Yeast and Moulds). The milk, water, swab/rinse sample were obtained in a multistage sampling technique whereby two categories of sampling units; farm gate bulk and bulking/collection centres.

3.2.3 Milk Sampling

3.2.3.1 Farm Gate Bulk

Pooled milk sample was taken at farm gate from the bulk milk destined for the group bulking centre. This was morning and evening milk at the farm. Before taking the sample, the milk handling container was shaken to mix the milk. A sample of 100mL of milk was poured into a sterile labelled screw-cap tube and stored in a cool box maintained at 8^0– 10^0 C using cooling elements that had ice. The collection of the milk samples was done in the morning at normal milking time. The milk samples were collected from 24 households from six milk bulking groups; Machite Nathenje, Lumbadzi, Majiga, Namwiri and Nkhweza.

3.2.3.2 Bulk Sample

This sample was taken from the milk bulking centres where milk from various production farms was pooled together for transportation to processor. This was taken as a processing point. The milk was brought in various containers including aluminium and plastic cans. Milk was poured into cooler tanks. Milk in each cooler tank was stirred to produce a homogeneous mixture before taking a representative sample. About 100mL of milk sample was taken and poured into a sterile labelled screw-cap bottle and stored in a cool box at 8^0 – 10^0 C using cooling elements. Five (5) bulk milk samples were taken from five bulking centres in five locations of Lilongwe. The collection points, milk bulking centres included Machite, Nathenje, Lumbadzi, Namwiri, and Majiga (the bulking centre of Nkhweza was not operational). The samples were transported to NCHSU laboratory within six hours and analytical work started immediately.

3.2.4 Swab sample for determining efficacy of the sanitation regime

Swabbing was done on the milk handling containers when the containers are cleaned and ready to be used in handling milk. Surface swabs for collecting microorganisms were done using a sterile cotton swab buds pre-wetted in peptone water at an area of 25 cm^2 in three replicates. Swabs were taken after a cleaning regime by rotating the cotton end in contact with the prepared milk handling container surfaces. The swabbed samples were then transferred to the 9ml 0.1% (w/v) buffered peptone water in a screw-cap tube and stored in a cool box at 8^0 C– 10^0 C. These were taken to the laboratory and shaken using a vortex for 2 minutes to dislodge the bacteria. Microbial analyses using standard methods and the biofilm forming microorganisms will be detected. Tissue culture plate method and tube method will be used to detect the biofilm forming microorganisms.

3.2.5 Water samples

Water samples from milk production areas were taken from farmer household. For well and borehole samples, 50ml samples were taken. The water was collected using a household cup and poured directly into a sterile screw cup glass bottle. The sampling container cup was aseptically replaced by wiping the cup and neck of the container with a paper towel that was soaked in 70% ethanol. The sample was kept in a cool box and transferred to the laboratory for microbial analysis.

3.5 Laboratory Analysis

3.5.1 Microbial Analysis

Laboratory analyses were conducted at Community Health Science Unit (CHSU) National Public Health (Microbiology) Reference Laboratory in Lilongwe, Malawi. The swabs from surfaces of milk-handling containers, water and milk samples were analysed for total viable count (TVC), total coliform count (TCC), and lactic acid bacteria count (LAB) using appropriate sterilized media. The samples were serially diluted in buffered peptone water before analysis.

3.5.2 Total Viable Count (TVC)

Milk-handling container surface swabs and milk samples were pour-plated on Nutrient agar (Oxoid, UK) and the plates were incubated at 37°C for 48 hours. One millilitre (1ml) of milk sample was serial diluted six-fold using buffered peptone water (Oxoid) then one ml of the sample milk was diluted in 9 ml of peptone water (ratio of 1:10) up to six dilutions. Sterile duplicate glass petri dishes were labelled according to the dilution index. One ml of the dilutions was aseptically withdrawn using a sterile 1ml pipette and delivered into an opened and sterile petri dish and closed. The same was done for a duplicate petri dish. This was repeated till all the dilutions were pipetted into their corresponding plates up to 10^{-6}. This was followed by pouring about 15 ml of Nutrient agar (Oxoid), which had been autoclaved at 121^0 C for 15 min, cooled and tempered in a water bath at 45^0 C. The sample and the agar were gently mixed by alternate clock and anti-clockwise rotations for about 3 min. and left to solidify on the bench for about 30 min. For milk samples the first dilutions are expected to have heavier growth, they were not used; instead the last three dilutions (10^{-4}, 10^{-5}, and 10^{-6}) were used for total viable counts while for the surface swab the first three dilutions were used (10^{-1}, 10^{-2}, 10^{-3}). The plates were inverted and incubated at 37 ^{0}C for 48 h. Finally, the colony counting was done using Dr. N. Gerber digital colony counter (Schneider and Co., Zurich) and counts recorded.

3.5.3 Total Coliform Count (TCC)

Appropriate triplicate serial dilution of milk-handling container surface swabs, water and milk samples were pour-plated on MacConkey agar (Oxoid, UK), incubated at 37°C for 24 hours and typical dark red colonies on the plates were considered as coliforms and counted. One millilitre (1ml) of milk sample was serially diluted six-fold using buffered peptone water (Oxoid) then one ml of the sample milk was diluted in 9 ml of peptone water (ratio of 1:10)

up to six dilutions. Sterile duplicate glass petri dishes were labelled according to the dilution index.

One ml of the dilutions was aseptically withdrawn using a sterile 1ml pipette and delivered into an opened and sterile petri dish and closed. The same was done for a duplicate petri dish. This was repeated till all the dilutions were pipetted into their corresponding plates up to 10^{-6}. This was followed by pouring about 15 ml of MacConkey agar (Oxoid), which had been autoclaved at 121^{o} C for 15 min, cooled and tempered in a water bath at 45^{0} C. The sample and the agar were gently mixed by alternate clock- and anticlockwise rotations for about 3 minutes and left to solidify on the bench for about 30 min. The plates were inverted and incubated at 37^{o} C for 48 h. For surface swabs the first dilutions were used for counting, while for the milk samples the last three dilutions (10^{-4}, 10^{-5}, and 10^{-6}) were used for total coliform counts

3.5.4 Lactic Acid Bacteria (LAB)

Samples of milk and surface swabs were serially diluted and pour-plated on MRS agar (Oxoid, UK). One millilitre (1ml) of milk sample was serially diluted six-fold using buffered peptone water (Oxoid) then one ml of the sample was diluted in 9 ml of peptone water (ratio of 1:10) up to six dilutions. Sterile duplicate glass petri dishes were labelled according to the dilution index. One ml of the dilutions was aseptically withdrawn using a sterile 1ml pipette and delivered into an opened and sterile petri dish and closed. The same was done for a duplicate petri dish. This was repeated till all the dilutions were pipetted into their corresponding plates up to $10^{-6}$and the plates were incubated at 37^{o} C for 48 hours and typical LAB counted.

3.5.5 Yeast and Moulds (Y/M)

Milk-handling container surface swabs, water and milk samples were pour-plated on Sabouraud Dextrose agar and the plates were incubated at 37°C for 48 hours, and colonies were counted and counts recorded.

3.6 Determination of Biofilm Indicator Organisms

3.6.1 Isolation of *Salmonella*

Surface swab samples, milk samples and water samples were enriched in buffered peptone water. The mixture of sample and peptone water was incubated at 37^{o} C for 24 h. After the incubation, the mixture was shaken gently to mix well, then using a sterile pipette, 1ml was transferred into 10ml Selenite broth (Difco). This was incubated in a water bath at 42^{o} C for 24h. After incubation, a loopful of the Selenite broth (Difco) culture was streaked on xylose lysine desoxycolate (XLD) agar (Oxoid). These were incubated at 37^{0} C for 24 h. The slow growers were incubated for 48 h. Colonies that appeared dark on XLD were taken to be non-lactose fermenters and were purified on MacConkey agar (Oxoid). The purified colonies on MacConkey agar were inoculated into the triple sugar iron (TSI) agar (Oxoid) slants by stubbing the butt and streaking the slant as illustrated in the Figure 6 below.

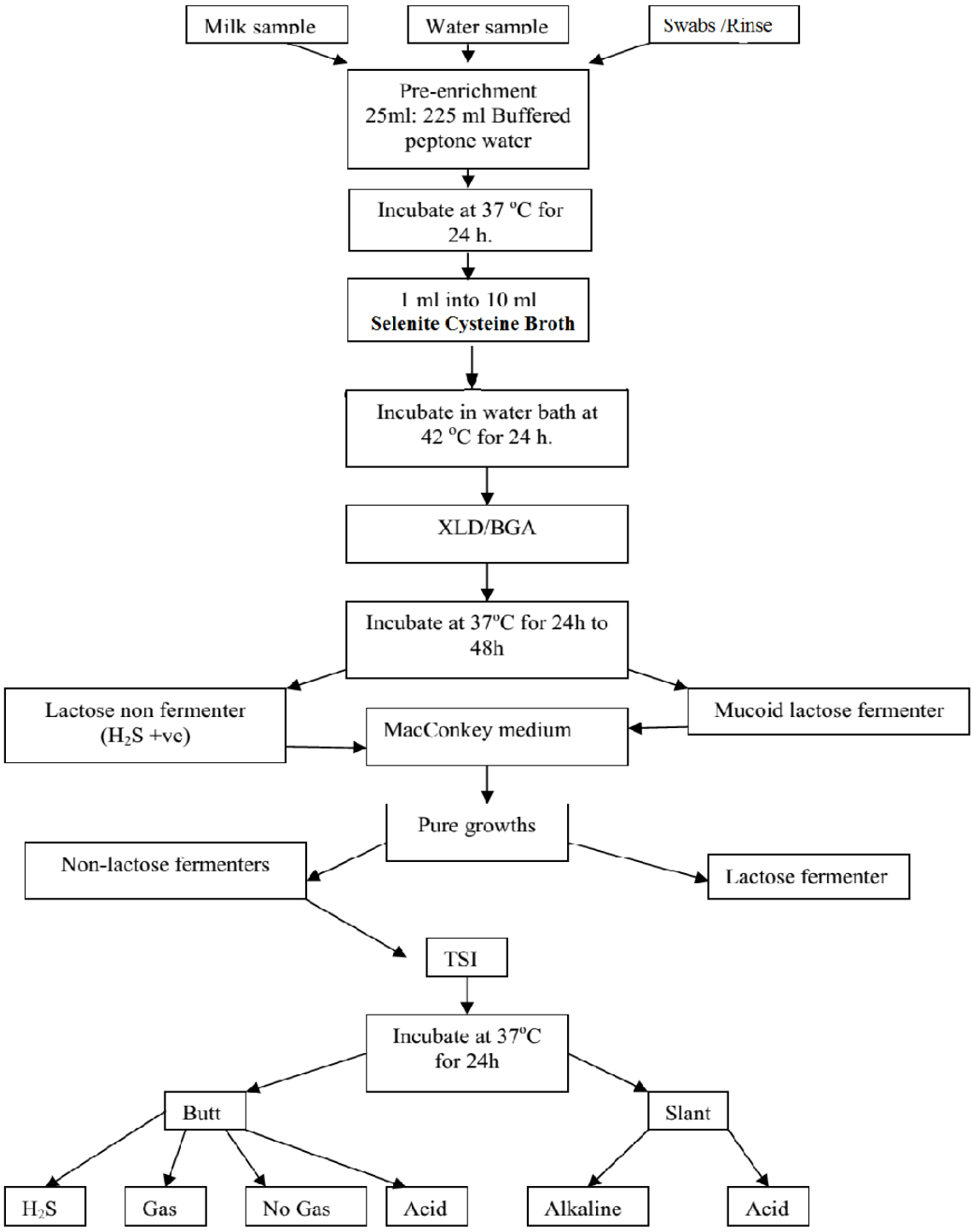

Figure 5. Flowchart of the isolation of *Salmonella*
Source; (Matofari, 2007)

3.6.2 Isolation of *Bacillus* and *Pseudomonas*

Milk sample, water sample, and surface swab samples were streaked directly on 5% sheep blood agar (Oxoid, blood agar base), incubated at 37^{o} C for 12 h. Growth of direct cultures was examined and sub-cultured. Selection of colonies from subcultures was done according to their predominance and homogeneity throughout the streak, and type of haemolysis. Special emphasis was given to the scrutiny of the slow growing and more fastidious colonies. All blood agar plates that showed none or scarce growth were re-examined after 48h and 72 h of incubation. Haemolytic colonies were sub-cultured onto blood agar whereas fast growing non-haemolytic colonies were sub-cultured on nutrient agar (Oxoid). Figure 7 below is a schematic procedure used for culturing of milk samples, water samples, and swab samples for microbial isolation and identification.

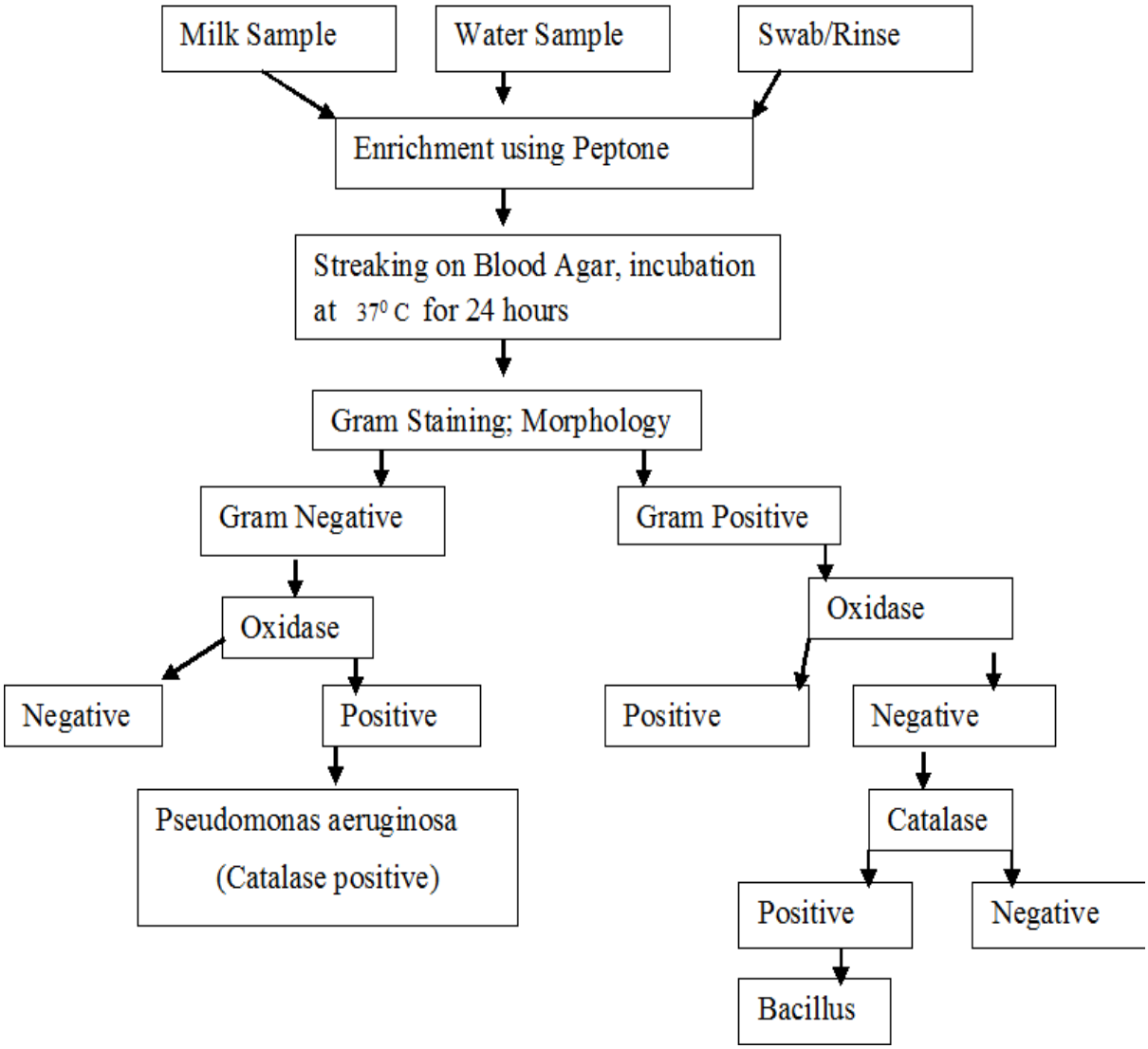

Figure 6. Flow chart for isolation of *Pseudomonas* and *Bacillus*

Source; (Matofari, 2007)

3.7 Biochemical Tests for the Identification of Typical Isolates

The typical colonies were further isolated and identified according to their morphological, physiological, and biochemical tests characteristics for Salmonella, Bacillus and Pseudomonas. The tests carried out were Gram reaction test, catalase test, oxidase test, Triple sugar Iron (TSI agar), Sulphur, Indole and Motility (SIM agar) test.

3.7.1 Catalase Test

Three to four colonies of the culture were picked using a sterile loop and transferred to a sterile glass slide. A drop of 3% hydrogen peroxide (H_2O_2) was transferred using a sterile pipette and mixed with the organisms on the glass slide using the same pipette. Effervescence indicated a catalase positive reaction.

3.7.2 Oxidase Test

The test was done to separate the oxidative and fermentative gram negative organisms. Pure colonies of the isolates (about 3 colonies) were spread on the test oxidase strip. Colour change to deep blue was positive for the test.

3.7.3 Indole Test

The test was performed on Gram negative bacteria to determine their ability to convert tryptophan into the indole. Pure colonies of isolates were suspended in peptone water medium and incubated 37^0 C for 24 h. One ml of Kovac‘s reagent was added and shaken gently. It was left to stand for 5 min. A pink to red colour development was positive for indole production.

3.7.4 Tube Method

(adopted from Saha *et al.*, 2014 and Mohamed *et al.*, 2016)

The principle: This is a qualitative method for biofilm detection. Microbial isolates are cultured in test-tube overnight. The biofilm formed is then stained with crystal violet. The stains are then classified as weak, moderate and strong based on eye sight.

A loopful of test microorganism isolates *Salmonella*, *Bacillus* and *Pseudomonas spp* were inoculated in 10 mL of trypticase soy broth with 1% glucose in test tubes. The test tubes were incubated at 37^{o} C for 24 h. After incubation, the tubes were decanted and washed with phosphate buffer saline (pH 7.3) and dried in an inverted position. The tubes were then stained with crystal violet (0.1%). Excess stain was washed with deionized water. Tubes were dried in inverted position. The strength of the stain in the test tube was scored, with reference to a blank test tube. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube. The amount of biofilm formed was scored as none, weak, moderate and high/strong.

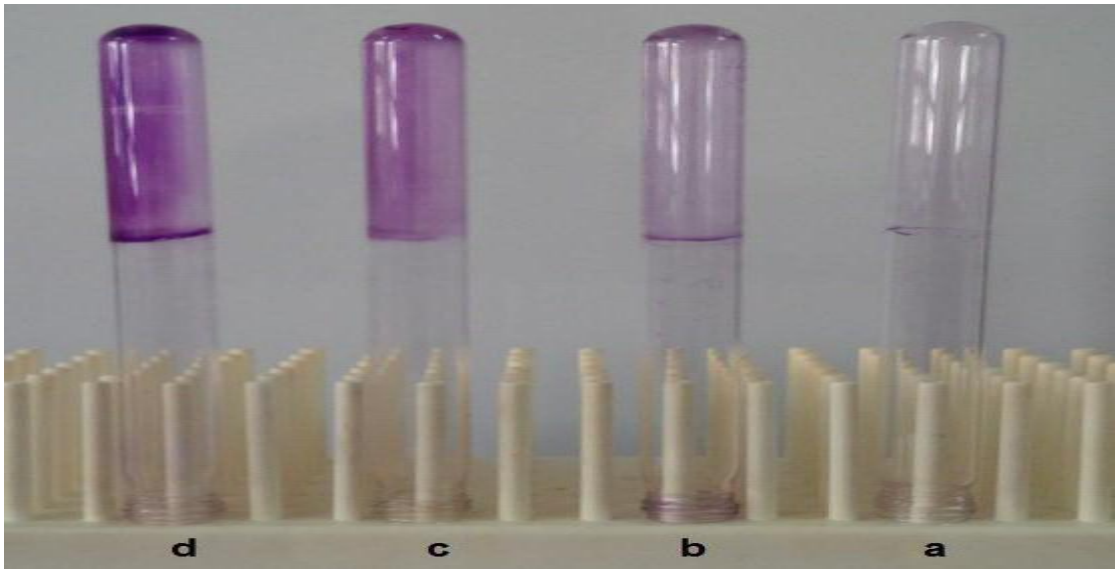

Figure 7. Detection of biofilm producers by tube method;

Where a; non biofilm producer,

b; weak biofilm producers,

c; moderate biofilm producers, and

d; strong/high biofilm producers.

Source; (Mohamed *et al*., 2016)

3.8 Statistical Data Analysis

Data obtained from the sanitation practices were analysed by means of general descriptive statistics and chi-square test for determination of independence using SPSS version 20 (SPSS, Inc., Chicago, IL, USA). The data obtained for the microbial counts was transformed into $\log_{10}$ before analysis. This data means were compared using Analysis of Variance (ANOVA) by the General Linear Model (GLM) of SAS version 9.1.3 (SAS Institute, Inc.; Cary, NC). Means comparisons were done using honest significant differences (HSD) at $P<0.05$.

CHAPTER FOUR

RESULTS

4.1 Hygiene Practices at Farm Gate and SME Centres

4.1.1 Socio Demographic Characteristics of the Respondents

Table 1 shows the socio demographic characters of the respondents. These parameters were assessed to evaluate how they affect the hygiene practices at the farm gate as well as the SME centres. The dairy industry is dominated with men up to 75 %, most of whom attended formal education to primary level (67%), and sell the milk through formal channel; milk bulking groups up to 94.3%.

Table 1. Socio demographic characteristics of respondent in different milk bulking groups

Site	N	Gender		Average Age	Average Number of cows	Amount of milk	Level of education			Selling Channel		
		Male	Female				None	Primary	Secondary	Formal	Informal	Formal & Informal
MBG1	87	**50 (57.5)**	37(42.5)	40.3±14.7	1	12±6	14(16.1)	**60 (69)**	13 (14.9)	**82 (94.3)**	4 (4.6)	1 (1.1)
MBG2	12	**9 (75)**	3(25)	47.4±12.6	1	11.3±5	3(25.0)	**8 (66.7)**	1 (8.3)	**11 (91.7)**	0	1 (8.3)
MBG3	36	**24 (66.7)**	12(33.3)	43.2±17.5	1	12±6	5 (13.9)	**24 (66.7)**	7(19.4)	**25 (69.5)**		11 (30.6)
MBG4	41	**30 (73.2)**	11(26.8)	46.7±15.7	1	14 ± 8	5 (12.2)	**27 (65.9)**	9 (22.0)	**26 (63.4)**	1 (2.4)	14 (34.1)
MBG5	54	**34 (63)**	20 (37)	43.7±15.4	2	12 ± 6	8 (14.8)	**32 (59.3)**	14 (25.9)	**39 (72.2)**	1 (1.9)	14 (25.9)
MBG6	26	**13 (50)**	13 (50)	51.4±12.2	1	12 ± 8	9 (34.6)	**14 (53.8)**	3 (11.5)		26 (100)	

*the number after ± is a standard deviation

**the numbers in brackets are percentages of the respondents in the category

MBG1- Machite MBG, MBG2-Nathenje MBG, MBG3- Lumbadzi MBG, MBG4-Majiga MBG, MBG5-NamwiriMBG, and MBG6- Nkhweza MBG

Note: the dairy farmers are mostly males (>50%) at an average age (40-50).

4.1.2 Milk Hygiene Practices

Table 2 shows the hygiene practices of the dairy farmers who belong to a bulking group that deliver milk to a particular bulking centre. This study showed that milking is done by hand (100%), with milking frequency of twice (98.4%) or once (1.6%) a day. Dairy farmers source water from boreholes up to 100%. The water was not treated before usage by up to 89% of the dairy farmers. Up to 75% of dairy farmers treated their water by boiling, 19.4% used chlorine and 11.5% used WaterGuard. All the dairy farmers washed their hands and udder before milking. . All the dairy farmers (100%) preserved evening milk at environmental temperature overnight.

Table 2. Hygiene Practices of dairy Farmers

Hygiene Practice	Category	MachiteMBG (N= 87)	NathenjeMBG (N=12)	LumbadziMBG (N=36)	MajigaMBG (N=41)	NamwiriMBG (N=54)	NkhwezaMBG (N=26)
Source of water	Tap (n (%))	4 (4.6)				7 (13)	
	Borehole	**79 (90.8)**	**12 (100)**	**35 (97.2)**	**39 (95.1)**	**47 (87)**	**21 (80.8)**
	River	4 (4.6)		1 (2.8)	2 (4.9)		5 (19.2)
Treatment of water	**No treatment**	**57 (65.5)**	**1 (8.3)**	**23 (63.9)**	**30 (73.2)**	**38 (70.4)**	**21 (80.8)**
	Water guard	6 (6.9)	1 (8.3)	3 (8.3)	1 (2.4)	2 (3.7)	3 (11.5)
	Chlorine	15 (17.2)	1 (8.3)	7 (19.4)	9 (22)	8 (14.8)	
	Boiling	9 (10.3)	9 (75)	3 (8.3)	1 (2.4)	6 (11.1)	2 (7.7)
Hand washing	**With soap**	**78 (89.7)**	**12 (100)**	**36 (100)**	**41 (100)**	**54 (100)**	**24 (92.3)**
	Using cold water	11 (12.6)		1 (2.8)		4 (7.4)	
	Using Warm water	**76 (87.4)**	**12(100)**	**35 (97.2)**	**41 (100)**	**48 (88.9)**	**26 (100)**
	Both cold and warm					2 (3.7)	
Udder washing	**With soap**	**79 (90.8)**	**12 (100)**	**27 (75)**	**40 (97.6)**	**46 (85.2)**	**21 (80.8)**
	Without soap	8 (9.2)		9 (25)	1 (2.4)	8 (14.8)	5 (19.2)
	Using cold water	5 (5.7)				3 (5.6)	
	Using warm water	**82 (94.3)**	**11 (91.7)**	**36 (100)**	**41 (100)**	**51 (94.4)**	**26 (100)**
	Both cold and warm		1 (8.3)				
Udder drying	**Yes**	**70 (80.5)**	**11 (91.7)**	**35 (97.2)**	**41 (100)**	**49 (90.7)**	**25 (96.2)**
	No	17 (19.5)	1 (8.3)	1 (2.8)		5 (9.3)	1 (3.8)
Preservation of milk	**Environmental Temperature**	**83 (95.40)**	**12 (100)**	**36 (100)**	**41(100)**	**51 (94.44)**	**26 (100)**
	Refrigeration	1 (1.1)				2 (3.7)	
	Do not store	3 (3.4)				1 (1.9)	
Sieving Milk	Yes	84 (96.6)	12 (100)	35 (97.2)	39 (95.1)	54 (100)	23 (88.5)

*The numbers in brackets are percentages of the respondents in the category

4.1.3 Milk Equipment and Milk Handling Practices

Table 3 shows milk handling equipment hygiene practices of dairy farmers. Up to 67% of the farmers use plastic containers for transporting milk while 92% preserve the evening milk on farm in plastic containers. Over 90% use warm water and detergent to wash the equipment and sundry.

Table 3. Milking equipment and hygiene practices of dairy farmers

Hygiene Practice	Category	MBG1	MBG2	MBG3	MBG4	MBTG5	MBG6
Milk handling containers	Aluminium	8 (9.2)	1 (8.3)	7 (19.4)	5 (12.2)	7 (13)	3 (11.5
	Plastic	**50 (57.5)**	**8 (66.7)**	**19 (52.8)**	**17 (41.5)**	**23 (42.6)**	**15 (57.7)**
	Both plastic and Aluminium	29 (33.3)	3 (25)	10 (27.8)	19 (46.3)	24 (44.4)	8 (30.8)
Bulking containers	Aluminium	28 (58.5)	1 (8.3)	15 (41.7)	24 (58.5)	29 (53.7)	8 (30.8)
	Plastic	**17 (41.5)**	**11 (91.7)**	**21 (58.3)**	**17 (41.5)**	**25 (46.3)**	**18 (69.2)**
Water to clean containers	Warm	**71 (81.6)**	**9 (75)**	**33.(91.7)**	**36 (87.8)**	**43 (79.6)**	**16 (61.5)**
	Cold	16 (18.4)	3 (25)	2 (5.6)	4 (9.8)	9 (16.7)	6 (23.1)
	Hot				1 (2.4)	2 (3.7)	4 (15.4)
	Both warm and cold			1 (2.8)			
Soap to cleaning containers	Yes	**80 (92)**	**12 (100)**	**36 (100)**	**41 (100)**	**51 (94.4)**	**23 (88.5)**
	No	7 (8)				3 (5.6)	3 (11.5)
Equipment Drying	Sun drying	**73 (83.9)**	**11 (91.7)**	**32 (88.9)**	**40 (97.6)**	**44 (81.5)**	**23 (88.5)**
	Using towel	14 (16.1)	1 (8.3)	3 (8.3)		8 (14.8)	2 (7.7)
	Both towel and sun			1 (2.8)		1 (1.9)	
	None				1 (2.4)	1 (1.9)	
Personnel hygiene training	Yes	**64 (73.6)**	**10 (83.3)**	**31 (86.1)**	**38 (92.7)**	**48 (88.9)**	**23 (88.5)**
	No	23 (26.4)	2 (16.7)	5 (13.9)	3 (7.3)	6 (11.1)	3 (11.5)

*The numbers in brackets are percentages of the respondents in the category

**MBG1- Machite MBG, MBG2-Nathenje MBG, MBG3- Lumbadzi MBG, MBG4-Majiga MBG, MBG5-NamwiriMBG, and MBG6-Nkhweza MBG

Note: Dairy farmers use plastic containers to handle milk.

4.1.4 Dairy Cattle Housing Characteristics

In the study area, most of the cows (54.7%) were housed in earth type floor barn and 45.3% were in concrete floor. Most of the respondents (69.1%) provided beddings to the cattle while (30.9%) did not provide beddings. In the barns, 77.3% of the respondents separated the feeding area from the sleeping are while 22.7% did not separate the feeding area from the sleeping area (Table 4).

Table 4. Dairy cattle housing characteristics

Parameter	Category	MBG1	MBG2	MBG3	MBG4	MBG5	MBG6
Floor	Concrete	22 (25.3)	5 (41.7)	24(66.7)	25(61)	37 (68.5)	3 (11.5)
	Earth	**65 (74.7)**	**7 (58.3)**	**12(33.3)**	**16 (39)**	**17 (31.5)**	**23(88.5)**
Beddings	Available	**38 (43.7)**	**10(88.3)**	**34(94.4)**	**34(82.9)**	**49(90.7)**	**12(46.2**
	Not available	49 (56.3)	2 (16.7)	2(5.2)	7(17.1)	5(9.3)	14(53.8)
Feeding area	Same as Sleeping area	33 (37.9)	2 (16.7)	1 (2.8)	5 (12.2)	5 (9.3)	12(46.2)
	Separate from sleeping area	**54 (62.1)**	**10(83.3)**	**35(97.2)**	**36(87.8)**	**49(90.7)**	**14(53.8)**

*The numbers in brackets are percentages of the respondents in the category

**MBG1- Machite MBG, MBG2-Nathenje MBG, MBG3- Lumbadzi MBG, MBG4-Majiga MBG, MBG5-NamwiriMBG, and MBG6- Nkhweza MBG

Note: the cattle enclosures are mostly on bare earth. Occasionally, feeding area is separated from sleeping area.

4.1.5 Hygiene Practices of Milk Bulking Centres

Table 5 below shows the hygiene practices of the milk bulking centres. All the bulking centres source water from boreholes. The water was not treated before usage by 50% of the

milk bulking centres. The bulking centres treated their water by using Waterguard (16.7%) and Chlorine (33.3%). Stainless steel cans and vats are used to handle milk except 50% of the bulking centres use them in combination with plastic containers. All containers are cleaned using cold water, detergents (100%), and 16.7 % are disinfected using Chlorine. The milk handling containers are sundried (16.67%) as well as dried using a towel (83%). Raw milk brought by individual farmers to the bulking centre is pooled in stainless steel vats and tanks and cooled to below 5 ^{o}C. The milk bulking centres conducted various quality tests prior to the acceptance of the milk (Table 5). These tests included density, sourness, organoleptic, temperature, and fat and rezasurin tests.

Table 5. Hygiene practices of milk bulking centres and processor

Hygiene Practice	Category	Frequency	Percentage (%)
Source of water	Borehole	6	100
Treatment of water	Water guard	1	16.7
	Chlorine	2	33.3
	No treatment	**3**	**50.0**
Quality test	Density, and sourness	2	33.3
	Density, sourness and Organoleptic test	2	33.3
	Density sourness, Temperature and Fat	1	16.7
	Density, sourness and Rezasurin	1	16.7
Milk containers	Stainless steels cans and vats	3	50.0
	Aluminium and Plastic containers	**3**	**50.0**
Cleaning containers	**Cold water**	**6**	**100**
	Detergent	6	100
	Disinfection	1	**16.7**
	No Disinfection	5	83.3
Container Drying	**Towel**	**5**	**83.33**
	Sundrying	1	16.67
Storage temperature	Below 5^{o} C	4	**66.7**
	Do not store	2	33.3

*Frequency is the number of respondents in the category, N (Number of respondents) =6

Note: Most bulking centres use non-potable water for equipment cleaning.

4.1.6 Association of Socio-demographic Characteristics and Hygienic Practices

Table 6 shows the chi-square table for the association between socio-demographic characteristics and the hygiene practices at $p<0.05$. There was a strong association ($p< 0.027$)

between some socio demographic characteristics of respondents and some of the hygiene practices of the respondents.

Table 6. Association of socio demographic characteristic and hygiene practices

		Gender		Level of Education			Personnel hygiene training		X^2	p-value
Parameter	Category	Male	Female	None	Primary	Secondary	Yes	No		
Use of equipment cleaning agent	Yes	156	87						5.883	0.015
	No	4	9							
Use of hand washing Agent	Yes	157	88						6.086	0.014
	No	3	8							
Use of hand washing Agent	Yes						209	36	12.191	0.001
	No						5	6		
Source of water	Borehole			44	147	42			21.434	0.002
	Tap			0	6	5				
	River			0	12	0				
Availability of animal beddings	Yes						154	23	4.869	0.027
	No						60	19		

Note: the hygiene practices of the farmers are affected by the socio-demographic characteristics of the respondents.

4.2 Determine Microbial Load and Type

4.2.1 Microbial Load

Table 7, below shows mean comparison of microbial load of samples of milk, water, swab and rinses. The microbial load of samples were significantly different at $p<0.05$.

Table 7. Means comparison of TVC, CC, Yeast and Moulds, and LAB of Sample

Sample Type	N	Log_{10} (cfu /ml)			
		TVC	CC	Yeast & Moulds	LAB
Milk	60	7.40 ± 0.18^{a}	6.06 ± 0.36^{a}	5.33 ± 0.38^{a}	5.73 ± 0.36^{a}
Rinse	16	5.43 ± 0.02^{b}	5.59 ± 0.07^{a}	4.85 ± 0.26^{a}	4.12 ± 0.30^{b}
Swab	48	5.27 ± 0.07^{b}	4.22 ± 0.28^{b}	2.46 ± 0.31^{b}	2.60 ± 0.30^{c}
Water	60	7.21 ± 0.33^{a}	5.23 ± 0.50^{ab}	1.73 ± 0.43^{c}	1.62 ± 0.41^{d}
Correlation coefficient between Log_{10} TVC and Log_{10} CC = 0.447 p <0.01					

*Means with the same letter superscript in a column are not significantly different at $p< 0.05$.

** The number after ± is a standard error

Note: the microbial count of the milk is significantly high, so is for water. The coliform count in the samples (milk, water rinses and swabs) is correlated to the total viable count, $p<0.01$.

Table 8, below shows the mean comparison of microbial load of milk, water, and swab/rinse samples of the dairy actors (at bulking centres and at dairy farmers' households). The log_{10} for total viable count, coliform count, yeast and moulds, and lactic acid bacteria of the milk, water, swab and rinse was significantly different ($p<0.05$) in samples from dairy farmers' household compared to the samples at the milk bulking centres.

Table 8. Means comparison of TVC, CC, Yeast and Moulds, and LAB for actors

Sample		Log_{10}(cfu/ml)			
	Actor	TVC	CC	Yeast & Moulds	LAB
Milk	MBG Centre	7.57 ± 0.45^{b}	5.34 ± 0.89^{b}	5.43 ± 0.94^{b}	6.95 ± 0.88^{a}
	Households	7.36 ± 0.20^{a}	6.21 ± 0.40^{a}	5.30 ± 0.42^{a}	5.48 ± 0.39^{b}
Water	MBG Centre	6.19 ± 0.79^{b}	4.36 ± 1.21^{b}	1.48 ± 1.06^{b}	0.00 ± 0.97^{b}
	Households	7.41 ± 0.35^{a}	5.56 ± 0.54a	1.77 ± 0.47^{a}	1.94 ± 0.43^{a}
Swab/Rinse	MBG Centre	5.23 ± 0.12^{b}	4.34 ± 0.48^{b}	2.48 ± 0.59^{b}	2.19 ± 0.52^{b}
	Households	5.34 ± 0.06^{a}	4.53 ± 0.25^{a}	3.21 ± 0.31^{a}	3.20 ± 0.28^{a}

*Means with the same letter superscript in a column for a sample type are not significantly different at $p< 0.05$

**The number after ± is a standard error

Note: Dairy farmers' households have significantly high microbial load.

4.2.2 Microbial Isolates Based on Gram- reaction

Table 9, below shows results of the Gram reaction of the microbial isolates from milk, water, rinse and swab samples. Of the 176 samples, Gram-negative rods dominated in about 83% of the samples. The Gram negative rods were significantly high at the bulking centres as well as the dairy farms. The dairy households had high, 82.95%, as compared to 70.6% Gram negative isolates from bulking centres

Table 9. Microbial isolates Grams reaction

Sample	N	Positive cocci	Positive rods	Negative rods
Milk	54	18 (33.3)	10 (18.5)	34 (63)
Rinse	16	-	4 (25.0)	16 (100)
Swab	46	2 (4.3)	14 (30.4)	40 (87)
Water	60	2 (3.3)	10 (16.7)	56 (93.3)
Actor				
Bulking centre	34	2 (5.9)	18 (52.9)	24 (70.6)
Households	142	20 (14.1)	20 (14.1)	122 (85.9)
Total	**176**	**22 (12.5)**	**38 (21.6)**	**146 (82.95)**

*The numbers in parenthesis are percentages of the isolates and the overall for the total,
**– is used to indicate that there were no microorganism detected under those Gram reactions

Note: Gram negative rods were dominant, with the households having high levels of gram negative rods compared to the bulking centres.

4.3 Determining Biofilm Indicator Microorganisms.

4.3.1 Biofilm Indictor Microorganisms

Table 10 below shows the incidence of biofilm indicators from the bulking groups. The biofilm indicator microorganisms which were isolated from samples; water, milk, and swab and rinse, from the six milk bulking groups included *Salmonella, Pseudomonas* and *Bacillus*.

Of the 182 samples 19.78% were containing *Salmonella* isolates. The biofilm indicator microorganisms were high (74.42%) at the farm level than the bulking centres. *Salmonella* is the biofilm indicator which was isolated at a higher rate (20%) of all the farm gate samples

Table 10. Biofilm indicator microorganism

Sample	N	*Bacillus*	*Pseudomonas*	*Salmonella*	**Total**
Milk	54	10 (16.7)	-	8 (13.3)	
Rinse	16	4 (25)	4 (25)	2 (12.5)	10 (5.5)
Swab	46	12 (26.1)	6 (13.0)	**14 (30.4)**	**32 (17.6)**
Water	60	6 (10.0)	8 (13.3)	12 (20.0)	26 (4.3)
Actor					
Milk bulking centre	38	16 (42.1%	-	6 (15.8%)	22 (25.58%)
Farm gate	150	16 (10.7%)	18 (12%)	30 (20.0%)	64 (74.42%)
Total	182	32(17.6)	18 (9.89)	**36 (19.78)**	

*The numbers in parenthesis are percentages of the biofilm indicator isolates and the overall for the total,

**– is used to indicate that there were no biofilm indicator microorganism detected

Note: *Salmonella* prevailed high in the samples, with up to 30.4 % presence in swab samples. Biofilm indicator microorganism are high at the dairy farmer households as compared to the bulking centres, *Salmonella* is highest followed by *Bacillus*, then *Pseudomonas*

4.3.2 Biofilm Formation Capacity of Biofilm Indictor Microorganisms.

Figure 9 below shows the categorization of biofilm forming capacity in the lab. The biofilm were categorized into high/strong, moderate, weak and none. There is a modification of the categories of the biofilm. The biofilms are categorised as none/weak, moderate and strong/high. However, during the analysis the samples categorized as weak had a strong pigmentation of the stain as compared to blank as well as those that were categorized as none.

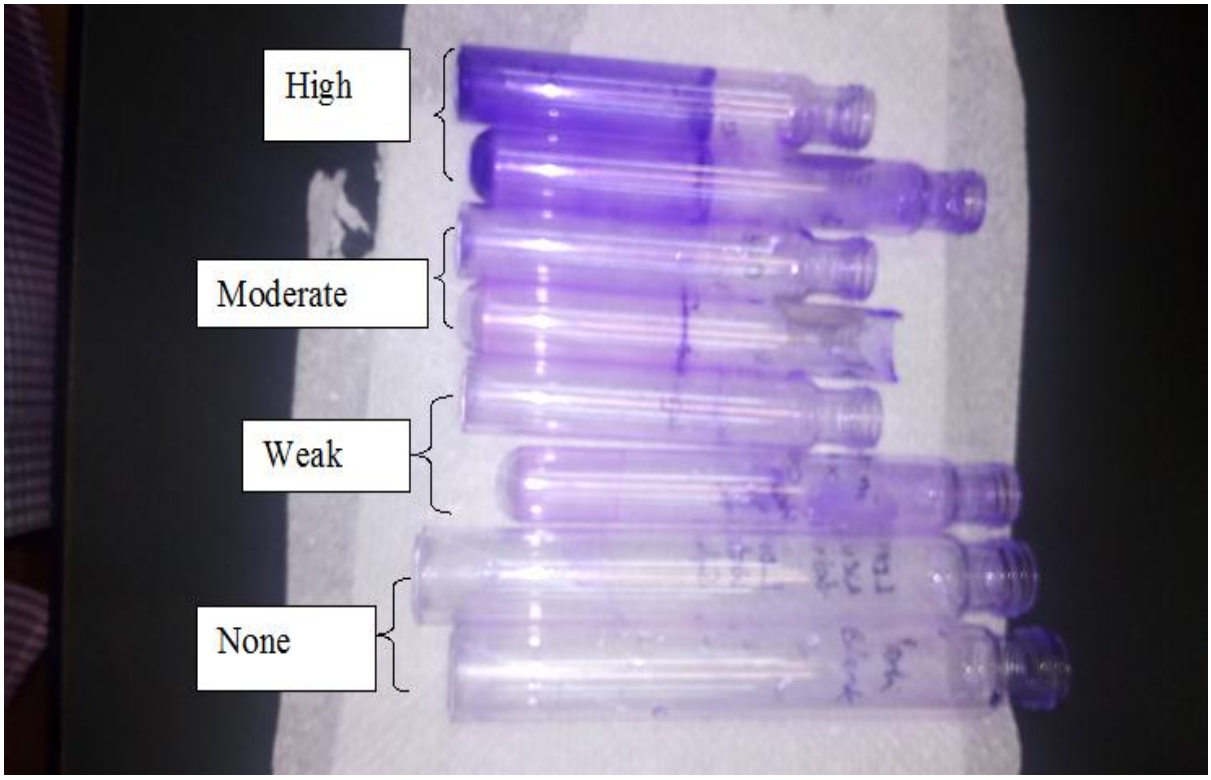

Figure 8. Biofilm forming capacity categories

Table 11 below shows the incidence of the biofilm forming capacity of isolates from milk water swab and rinse samples. The microbial isolates formed 39.5% and 16.3% moderate and strong/high biofilm, respectively. The microbial isolates from water and swab samples formed up to 85.7 % of the strong biofilms. The microbial isolates from farm level and bulking centres formed strong biofilms 83.33%, 16.67%, for the farm level and bulking centres, respectively.

Table 11. Biofilm forming capacity of microbial isolates from samples

Sample	N	None	Weak	Moderate	High/Strong
Milk	18	2 (11.1)	8 (44.4)	6 (33.3)	2 (11.1)
Rinse	10	-	-	10 (100)	-
Swab	32	8 (25.0)	10 (31.3)	8 (25)	6 (18.8)
Water	26	8 (30.8))	2 (7.7)	10 (38.5)	6 (23.1)
Actors					
Milk bulking centres	38	6 (15.8%)	2 (5.3%)	8 (21.1%)	2 (5.3%)
Households	150	12 (8%)	18 (12%)	26 (17.3%)	12 (8%)
Total		**18 (20.93%)**	**20 (23.26%)**	**34 (39.53%)**	**14 (16.3%)**

*The numbers in parenthesis are percentages of the biofilm forming capacity of isolates and the overall percentages for the total,

**– is used to indicate that there were no isolate detected at that biofilm capacity

Note: Biofilm indicator microorganisms formed moderate biofilm up to 39.5% of the isolates.

The majority of the biofilm indicator microorganisms were identified at households

CHAPTER FIVE
DISCUSSION

5.1 Hygiene Practices at Farm Gate and SME Centres.

5.1.1 Socio-demographic Characteristics.

The study results in table 1 showed that the majority of the respondents were males. The dairy cows are passed-on from one member to the other in milk bulking groups and membership to the group requires payment of registration fee (Sindani, 2012). Female headed families might have difficulties to pay the registration fee. Resultantly, few females were registered in this study. Furthermore, the mode of payment in bulking groups is on monthly basis providing a mode of saving to the famers. This provides a reliable source of money for the household. Therefore, males are involved to provide for their families. Though a high number of males are registered, the females provide the much needed labour at the household such as feeding the dairy cow and cleaning milk equipment.

The majority (75.8%) of farmers sell the milk through the formal channel (milk bulk centres), this is a result of one of the requirement of the members of a bulking group; to deliver milk at the bulking centre. The farmers who acknowledged to sell milk to both the formal and informal channels sold the milk to the informal channel only when it has been rejected at the bulking centre, after failing to meet requirements at the bulk centre (Revoredo-giha, *et al.*, 2016).

There were a lot of respondents in the illiterate and primary school level. The respondents might have not attended formal education system and/or attended school to primary level because of lack of money to procure school resources. These resources include school uniform, exercise books, school bag. Therefore, the parents refused them to attend (National Statistical Office, 2012)

5.1.2 Hygiene Practices

The study as shown in table 2 has showed that the majority of dairy farmers accessed water from boreholes, which was used without any treatment. This is associated with microbial contamination to milk handling equipment and the animal udder, hence the milk (Taulo, *et al*., 2008). There were few famers who indicated that they treat the water with chlorine. The water treatment was done when chlorine was provided by the local health centres. Those who treat the water by boiling, they are strained by the scarcity of source of energy. Consequently, boiling is done periodically.

The dairy farmers have indicated to practice hand washing and udder washing with soap and warm water. However, the concentration of the soap used, temperature, time and manual effort applied during the cleaning process affect the effectiveness of the cleaning regime (Parker, 2007). In this study, the actors were not aware about the effective concentrations of the detergent/soap and the cleanliness of the towel used for drying the udder, hence their contribution to high microbial loads in the milk. After cleaning, disinfection is necessary to minimize microbial contamination to 99.9% (Schmidt, 2012). However, all the dairy farmers were not disinfecting the udder before and after milking. This could enhance milk contamination in the udder even before milking. The udder is dried after cleaning and the cleanliness of the towel has an effect the cleanliness of the udder. Usage of a dirty towel to clean the udder contaminates the udder. In addition to the cleanliness of the towel, usage of the same towel on more than one animal further enhance cross contamination between the animal udders. This enhances the chance of milk contamination in the udder even before milking (Cook & Reinemann, 2007).

Moreover, other factors contributing to high microbial loads include the bulking of evening and morning milk, lack of cold chain facilities and time taken to deliver the milk (Wanjala *et al.*, 2018). Most farmers bulked evening milk with morning milk but they stored the evening milk in a water bath which could not attain refrigeration temperatures to reduce the microbial growth rate. The evening milk therefore could have high microbial numbers and thus contaminate the morning milk, despite the swift delivery of the milk within 2 hrs of morning milking.

Results showed that plastic bucket containers were mostly used to handle milk and not the recommended aluminium cans. Moreover, all the actors cleaned the milk handling containers using soap and sundried them though none of the actors disinfected the equipment after washing. Plastic containers are difficult to thoroughly clean as compared to the aluminium containers thus; more microbes will remain on plastic containers. Resultantly, the microbes form biofilms which are persistent source of contamination to milk (Orwa *et al*., 2017). Previous studies have shown that the milk microbial quality along the value chain is affected by contamination of the containers handling the milk (Wafula *et al*., 2016; Welearegay *et al*., 2012). In this study, therefore, the milk handling containers could have contributed to the microbial loads recorded.

The study has shown that the milk handling containers are sundried before usage, this is true during the milking of milk in the evening, but the morning milking the containers are used

without drying. This practice introduces microbe in the milk from the water. The results of this study have shown the poor hygienic practices along the dairy value chain. This agrees with findings of (Sindani, 2012).

The environment in which the milk-producing animal is kept especially the sleeping place is very important in hygienic milk production (Oumer *et al*., 2017). Most dairy farmers provided a separate sleeping area from the feeding area and provided beddings and a higher proportion of them had concrete floors, which were expected to lower microbial contamination (Gashaw and Gebrehiwot, 2018; Oumer *et al*., 2017). However, the frequency of changing beddings and cleaning of the dairy barns could affect microbial counts. Moreover, the use of earth floor for sleeping increases the chances of contamination of the udder as the animals lay of the floor since soil is a natural niche for microorganisms. This is because soiling of the sleeping places by feeds, urine and faecal matter for those animals that had the same sleeping and feeding stall could highly contaminate the milk as similar studies have shown that the hygienic status of the stall where milk animals sleep is very important since it predisposes that animal's udder to dirt (Saran, 1995). It has been reported that beddings' microbial counts and type is correlated with microbial counts and type on the animals' teat ends (Zdanowicz *et al*., 2004). Apart from milk contamination, studies have also shown that some bedding materials like sand and wood products contribute to infections of the udder like mastitis (Munoz *et al*., 2006). Therefore, partitioning the feeding and sleeping places and provision of easy-to-clean concrete floors and bedding materials may not necessarily reduce microbial contamination if hygienic practices in the barn are not met.

5.2 Determine Microbial Load and Type.

5.2.1 Microbial Load for Milk Samples

The microbial load for milk samples collected from dairy actors in the six milk bulking groups in Lilongwe was significantly higher compared to the East African standards (0- 6 $\log_{10}$ cfu/mL).

The higher microbial contamination of the milk can be associated with the postharvest handling of the milk (Mhone, *et al*., 2011). The milk from the udder of a health udder is assumed to be sterile, however the milk is a high source of nutrient hence it support even the microbial life. The microbial load determined in the milk samples would have emanated from the udder, and external sources. The external sources may include water, personnel and equipment. The hygiene practices of the personnel during milking, storage and handling after milking, type of containers used to handle milk and the cleaning regimes are the major sources of microbial contamination of milk. The udder is contaminated by microorganisms which penetrate the udder teat from the surface where the udder touch in barn where the animals are kept, water used to clean the udder, cloth used to dry the udder after washing and the personnel hands when milking

The study has shown that the farmers' hygienic practices at the farm level are not standard. The source of water used at the farm level is highly contaminated. This water is used to clean the udder, equipment as well as the hands of the farmer before milking without treatment. Therefore the udder, equipment and the hands of the farmers are contaminated by microorganisms. The microbial contamination of the udder, equipment and hands cleaned are likely to have contributed to the higher microbial counts in milk (Matofari, 2007; Kashongwe *et al.,* 2017). The microbial count of the equipment rinses as well as the swabs confirms the heavy microbial contamination on the equipment surface. Milk being a high nutrient source to microorganisms will propagate the microbial growth of microorganisms on the equipment surface. The microorganisms will therefore, form biofilm on the equipment surface.

Furthermore, the animals are housed in earth floor, such that the udder, tail and hind legs are soiled with dung and mud. These are sources of microorganisms. Therefore the microbes are transferred from the skin of the udder, tail and hind legs to the milk during milking, acting as another source of microbial contamination.

Dairy farmers keep evening milk overnight (12 hours) at an environmental temperature. The uncontrolled temperature propagates the growth in numbers of the microorganisms in the milk. This further enhances deterioration of the milk.

Milk samples from milk bulking group 6, showed an overgrowth on media plate (too numerous to count; TNTC) as shown in a table in appendix G. The hygiene practices of the dairy farmers, from Milk bulking Group 6, contributed to this microbial growth. The bulking centre in this bulking group is not operational. Resultantly, the farmers sell the milk directly to consumers and there are no tests done on the milk before selling or buying. The milk is sold at a lower price as compared to the payment which the dairy farmer used to get from the bulking centre. As a result, the farmers adulterate the milk with water, to meet the anticipated profit as well as exploit the consumer. The water added to milk is not treated; this is a source of microorganisms contaminating milk.

5.2.2 Microbial Load for Water Samples

The study has shown microbial load of water samples is 7.21 ± 0.33 $\log_{10}$ cfu/ml. The microbial load of the water is significantly high as compared to the Malawi Bureau of Standard and World Health Organization for United Nations (WHO), and East African Standards acceptable level of microbial load for portable water (0 cfu/100ml, 0cfu/ml) (Taulo, *et al*., 2008; Onyango, *et al.*, 2018).

The possible cause of the microbial contamination may be from poor environmental sanitation such as faecal deposition in the open. In addition, the open sourced water are exposed to animals which are carriers of microorganisms. Resultantly, there is cross contamination of microorganisms from the environment and animals to the water sources. The dairy farmers source water from sources which are far from the household resulting in storage of the water before use. The water is stored in containers without treatment as shown by the majority of the respondents not treating the water before use. The water storage containers are cleaned after several days, leaving organic sediments to settle at the bottom of the containers, to serve as a source of nutrient for microorganisms. The freshly drawn water is mixed with stored water. Furthermore, personnel hygiene practices at household in using the water from the storage containers may be the source of microbial contamination of the water. The cups and hands dipped into the storage vessel is another source of contamination (Taulo *et al.*, 2008). For instances, the hygiene of the room where the water is stored, the access of the water storage container to children, could be another source of microbial contamination.

Table in appendix G shows a significant variation in the microbial contamination of water from bulking groups. The significant variation in the microbial load of the water samples from the different milk bulking groups may have resulted from the differences in the hygiene

practices in fetching and keeping the water at the house hold. The milk bulking centres have lower microbial load as compared to the household because the bulking centre fetch the water and use without storing. Therefore the possible cause of the microbial contamination may be the environment to the water source, container hygiene and the personnel hygiene when fetching the water.

5.2.3 Microbial Load of Swabs and Rinses

The effectiveness of the cleaning process of the containers was evaluated by determining the microbial load and type of microorganisms on the containers surfaces. The microbial load of microorganisms on the container surfaces was significantly high as compared to the minimum load of microorganisms after cleaning, 1 colony per square centimetre (Eeast African Standard, 2001). This might have originated from the quality of water used to clean the containers and the procedure followed in cleaning. The study documented that the dairy farmers cleaned the containers with water sourced from a borehole and was not treated by majority of the famers. During the administration of the questionnaires, the farmers stated that the cleaning of the containers involved usage of soap without prior rinsing. This may result in making the cleaning process require more detergent and water as compared to when rinsing is done first prior to application of soap. Resultantly, milk fats and organic residues attach on the milk handling containers surface and harden becoming difficult to remove. Furthermore, the concentration of the soap applied during cleaning is not known, as the soap is applied until there is foaming. This may not be effective to remove the fat and organic residues on the surface of the container (Parker, 2007). Thus, the surface of the milk handling containers was likely to have nutrient residues.

The farmers in bulking group 6 cleaned the milk handling vessels awhile after emptying the vessel of milk. The milk residues attached strongly to the surface, becoming difficult to remove. Consequently, the container surface had milk residues on the surface serve as a source of nutrients for the microorganism on the surface of the containers (Bekuma & Galmessa, 2018).

5.2.4 Microbial Isolates Gram Reactions

The microbial isolates from the milk samples comprised of 62.96% of gram negative rods. This group includes enterobacteriaceae such as *Salmonella* spp. and *Pseudomonas* spp. could have emanated from the soil (excrete in the barn), water and the personnel milking the cows. High numbers of *Coliforms* in this study accounts for the high incidences of gram negative rods. *Coliforms* also show the hygienic conditions under which the milk was produced; further signifying the impact of the hygiene practices at the farm level on the microbial quality of milk (Oliver, *et al*., 2005; Marchand *et al*., 2012).

Salmonella and *Pseudomonas* species are some of the pathogenic and spoilage gram negative microorganisms isolated. *Pseudomonas* species produce heat stable proteases and lipases which are responsible of off-flavours in milk as well as sweet curdling after pasteurization (Sørhaug & Stepaniak, 1997; Marchand *et al*., 2009). These microorganisms are isolated in milk which has undergone poor refrigeration conditions. The occurrence of *Salmonella* confirms the possible faecal contamination of the milk water and milk handling containers during milking.

Spores of *Bacillus* species were detected. *Bacillus* species are available in nature; soil air water and on the animal feeds. The animals' tail, udder and hind legs are normally soiled with dung and mud. Thus, spores of *Bacillus* species are attached to the skin of the animal, and are transferred from the skin and hair of the animal during milking to the milk. *Bacillus* species produce stable extracellular protease and lipase enzymes which cause spoilage to milk and milk products (Marchand *et al*., 2012)

5.3 Determination of Biofilm Indicator Microorganisms

The biofilm indicator organisms isolated from the water, swab/rinse and milk were tested for the ability to form biofilm using tube method. The isolates formed high/strong biofilm, moderate, weak and some of the isolates had no capacity to form biofilms. The isolates from swabs demonstrated higher percentage of high/strong biofilm forming capacity, followed by isolates from water and lastly isolates from milk samples. The swabs had the highest number of biofilm forming isolates as compared to those in water and milk because the microbes form biofilm when they attach to surfaces and there is a strong difference in the attachment of microbes which are 'surface naïve' and 'surface sentient'(Armbruster and Parsek, 2018). The surface sentient are microbes with a gene which is passed from the parent to the daughter cells to sense a surface and attach while surface naïve cells do not have genes to sense the surface and attach. In free swimming, or planktonic culture, the bacterium has a single flagellum. However, upon surface attachment several lateral flagella sprout. The daughter cell from this 'surface sentient' cell has therefore the lateral flagella attached to its cell, therefore easy attachment. The bacteria uses multigenerational memory coupled on genes to adaptively adhere to surfaces (Lee *et al*., 2018). Therefore, there is high likelihood to find the surface sentient at the surface of the milk handling container (swab) as compared to the free microbes in the milk and water. Furthermore the surface has a high concentration of organic substances to serve as food for the microbes. The cleaning process of the containers was not meeting the standard as the farmers were not disinfecting the containers after cleaning. Thus, there were microbes on a surface with high level of organic substances. Consequently the biofilms were high on the surface than in the liquid medium.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

From the results of this study, the following are conclusions;

1. The actors in the milk value chain from farm to bulking centres do not apply adequate sanitation practices.
2. The microbial load of the water, surface container, and milk are significantly higher as compared to the standard microbial load. Gram-negative rods dominated microbial load at the farm and reduced towards the bulking centre of the chain
3. Biofilm indicators were present in the Gram-negative rods isolated, including *Salmonella*, *Pseudomonas* and *Bacillus*. Microbial Isolates from rinses showed highest potential to form biofilms followed by water and swab samples.

Recommendations

1. Capacity building for dairy actors on hygiene practices, especially farmers and service providers at the bulking centres. .
2. Infrastructure along the dairy value chain to be improved
3. The main spoilage organisms that have shown potential for biofilm formation in the rinses, swabs and water should be screened and characterized.

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APPENDICES

APPENDIX A. Hygiene practices at farm gate

Farmers Questionnaire

Date …… Questionnaire code……..

To assess the risk practices (at the farm level) that may lead to the development of biofilms on the milk handling containers.

I am Richard Banda, a student of Egerton University, Kenya, registration number KM16/11666/16. I am pursuing a Master of Science in Food Science programme in the Faculty of Agriculture, department of Dairy, Food Science and Technology (DAFTEC). I am currently undertaking a research study assessing the risk practices (at the farm level) that may lead to the development of biofilms on the milk handling containers. I request your participation in responding to the following questions.

Section 1: General Household characteristics

A1.Name of respondent_____

A2.Gender Male [ ]

Female [ ]

A3.Age: <30years [ ] 30-40 years [ ] 40-50years [ ] 50-60years [ ] >60years [ ]

A4.Milk bulking group _____

A5.House hold head level of Education

(1) None (2) Primary (3) secondary (4) tertiary

A6. How many cattle do you have on your farm? _____

A7. How many kilograms of milk are produced by your farm per day [ ]

A8. How much sold [ ] Home consumption [ ]

A9. Through which channel do you sell your milk?

(1) Formal (cooperative) [ ]

(2) Informal (trader, direct to consumer) [ ]

Section 2: Animal Welfare

A11. Animal house: Floor type

(1) Concrete [ ]

(2) Earth [ ]

A12. Beddings available

(1) Yes [ ]

(2) No [ ]

A13. Sleeping area

(1) Same as feeding [ ]

(2) Separate [ ]

Section 3: Water

A14.Source of water

[ ] Tap

[ ] borehole / well

[ ] River [ ] other source

…….

A15. Do you treat the water?

[ ] Yes

[ ] No

A16. If yes how do you treat

……...……

Concentration …….

Section 4: Animals Udder Preparation

A17. Which water do you use in washing the udder?

[ ] Warm water

[ ] Cold water

A18. Do you use any cleaning agent when washing the udder?

[ ] Yes

[ ] No

If yes which one? ……..

A19. After cleaning do you use a disinfecting agent?

[ ] Yes

[ ] No

If yes which one? ……..

A20. When drying the udder what do you use?

[ ] Single towel

[ ] Separate towels

[ ] None

Section 5: Equipment cleaning

A21. What is the type of your milking equipment?

[ ] Aluminium

[ ] Plastic

[ ] Both plastic and Aluminium

[ ] Other, specify ……..

A22. What is the type of your milk bulking equipment?

[ ] Aluminium

[ ] Plastic

[ ] Other, specify ……..

A23. Which type of cleaning do you use in cleaning the equipment?

[ ] Manual cleaning

[ ] Cleaning in place (CIP)

[ ] Other, specify……

A24. Which water do you use in washing the equipment?

[ ] Warm water

[ ] Cold water

[ ] Hot water

A25. Do you use any cleaning agent when washing the equipment?

[ ] Yes

[ ] No

If yes which one? ……

A27. After cleaning do you use a disinfecting agent?

[ ] Yes

[ ] No

If yes which one? …… Concentration …….

A28. Do you dry the equipment after cleaning?

[ ] Yes

[ ] No

If yes, which method do you use? …….

Section 6: Milkers Preparation

A29. Have you ever undergone training on hygiene milk production?

[ ] Yes

[ ] No

A30. Which water do you use in washing the hands?

[ ] Warm water

[ ] Cold water

A31. Do you use any cleaning agent when washing the hands?

[ ] Yes

[ ] No

If yes which one? ……..

A32. After cleaning do you use a disinfecting agent?

[ ] Yes

[ ] No

If yes which one? ……..

Section 7: After Milking

A33. Do you sieve milk before bulking?

[ ] Yes

[ ] No

A34. Do you cool milk or deliver it within 2 hours after milking?

[ ] Yes

[ ] No

Where do you take (sale) your milk?

[ ] Collection centres

[ ] Milk shops

[ ] Sell direct to consumers

[ ] Milk Bulking Group (MBG)

[ ] Other, specify …….

How long does it take for you to transfer milk to that place?

[ ] within 2 hours

[ ] within 4 hours

[ ] within 6 hours

[ ] More than 8 hours

[ ] Overnight

Observation checklist

……
……
……
……
……
……
……
……

Follow-up (check list)

1. Water treatment,

2. Udder wash,

3. Milking and bulking equipment,

4. Milker’s preparation.

APPENDIX B. Hygiene practices at SME

MBGs and Processors Questionnaire

Questionnaire code…….. Date ……

Section 1: General characteristics

D1.Name of the MBG/ processor_____

D2. How many Kgs of milk do you receive in a day ……

D4. What is the source of your milk?

[ ] Farmers

[ ] Milk brokers

[ ] Other, specify ……

D5. What are the quality parameters that you check?

.....

.....

.....

D6. At what temperature do you store milk? …….

Section 2: Water

D7. What is the source of water?

[ ] Tap

[ ] borehole / well

[ ] River

[ ] other source …….

D8. Do you treat the water?

[ ] Yes

[ ] No

D9. If yes how do you treat …… ……

Concentration ……

Section 3: Equipment Sanitation

C11. What is the type of your milk handling equipment?

[ ] Aluminium cans

[ ] Plastic jerry- cans

[ ] Plastic buckets

[ ] Other, specify ……

A23. Which type of cleaning do you use in cleaning the equipment?

[ ] Manual cleaning

[ ] Cleaning in place (CIP)

[ ] Other, specify……..

A24. Which water do you use in washing the equipment?

[ ] Warm water

[ ] Cold water

[ ] Hot water

A25. Do you use any cleaning agent when washing the equipment?

[ ] Yes

[ ] No

If yes which one? ……..

Which type of mechanical force do you use while cleaning?

[ ]……..

[ ]……

[ ]……

A27. After cleaning do you use a disinfecting agent?

[ ] Yes

[ ] No

If yes which one? …… Concentration …….

A28. Do you dry the equipment after cleaning?

] Yes

[ ] No

If yes, which method do you use? ……

Observation checklist

……

……

……

……

……

……

……..

Follow-up plan

1. Water treatment

2. Equipment preparation

3. Training

APPENDIX C. Analysis of Variances, Mean of square; Water samples

Analysis of Variances, Mean of squares of microbial load Log_{10} TVC

Source of Variation	DF	R^2	CV	Error	Log TVC	F-Value
Site	5	0.3156	30.5401	4.8483	24.144	0.0008
Actors	1	0.0322	35.0423	6.3832	12.3099	0.1702
Sample ID	29	0.9996	0.9631	0.0048	13.1858	< 0.0001

Analysis of Variances, Mean of squares of microbial load Log_{10} CC

Source of Variation	DF	R^2	CV	Error	Log CC	F-Value
Site	5	0.1783	67.6546	13.1451	30.8033	0.0536
Actors	1	0.0136	71.5218	14.6908	11.7849	0.3741
Sample ID	29	0.9996	1.9389	0.0108	29.7768	< 0.0001

Analysis of Variances, Mean of squares of microbial load Log_{10} Yeast and Moulds

Source of Variation	DF	R^2	CV	Error	Log Yeast and Moulds	F-Value
Site	5	0.6949	111.2881	3.6882	90.7585	< 0.0001
Actors	1	0.0011	194.3245	11.2452	0.7301	0.7998
Sample ID	29	0.9999	0.2366	0.0000167	22.5156	< 0.0001

Analysis of Variances, Mean of squares of microbial load Log_{10} LAB

Source of Variation	DF	R^2	CV	Error	Log LAB	F-Value
Site	5	0.7705	97.4599	2.4907	90.3144	< 0.0001
Actors	1	0.0537	190.9595	9.5621	31.4669	0.0748
Sample ID	29	0.9999	0.8130	0.000173	20.2092	< 0.0001

APPENDIX D. Analysis of Variances, Mean of square; Swab and Rinse

Analysis of variances, mean of squares table; Log TVC

Source of Variation	DF	R^2	CV	Error	Log TVC	F-Value
Site	5	0.2592	7.4685	0.1574	0.6389	0.0032
Actors	1	0.0099	8.3509	0.1968	0.1232	0.4319
Sample ID	29	0.9986	0.4201	0.0005	0.4244	< 0.0001

Analysis of variances, mean of squares table; Log CC

Source of Variation	DF	R^2	CV	Error	Log CC	F-Value
Site	5	0.1401	38.1255	2.9324	5.5404	0.1101
Actors	1	0.0021	39.7236	3.1834	0.4116	0.7204
Sample ID	29	0.9988	1.8522	0.0069	6.8119	< 0001

Analysis of variances, mean of squares table; Log Yeast and Moulds

Source of Variation	DF	R^2	CV	Error	Log Yeast and Moulds	F-Value
Site	5	0.3599	60.3677	3.3988	22.16775	< 0.0001
Actors	1	0.0191	72.2802	4.8725	5.8729	0.2765
Sample ID	29	0.8832	33.6788	1.0578	9.3793	< 0.0001

Analysis of variances, mean of squares table; Log LAB

Source of Variation	DF	R^2	CV	Error	Log LAB	F-Value
Site	5	0.4298	52.5883	2.4564	21.4838	< 0.0001
Actors	1	0.0452	658226	3.8483	11.2941	0.0917
Sample ID	29	0.9512	20.0949	0.3587	8.1964	< 0.0001

APPENDIX E. Analysis of Variances, Mean of square of Microbial load of Milk samples

Analysis of Variances, Mean of squares; Log TVC

Source of Variation	DF	R^2	CV	Error	Log TVC	F-Value
Site	5	0.8751	7.1162	0.2772	20.9809	< 0.0001
Actors	1	0.0028	19.4053	2.0609	0.3345	0.6885
Sample ID	29	0.9986	0.4201	0.0005	0.4244	< 0.0001

Analysis of Variances, Mean of squares; Log CC

Source of Variation	DF	R^2	CV	Error	Log CC	F-Value
Site	5	0.5199	33.4469	4.1124	48.0974	< 0.0001
Actors	1	0.0135	46.2615	7.8673	6.2566	0.3762
Sample ID	29	0.9986	0.4201	0.0005	0.4244	< 0.0001

Analysis of Variances, Mean of squares; Log Yeast and Moulds

Source of Variation	DF	R^2	CV	Error	Log Yeast and Moulds	F-Value
Site	5	0.2589	49.8726	7.0644	26.6497	0.0053
Actors	1	0.0002	55.8915	8.8724	0.1264	0.9054
Sample ID	29	0.9986	0.4201	0.0005	0.4244	< 0.0001

Table; Analysis of Variances, Mean of squares of microbial load for Milk; Log LAB

Source of Variation	DF	R^2	CV	Error	Log LAB	F-Value
Site	5	0.2173	45.5907	6.8160	20.4410	0.0184
Actors	1	0.0381	48.7688	7.7994	17.9047	0.1352
Sample ID	29	0.9986	0.4201	0.0005	0.4244	< 0.0001

APPENDIX F: Means comparison of TVC, CC, Yeast and Molds, and LAB of the Sample Type in a site

Sample			Log_{10} (cfu /ml)			
	N	Site	TVC	CC	Yeast &Moulds	LAB
Milk	12	MBG1	4.87 ± 0.15^{c}	4.57 ± 0.59^{b}	3.89 ± 0.77^{b}	4.15 ± 0.75^{b}
	10	MBG2	8.24 ± 0.16^{a}	8.15 ± 0.64^{a}	5.98 ± 0.84^{ab}	5.79 ± 0.83^{ab}
	10	MBG3	7.36 ± 0.16^{b}	7.31 ± 0.64^{a}	5.90 ± 0.84^{ab}	5.64 ± 0.82^{ab}
	10	MBG4	7.73 ± 0.17^{b}	2.78 ± 0.64^{b}	4.10 ± 0.84^{b}	4.83 ± 0.83b
	10	MBG5	8.43 ± 0.17^{a}	5.86 ± 0.64^{a}	4.54 ± 0.84^{b}	6.32 ± 0.82^{a}
	8	MBG6	TNTC	TNTC	TNTC	TNTC
Water	12	MBG1	7.68 ± 0.64^{a}	5.37 ± 1.04^{a}	0.00 ± 0.55^{b}	0.00 ± 0.46^{b}
	10	MBG2	4.26 ± 0.70^{b}	4.10 ± 1.15^{a}	0.20 ± 0.61^{b}	0.20 ± 0.49^{b}
	10	MBG3	6.78 ± 0.69^{ab}	6.78 ± 1.14^{a}	0.00 ± 0.60^{b}	1.07 ± 0.49^{b}
	10	MBG4	8.04 ± 0.70^{a}	4.48 ± 1.15^{a}	1.69 ± 0.61^{b}	1.46 ± 0.50^{b}
	10	MBG5	8.18 ± 0.69^{a}	3.56 ± 1.15^{b}	1.67 ± 0.60^{b}	0.20 ± 0.49^{b}
	8	MBG6	TNTC	TNTC	TNTC	TNTC
Swab/Rinse	12	MBG1	5.45 ± 0.11^{a}	4.90 ± 0.49	3.05 ± 0.53^{a}	3.00 ± 0.45^{b}
	14	MBG2	4.89 ± 0.11^{b}	4.82 ± 0.45	2.33 ± 0.49^{ab}	1.90 ± 0.42^{b}
	10	MBG3	5.41 ± 0.13^{a}	4.31 ± 0.54	0.91 ± 0.58^{ab}	1.18 ± 0.49^{bc}
	10	MBG4	5.42 ± 0.12^{a}	3.28 ± 0.54	3.27 ± 0.58^{a}	3.78 ± 0.50^{ab}
	10	MBG5	5.39 ± 0.13^{a}	4.14 ± 0.54	4.05 ± 0.58^{a}	3.47 ± 0.49^{ab}
	8	MBG6	TNTC	TNTC	TNTC	TNTC

APPENDIX G; Plates Showing Hygiene Practices

Plate1 1: Milking process

Plate 2: Milk handling containers at farm

Plate 3: Cooler tank at MBG

Plate4: processor collecting milk from MBG

Plate5: Dairy farmers at MBG

APPENDIX H: Research Permit

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Ministry of Agriculture & Food Security,
Department of Animal Health and
Livestock Development,
P. O. Box 2096
LILONGWE - MALAWI

From: The Director 23/04/2018
Department of Animal Health and Livestock development
P.O. Box 2096
Lilongwe

To: The Officer In-Charge
CHSU

Dear Sir/Madam,

Subject: LETTER OF INTRODUCTION FOR RICHARD BANDA.

Mr Richard Banda, is doing a study on Hygiene Practices of Small and Medium Enterprises in Milk Processing plants and their influence on Development of Biofilms in Lilongwe as a partial fulfillment to attain his Masters Degree in Food Science at Ergerton Univeristy, Nairobi Kenya.

The purpose of this letter is to request your institution to assist him in analysing his samples which will involve culture, isolation and identification of the possible microorganisms that he is likely going to find from the milk samples he is going to collect.

We find this study to be very important since it will generate information that will help us in understanding more about food borne pathogens found in milk which are of public health importance.

Looking forward to any assistance that your institution can help.

23 APR 2018

Dr. Gilson R. Njunga
Acting Deputy Director, Research and Investigations.