

**INFLUENCE OF CONTAINER DESIGN MATERIAL, CLEANING REGIME,
WATER QUALITY AND PERSONNEL HYGIENE ON BIOFILM
FORMATION IN FARM-LEVEL MILK CONTAINERS IN KAJIADO
COUNTY, KENYA.**

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**A Thesis Submitted to the Graduate School in Partial Fulfilment of the
Requirements for the Award of the Degree of Master of Science in Food Science
and Technology of Chuka University.**

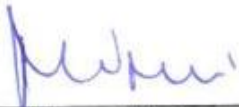
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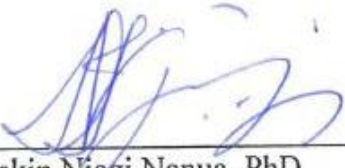
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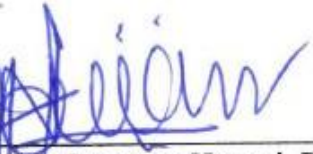
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DEDICATION

This thesis is dedicated to my wife Caroline for the emotional support and endurance during my long education journey.

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ABSTRACT

Food safety and the reduction of food losses remain critical concerns, particularly due to the consumption of milk contaminated with microorganisms. Milk is highly susceptible to contamination from microbes that form biofilms on milk handling equipment. Biofilms are heterogeneous and complex, making them difficult to eradicate. Therefore, this research aimed to investigate the influence of container design material and cleaning regime on biofilm formation in farm-level milk handling containers. The study employed a completely randomized design focusing on mazzicans and jerry cans. Chi-square tests, Fisher's Exact test, logistic regression and robust permutation tests were used to analyze the data using R Core Team software version 4.4.0 of 2024. Bacterial multispecies comprising of *Staphylococcus aureus* (71%), *Bacillus cereus* (60.5%) and *Pseudomonas aeruginosa* (27.6%) were reported to co-exist as biofilms. Chi square test of independence revealed that there is association between cleaning regime and biofilm formation ($P < 0.001$). Logistic regression tests further revealed that using liquid detergent significantly reduces the likelihood of biofilm formation compared to the bar soap regime with a odds ratio of 0.01 ($P < 0.001$). Fisher's Exact test also revealed that there is an association between water quality, personnel hands and biofilm formation with an odds ratio of 0.203 ($P = 0.03792$). The odds ratio of 0.203 also revealed that biofilm formation is less likely in water compared to personnel hands. Logistic regression test also revealed that there was no significant influence of container design material on biofilm formation with an odds ratio of 0.84 ($P = 0.768$). It was concluded that biofilm is a potential food safety concern and a source of spoilage of milk products in Kajiado County thus a contributor of food waste and loss leading to economic losses. It is recommended that cleaning regime needs to be standardized since it is a contributing factor to proliferation of biofilms. Stringent water quality and personnel hygiene practices are required also to avert biofilm formation. Future studies are recommended to determine changes in biofilm formation on different containers under varying conditions.

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ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
CI	Confidence Interval
CIDP	County Integrated Development Plan
CFU	Colony Forming Unit
CRD	Completely Randomized Design
DNA	Deoxyribonucleic acid
EPS	Extracellular Polysaccharide
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GLM	General Linear Model
GMP	Good Manufacturing Practices
GPRS	General Packet Radio Services
HACCP	Hazard Analysis Critical Control Point
H ₂ S	Hydrogen Sulphide
ISO	International Organization for Standardization
KDB	Kenya Dairy Board
MCPPB20	Monte Carlo Permutation Procedure test
MIC	Minimum Inhibitory Concentration
MTS	Milking and Transportation Containers
NACOSTI	National Commission for Science, Technology and Innovation
NGO	Non-governmental Organization
ODK	Open Data Kit
OR	Odds Ratio
RPM	Revolutions Per Minute
TSI	Triple Sugar Iron
TVC	Total Viable Count
USD	United States Dollar
VBNC	Viable But Non Culturable
WHO	World Health Organization
XLD	XyloseLysineDesoxycholate

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Over the past decades, the dairy industry has faced several challenges in ensuring food safety. In this study, focus was on milk-handling practices at the farm level in rural Kenya. It is estimated that 65-80% of all infections are biofilm related and that most microbes exist in form of biofilms (Jamal *et al.*, 2018; Ren *et al.*, 2015). Biofilm-forming bacteria are major sources of both pathogenic and spoilage microorganisms in the dairy industry (Bhunja, 2018; Elmoslemary *et al.*, 2009). These include pathogenic microorganisms namely *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica enteritidis* and Typhimurium serotypes whereas spoilage microorganisms include *Pseudomonas aeruginosa* (Hickey *et al.*, 2015; Oliver *et al.*, 2009). Biofilms affect the safety and quality of milk and milk products (Moretro and Lansrud, 2017). According to a study in Nairobi (Wanjala *et al.*, 2017), there was high detection of acidity in milk samples handled in plastic containers at 67.6% compared to aluminium containers at 43.8%. The study also showed that 96% of milk sold had total viable count (TVC) above recommended thresholds by Kenya Bureau of standards. Plastics and other surfaces contribute to biofilm formation which can lead to food spoilage and foodborne diseases (Cholewińska *et al.*, 2022; Joseph *et al.*, 2001). Milk marketed in Kenya has been found to have low bacterial quality thus posing danger to consumers (Alonso *et al.*, 2018). Regulatory framework governing farm-level use of food-grade milk handling containers in Kenya has shown non-compliance, with most informal small-scale farmers preferring use of cheap plastics (Nyokabi *et al.*, 2021). Policy and practice regarding use of milk handling containers still shows huge gaps with food safety concerns raised in both formal and informal sector (Alonso *et al.*, 2018). Microorganisms in mixed-species biofilm are complicated to study and targeted research efforts are still rather unexplained (Cappitelli *et al.*, 2014; Yuan *et al.*, 2020).

Biofilm refers to a group of bacterial or fungal cells that are held together by extracellular matrix. This is an emerging contemporary area, which is a major public health concern (Hall-Stoodley and Stoodley, 2004; Rather *et al.*, 2021). It's adaptation to the surfaces and survival contribute to high degree of resistance to cleaning and disinfection programs (Høiby, 2017). Regular and thorough cleaning is essential to

prevent the establishment and maturation of biofilms. Microorganisms have been shown to withstand against cleaning and disinfection to form biofilms with even modern disinfectants not always effective (Yushina *et al.*, 2024). Cleaning and disinfection have been reported not to completely eradicate microorganisms (Somers and Wong 2004). Bacteria biofilms are reported to be 100-1000 times more resistant to various cleaning methods with the efficacy of the cleaning agents varying (Bénézech and Faille, 2018; Jha *et al.*, 2022). Water, soil and air pollution are some of the environmental contaminants of food products occurring from production to distribution. Water quality directly impacts the initial microbial load that can colonize surfaces and form biofilms (Galie *et al.*, 2018; Sharif *et al.*, 2018; Simoes *et al.*, 2010). Proper hygiene practices, including hand washing and the use of protective gear, reduce the transfer of microorganisms to surfaces. Minimizing cross-contamination from personnel to containers and equipment is essential for biofilm prevention (Evans *et al.*, 2022).

Plastics have been reported to be the main containers used in handling milk in Kenya and other East African countries (Kurwijila *et al.*, 2016; Omore *et al.*, 2005). Plastics form the most common containers used in informal markets in Kenya (Nyokabi *et al.*, 2021). Plastic containers are difficult to clean and sanitize and have the potential to harbor microbes (Knight-Jones *et al.*, 2016; Wanjala *et al.*, 2017). According to a study (Orregard 2013), the most common methods used in the dairy value chain to clean milk handling containers are the use of bar soap with hot, warm or cold water sometimes with a scourer. One of the considerations in choosing milk handling container is the ease of cleaning which is hardly met by these plastic containers.

Properties of food contact materials have been shown to contribute to biofilms. These includes chemical composition, roughness and surface charge (Chaturongkasumrit *et al.*, 2011). Design material of food handling containers and equipment has been reported to contribute to biofilms (Marriott *et al.*, 2018). Plastic containers sanitization and scrubbing has been reported to ineffectively remove surface bacteria thus contributing to biofilms (Clayborn *et al.*, 2015). Plastics' chemical composition varies from polyethylene, polypropylene, polystyrene, polyvinylchloride, polyethylene terephthalate, polycarbonate, polymethyl methacrylate, polyurethane to polyamide (Fenichell, 1996). The hydrophobic nature of plastics makes them have a greater surface

adherence by microorganisms (Tang *et al.*, 2011). Cleaning efficiency of these plastics is also determined by the texture and shape of equipment. A new and more affordable food grade plastic container (the “Mazzican“) that was developed and shown to be an appropriate vessel for improving the hygiene of milk handling during milking and transportation to the market in other countries of East Africa is another type of plastic milk handling container (Kurwijila *et al.*, 2017).

The interplay of container design material, cleaning regime, microbiological water quality, and personnel hygiene influences biofilm formation. Effective strategies to mitigate biofilms require a multifaceted approach that considers all these factors. The potential role of biofilm formation in plastic food-grade farm-level milk handling containers requires more studies to generate new information on how cleaning regime, container design material and other risk factors like water quality and personnel hygiene contribute to biofilms in plastics. This could help in strengthening regulatory framework governing food-grade farm-level milk handling containers. Against this backdrop, the current research was carried out to determine the influence of container design material, cleaning regime, microbiological water quality and personnel hygiene on biofilms formed in farm level food-grade mazzican and jerry cans in Kajiado County, Kenya.

1.2 Statement of Problem

Despite the stringent hygiene protocols in dairy production, biofilm formation in milk handling containers remains a significant challenge. Biofilms, which are microbial communities attached to surfaces, pose serious risks as they can harbor spoilage and pathogenic bacteria. Biofilms are difficult to remove once formed leading to contamination of milk products thus compromising on food safety and public health. Several factors: design material, cleaning regimes, quality of water and hygiene practices of personnel handling the containers can influence biofilm formation. The influence of these factors on biofilm formation in farm level milk handling containers is not fully known. This research sought to investigate the influence of container design material, cleaning procedures, water quality and personnel hygiene on biofilm formation in farm level milk handling containers.

1.3 Objectives of the study

1.3.1 General Objective

The general objective of this study is to investigate the influence of container design material, cleaning regime, microbiological water quality and personnel hygiene on biofilm formation in farm level milk handling containers of small-scale farmers in Kajiado County.

1.3.2 Specific Objectives

- i. To assess the effect of cleaning regime on biofilm formation in farm level milk handling containers.
- ii. To determine the influence of microbiological water quality on biofilm formation in farm level milk handling containers.
- iii. To determine the influence of personnel hygiene on biofilm formation in farm level milk handling containers
- iv. To determine the influence of container design material of farm level milk handling containers on biofilm formation.

1.4 Hypotheses

Based on the above objectives, this study tested the following hypotheses:

- H₀1: Cleaning regime has no statistically significant effect on biofilm formation on farm level milk handling containers.
- H₀2: Microbiological water quality has no statistically significant influence on biofilm formation on farm level milk handling containers.
- H₀3: Personnel hygiene has no statistically significant influence on biofilm formation on farm level milk handling containers.
- H₀4: Container design material has no statistically significant influence on biofilm formation on farm level milk handling containers.

1.5 Justification of the Study

Food safety and public health is a concern due to consumption of contaminated milk. Milk is highly susceptible to contamination from microbes that form biofilms on milk handling equipment. Biofilms harbor microorganisms that can lead to foodborne diseases. Understanding the factors that influence biofilm formation helps enhance

safety and reduce health risks associated with milk consumption. Contamination of milk can lead to spoilage, reducing shelf life resulting to economic losses. Food waste and loss contributes to food and nutrition insecurity. Understanding the factors that contribute to these losses helps in minimizing spoilage, improving product quality and ensuring milk remains safe for marketing over extended period. Regulatory compliance to strict hygiene and safety standards helps in avoiding potential fines, recalls or reputational damage. Study site chosen was Kajiado since it has reported high incidence of milk spoilage and has presence of high number of jerry cans and mazzicans. Besides, past similar studies were done in other setups, thus Kajiado as a pastoral setup was chosen to compare findings across different geographic locations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Dairy Industry

2.1.1 Global Dairy Sector Production Capacity and Revenue.

The dairy industry has developed significantly over recent decades and is one of the fastest growing food production sectors in the world compared to other food commodities (Gao and Haworth, 2016). Dairy industry has experienced tremendous growth in the past (Emond, 2019). There have been a lot of innovations in both production systems, handling, processing techniques and processes. These have contributed to growth in dairy farmers' volumes of milk produced, value-added, marketed and consumed. Increasing production capacity of livestock resources highly depends on mechanisms geared towards enhancing hygienic milk handling practices. Milk, which is a nutrient-dense product harbors both spoilage and pathogenic microflora that has a bearing on the income and health status of the consumers of the products.

It is estimated that the value of milk sales grew to USD 720 billion in 2019 and expected to grow further to USD 1032 billion in 2024 (Lohumi *et al.*, 2018). Globalization has contributed to consumption of milk and milk products across the world with expanded international trade among various countries. Dairy products exported globally in 2020 are estimated to be worth over USD 55 billion, cheese and butter leading (Bojovic and McGregor 2023; Lohumi *et al.*, 2018). With increased international trade and volumes of milk and milk products consumed, it calls for stringent measures to be applied at all levels from farm to folk in observing safety and quality standards.

Global dairy sector has seen expansion of small-scale production in several countries contributing to increased international trade for milk and milk products (Bojovic and McGregor, 2023). Global milk production is approximated to have reached to 906 million tonnes in 2020 with Asia, Europe, and America reported to be the highest producers. Asia approximated to have produced 379 million tonnes with India alone producing 195 million tonnes due to increased operational and production efficiency (Grace *et al.*, 2020). Europe produced 236 million tonnes with North America producing over 111 million tonnes.

2.1.2 Dairy Sector in Sub-Saharan Africa

Globally, one hundred and fifty million farming households comprising of approximately 750 million people are engaged in milk production with majority drawn from developing countries (Sraïri *et al.*, 2019). Cow's milk accounts for over 80% of milk produced in the region with 50% being contributed by East Africa countries (Opoola *et al.*, 2019). Africa produced 49 million tonnes of milk in 2020, Algeria reporting increase whereas Kenya, Ethiopia and South Africa reported decline due to drier and warmer weather and increased feed prices (Grace *et al.*, 2020). Africa milk production forecast in 2022 was 51 million tonnes affected mainly by extreme weather conditions in Kenya, Ethiopia and South Africa. Improvement in animal breeds is one of the contributing factors to growing volumes of milk in the region (Ojango *et al.* 2017). It is estimated that over 80% of milk produced in sub-Saharan Africa is marketed whereas the remaining 20% consumed by the farming households (Merem *et al.*, 2022). This calls for stringent measures in ensuring hygienic handling practices are embraced by the handlers in the informal sector. Milk losses and spoilage is mainly due to poor milk handling and storage (Nyokabi *et al.*, 2021; Staal, 2006). This means infrastructural and capacity development for different actors in the value chain needs to be enhanced to avert this condition.

In sub-Saharan Africa, dairy sector is regarded as the leading with huge potential for poverty eradication and improving food and nutrition security. Raw milk production is mainly by smallholder farmers thus forming the biggest source of employment for the rural population (Zavala and Revoredo-Giha, 2022). Milk production in sub-Saharan Africa is highly dependent on rain-fed fodder and pasture production hence the industry experiences varying trends of milk availability throughout the year. There is growing demand for milk and milk products, Kenya and South Africa being leaders in milk production (Sumner, 2020). Traditional systems have dominated the region though evolving into modern farming systems and improved breeds, which are contributing to increased milk production.

2.1.3 Dairy Sector in Kenya

According to Kenya Dairy Board (KDB), it is estimated that a total of 5.2 billion litres of milk is produced annually, which is mainly produced by over 1.8 million small scale

farmers. The small-scale dairy farmers produce over 90% of the milk, which is marketed mainly through informal channels (Zavala and Revoredo-Giha, 2022). Marketing of milk is undertaken both formally and informally with informal marketing taking lead (Bingi and Tondel, 2015). Kenyan per capita milk consumption is the highest in sub-Saharan Africa at 110 litres (Bahta *et al.*, 2023; Rademaker *et al* 2016). This means that there is ready market for milk and milk products. This high milk consumption means higher chances of transmission of foodborne diseases in milk if hygienic measures are not adhered to. According to the Kenya Dairy Master Plan 2010-2030, Ministry of livestock and development (2010), the dairy industry has grown at a rate of 3-4% annually. Its continued growth is a key factor in attaining the national development goals as spelt out in Vision 2030. Most of the commercial production is concentrated in central and northern Rift Valley areas, but farmers in other areas are increasingly taking up small-scale dairy farming.

The dairy industry is regulated by the Kenya Dairy Board, established in 1958 by the Dairy Industry Act Cap. 336. It is from this Act that the Board derives its mandate of regulating, developing, and promoting the dairy industry in Kenya. Despite having well laid out regulatory requirements, enforcement remains a big challenge with incidences of foodborne diseases emanating from consumption of milk and milk products being on the rise (Rademaker *et al.*, (2016). Current information on the role and impact of biofilm formation in plastic raw milk handling containers is required. Informal milk marketing channels are the most common methods in Kenya (Kembe *et al.*, 2008; Ongwech *et al.*, 2022). This means that due to lack of stringent measures to ensure hygiene and safety of the milk handled in this space, consumers may be at risk of foodborne diseases. With small scale producers dominating the highly fragmented market, enforcement of milk handling hygiene and safety measures remains a big challenge. Most of the small-scale farmers find ways of evading the enforcers by using marketing channels and timing for selling their product, which is unrealistic for the enforcers.

Kajiado County, which occupies arid and semi-arid areas of Kenya is one of the highest pastoral communities' counties with the highest milk production especially during rainy seasons (Kajiado County Integrated Development Plan (CIDP) 2018-2022). With inadequate milk handling infrastructure, just like other areas in the country, milk

business is dominated by informal actors. Pastoral systems are associated with high mobility in search of pasture and water. This includes moving towards remote areas with no access to regular water and milk cooling systems. Unhygienic milk handling conditions are aggravated by the high temperatures experienced in semi-arid areas in most months of the year. This means that failure by the milk handlers to observe hygienic milk handling compromise largely the health of the consumers. Low milk quality standards are also attributable to lack of quality testing by the handlers in the informal sector (Muriuki, 2011). With most of the farmers in the area handling their product using plastic jerry cans, greater attention is required in these areas to deter formation of biofilms.

2.1.4 Milk Handling Systems by Largescale Farmers

Largescale farms account for less than 20% in Kenya and are usually situated in rural setups. Most of largescale farmers are endowed with resources thus have huge investments in utensils, infrastructure and equipment that are superior in maintaining milk quality and safety (Rademaker *et al.*, 2016). With mechanized milking equipment, transportation and storage of milk by large producer farmers, minimal handling of milk by personnel, automated cleaning systems, better knowledge and skills translate to higher quality standards of milk handled in these large farms (Onono *et al.*, 2013). Large scale farmers have financial muscles to acquire extension services from both government and private practitioners unlike small-scale farmers. This means that they can get appropriate knowledge and skills regarding clean milk production.

Good dairy farming practices, which entail animal health, milking hygiene, feed and water and environment are majorly embraced by largescale farmers (Njarui *et al.*, 2016). This results into better quality and safety of raw milk and finished products emanating from these farms unlike for small scale farmers. Unlike small scale farmers where milk is marketed in the informal milk trade that mainly consists of small-scale operators like hawkers, brokers, self-help groups, neighbours and business establishments like hotels, large scale farmers either process or sell directly to major processors (Muriuki, 2011). This means lower chances of cross-contamination as milk changes ownership unlike in the case of small-scale farmers. Besides, large scale farmers take little time before

subjecting their milk to cooling systems thus may be of superior quality compared to milk handled by small scale farmers.

2.1.5 Milk Handling Systems by Small Scale Farmers

Low to middle income countries' milk production systems are mainly through smallholder farmers. Milk quality issues in small scale dairy farmers are mainly due to poor and unhygienic handling practises with informal milk marketing systems contributing to this (Wafula *et al.*, 2016). Improper handling, poor hygiene and sanitation by small scale farmers are contributed by lack of skills and knowledge on milk standards and food safety (Omore and Baker, 2009). Farmers should adopt practises that suit their financial status, are technically sound and fit their social and cultural norms. This, therefore means that there should be intensive stakeholder engagement during design material of milk handling equipment and policy formulation (Alonso *et al.*, 2018). This will ensure high adoption of new methods and equipment that promote milk quality and food safety. These being some of the drivers of milk quality especially in Africa, with most of milk produced by small scale farmers, there is need to address root causes of poor milk handling and hygienic practises (Alonso *et al.*, 2018).

Current milk production, handling, and consumption in the country expose populations to health risks due to unhygienic milking environments, the use of plastic containers, and use of untreated water (Nyokabi *et al.*, 2021). This is aggravated by the fact that most of the small-scale farmers channel directly their produce to consumers and retailers through informal marketing systems (Kiambi *et al.*, 2020). The low quantity of milk produced and low investment capacity by small-scale farmers contribute to lack of cooling systems leading to lower chances of maintaining milk quality. Poor road networks also affect quality of milk handled by small-scale farmers since they take long time before ferrying the raw milk to the markets or milk collection/cooling centres. Therefore, poor milk handling practises and unhygienic methods coupled by lack of cooling systems are some of the contributing factors to proliferation of microbes in milk handled by small-scale farmers. These are the primary contributors to biofilm formation in milking, milk storage, transportation, and processing equipment.

Accelerated and consolidated expansion of milk production and trade within the country can be achieved by realizing the productive potential of smallholders. However, despite high adoption of dairy farming in many regions of the country, food safety and quality remain the highest challenge due to inappropriate milk handling methods (Alonso *et al.*, 2018). Thus, sustainability of the increased dairy production must be supported by a corresponding increase in hygienic milk handling practices especially by small scale farmers. Without access to the right milk handling and storage containers, smallholder dairy farmers lose up to 30% of their product and the income they could have made from it. The informal milk marketing channels handle approximately 80-90% of East and Central Africa milk (Zavala and Revoredo-Giha, 2022; Ongwech *et al.*, 2022). Small-scale milk producers account for over 90% of total milk production. Most small-scale farmers use plastic containers as the main milk handling, storage, and transport containers, which are difficult to clean and disinfect thus contributing to poor milk quality (Ndungi *et al.*, 2021).

Kenya dairy sector policies and regulations are considered as barriers to informal milk sector which is dominated by small scale farmers (Kiambi *et al.*, 2020). Informal milk marketing is attractive to small scale farmers because of the high demand for raw milk (Alonso *et al.*, 2018; Nyokabi *et al.*, 2018). Complex, costly and highly fragmented regulatory framework of Kenya's dairy sector has been identified as one of the barriers to compliance with a mismatch between policy and practice (Alonso *et al.*, 2018). Criminalization and hefty penalties to non-compliance especially by the informal milk marketing channels has been reported as an unsustainable way of dealing with non-compliance issues (Kiambi *et al.*, 2020; Omore and Baker, 2009). This means that other innovative ways of regulation should be sought.

2.2 Cleaning and Sanitization for Milking and Milk Handling Containers

Cleaning processes for milk handling, storage, transport, and processing equipment usually vary (Orregard, 2013). Depending on the design material of the containers, it may call for different methods or detergents for cleaning. Sensory tests such as smelling unpleasant odor and feeling greasy surfaces have been used to determine the visible sanitation defects, but microbiological evaluations are standardized methods of guaranteeing sanitation programs (Holah *et al.*, 2002). This means that hygienic

conditions of food-contact surfaces including those of plastics can be better determined through microbiological tests rather than sensory tests. Some methods used in cleaning may be abrasive with flow conditions also varying. The choice of cleaning method and detergent used may influence the stability of biofilms formed (String *et al.*, 2020). Small scale farmers especially in developing countries like Kenya use methods that have not been studied or approved scientifically. The determination of effective cleaning and sanitation programs as a control of biofilm formation is still a complex issue (Unlu, 2020).

Cleaning procedures and design material of equipment play a big role in formation of biofilms (Matthews *et al.*, 2017; Schmidt and Ericson, 2005; Silva *et al.*, 2010). Studies have been carried out on adherence of certain microorganisms on stainless steel, marble, polyvinyl chloride, polyurethane, silicone-coated cloth and granite food-processing contact surfaces. These have shown how interaction between the microbial mix can either aid or deter formation of biofilms compared to various cleaning detergents and contact time. This therefore calls for generation of new information on how plastics interact with various microorganisms and how their cleaning and design material may influence formation of biofilms.

The effectiveness of cleaning influence the nature, age and composition of the biofilm in milk with the composition of the cleaning agent, its concentration, contact time, temperature, abrasion forces and properties of the milk contact material contributing greatly to biofilm action (Lelievre *et al.*, 2001; Ostrov *et al.*, 2016). Use of mechanical scourers during cleaning practices especially for plastic containers may also increase roughness which has been reported to enhance opportunity for biofilm formation (Spengler *et al.*, 2019). Effective removal of biofilms in milk means disruption even before introduction of a cleaning agent. This may be influenced by the design material of the milk handling containers, processing, or transport equipment. Though Hazard analysis critical control point (HACCP) and good manufacturing practices (GMPs) have been adopted widely as ways of ascertaining food safety and quality, biofilms formation remains a challenge (Shi and Zhu, 2009).

2.2.1 Prevention and Control of Biofilm Formation in the Dairy Industry.

Microbial cells present in biofilms have proved difficult to eradicate. This is due to the complex nature that makes them resistant to environmental stress factors including antimicrobials (Rossi *et al.*, 2016; Yin *et al.*, 2021). Detection of biofilms is also key towards their mitigation. Most cost-effective methods of detection employ agar plating techniques, which may have some shortcomings. Some microorganisms including *L. monocytogenes* exhibit viable but non-culturable (VBNC) phenomenon at low metabolic activity (Schottroff *et al.*, 2018). This therefore means its quantification may give inaccurate results, thus, even its control using novel technology may be misguided. Therefore, even when weak biofilms are detected by agar plating methods, stringent control measures need to be taken to mitigate as this may be a serious public health concern. There has been increase in antimicrobial resistance coupled with ineffective control of biofilms using conventional cleaning methods and other sanitizing regimes (Marques *et al.*, 2007).

Colonization of microbial cells on solid surfaces is a multi-stage process involving physicochemical and biological factors. Biofilm formation process involves an initial reversible attachment, transformation to irreversible attachment, early development of biofilm, maturation, and dispersion of cells from biofilm to the surrounding space (Van Houdt and Michiels, 2005). This requires new strategies for the control of pathogenic and spoilage microorganisms encapsulated within biofilm matrix. The use of enzymes as innovative anti-biofilm agents because of their ability to disintegrate extracellular polymer substances matrix components, which includes polysaccharides, proteins and microbial DNA has been one of the proposed ways of dealing with the highly resistant biofilms in the food industry. Different enzymes have been applied in controlling biofilm formation with their mode of action, and effects of combining various enzymes having been widely studied (Molobela *et al.*, 2010; Nagraj and Gokhale, 2018).

In-depth advantages and disadvantages of using enzymes and other novel agents as an anti-biofilm strategy has been investigated and strategies drawn that may be unsustainable especially for small scale farmers and cottage level processors (Zhu *et al.*, 2022). Other anti-biofilm chemical oxidation agents that have been used include ozone and iodine that have effect on equipment surfaces and do not fully penetrate

biofilm structure. Other biofilm agents combine oxidizer, hydrogen peroxide, phase transfer catalyst and ammonium that are not cost-effective. Quorum sensing inhibition in biofilms, use of bacteriocins, bacteriophages, biosurfactants and essential oils are also novel ways of controlling biofilm menace (Brackman and Coenye, 2015). The various methods and agents that have been widely studied so far may not be applicable in plastic milk handling containers mainly because of the diverse chemical mix and the design material of the plastic containers. The interaction between these agents and plastics is also not well known.

2.2.2 Biofilm Formation Mechanisms and Composition

Biofilms refer to surface-attached microbial communities containing bacterial and/or fungal cells in extra cellular matrix (Abdallah *et al.*, 2014; Bayles, 2007). This matrix comprises of polysaccharides, proteins, lipids and DNA (Bridier *et al.*, 2011 ; Jamal *et al.*, 2018; Su *et al.*, 2022). Conditioning film, a precursor to biofilm, is a thin layer that develops on surfaces especially plastics, when it interacts with water thus contributing to adhesion of microorganisms (Bhagwat *et al.*, 2021; Talluri *et al.*, 2020). Microbial cells in the biofilms are protected from environmental factors thus their proliferation has serious pathogenic and spoilage effects on food products (Pagan and Garcia-Gonzalo, 2015; Saha 2004). Biofilms have also been shown to contribute to damaged equipment surfaces, disease outbreaks and increased energy costs (Brooks and Flint, 2008; Moretro and Langsrud, 2017; Yuan *et al.*, 2018). Biofilm composition differs but mainly comprise of 97% water and about 2-5% cells (Choudhary and Singh, 2020; Sutherland, 2001; Vatansever, 2018). This therefore means that they are highly hydrated with this acting as a media of nutrient circulation.

Biofilms have been shown to alter organoleptic properties of products especially in the dairy industry through formation of either micro or macro-colonies. Some of these biofilms can produce lipases, proteases that may affect the contact surfaces as well as quality of raw and finished products (Carrascosa *et al.*, 2021). Though only a few of bacterial cells can adhere to surfaces as well as some surviving cleaning and disinfection, bacterial species that form biofilm have also been shown to have genomic variations with genes involved in their formation leading to completely different biofilms under different growth conditions (Yuan *et al.*, 2018). This heterogeneity is

one of the aspects of biofilm formation that makes eradication of biofilms in the food industry a complex issue. This therefore means that formation of biofilms in plastics used in storage and transportation of raw milk may have different interactions compared to other food contact surfaces. Besides, different environmental conditions (temperature, pressure, variations in climatic conditions), food matrix components, attachment surface, bacterial cells involved, electrostatic charging of surfaces, cleaning regimes may also have variations in biofilm formation and subsequently lead to varying foodborne infections (Pinto *et al.*, 2019).

Composition of biofilms have been shown to be mixed bacteria that may take a few hours from 2 - 24 days to form thus making a complex structure. This is difficult to eliminate in the food industry (Giaouris *et al.* 2012). Exponential growth phase for biofilms on stainless steel for some bacterial species has been shown to have short contact time of as low as 2 hours with mature stage reported at 4 hours and continuously constructed until 48 hours (Dula *et al.*, 2021). The varying growth conditions for various biofilm bacterial species in different contact surfaces calls for further research on plastics widely used in handling milk products in the country. This therefore means that biofilms contribute to serious quality and safety issues in the food industry. Planktonic cells of same species differ greatly compared to same species forming biofilm thus tolerance to physical and chemical treatments differ also greatly. It is therefore imperative that greater mitigation measures should be employed towards biofilms.

2.2.3 Biofilm Resistance to Cleaning and Sanitation

Bacterial resistance to antibiotics is a current area of concern and especially biofilms (Rossi *et al.*, 2016; Limoli *et al.*, 2015). Biofilms in dairy sector have been found to be resistant to disinfectants and difficult to remove (Abdallah *et al.*, 2014; Huang *et al.*, 2020). Many commercial disinfectants used in dairy farms have also been shown to have limited control of biofilm formation (Marchand *et al.*, 2021). Bacteria resistance to disinfectant for planktonic phase varies with biofilm resistance. Gram-positive bacteria have been shown to better resist than gram-negative ones with resistance varying between strains of same species (Bridier *et al.*, 2011). Bacteria comprising biofilm structures are 10-1000-fold more resistant to antibiotics than the planktonic phase (Mah and O'Toole, 2001). The mechanisms are not well known for this variation,

but hypotheses include slow diffusion of antibiotics to inner biofilm layers, slow growth state due to lack of nutrients and accumulation of harmful metabolites thus survival, and a subpopulation of cells of biofilms resembling spore formation process thus have a high resistant characteristic to protect them from antibiotics (Maric and Vranes, 2007).

Antibiotics available for deterrence of biofilms have higher minimum inhibitory concentration (MIC) and minimum bactericidal concentration. Efflux pump inhibition is one of strategies to inhibit resistance. Quorum sensing mechanisms inhibition can also solve this menace of resistance (Singh *et al.*, 2017; Ye *et al.*, 2022; Zamani *et al.*, 2018). Some phytochemicals like tannins act as chelating agents thus control bacterial resistance. Increasing prevalence of resistant strains of bacteria with reduced susceptibility to antibiotics necessitate need for new ways of controlling infections (Ventola, 2015). Cleaning of plastic milk handling containers especially by the pastoralist communities in Kenya use herbs that have phytochemicals present. Smoke from specific tree species has been shown to have antimicrobial properties. These include *Olea Africana*, *Acacia nilotica*, *Balanites aegyptiaca* and *Combretum spp.* (Wayua *et.al*, 2012). This may influence biofilm formation process. Therefore, this study on farm-level plastic milk handling containers sought to understand whether bacterial biofilm formation may be aggravated or deterred through cleaning and sanitation regimes employed by pastoralists.

2.2.4 Factors Causing Increased Biofilm Resistance to Cleaning Regimes

Harsh environmental factors used during cleaning and sanitation have been found to have little or no effect on biofilms (Dynes *et al.*, 2009; Matthews *et al.*, 2017). Microbial cells adapt to harsh environments with subsequent exposures making them tolerate such or harsher conditions (Singh *et al.*, 2017). Quorum sensing system, which differs for both gram-positive and gram-negative bacteria plays a major role in this process regulated by microbial population (Rutherford and Bassler 2012). With this diversity of microbial colonization also varying, this resistance phenomenon is a complex issue that biofilms in milk need to be controlled right from farm to processing to avert foodborne diseases after detachment (Zhao *et al*, 2017). It has also been shown that upon colonization of a biofilm by microbial cells, subsequent attachments can happen by

other microbial cells (Norwood *et al.*, 2000). This poses a greater risk of stability and resilience of biofilms in milk.

Multispecies of microbial biofilms increase resistance as compared to mono-species microbial cells. Modifications in the extracellular polysaccharide either in composition or quantity has also been shown to affect the resistance ability of the biofilms. This alteration can be caused by either physical, biological or chemical activity (Dynes *et al.*, 2009).

2.3 Microbiological Quality of Milk and Risk Factors in Biofilm Formation in Handling Containers

Food losses and wastages through spoilage of milk and milk products have heavily costed the dairy sector globally. Spoilage and pathogenic microorganisms are of particular concern. Both gram-positive and gram-negative bacteria have been identified in milk and milk products at various stages of the value chain (Lafarge *et al.*, 2004). Gram-negative bacteria usually account for the biggest share with environmental conditions at various stages affecting the numbers and type of microbial colonization. During these stages, microbial cells can form biofilms, multiply to high numbers with some of them producing heat stable enzymes aggravating milk spoilage (Marchard *et al.*, 2009). This means that their impact can be felt post-processing all the way to consumption. This is a great public health risk for populations where foodborne disease burden is growing day by day. Other undesirable sensory parameters may also result due the enzymes or microbial cells present in biofilms.

The environmental conditions during milk handling, transport or processing stages determine the predominance of specific microorganisms in the end-product. Microbiological studies on raw milk have revealed that milk handling containers determine the microbiological quality of milk (Orwa *et al.*, 2017). This means that the improper use of farm-level food-grade mazzicans and aluminum milk handling containers by food handlers may have a direct correlation on formation of biofilms and incidences of food poisoning reported in the Country. Biofilms formed pose a greater risk if subsequent milk passes through same equipment or areas which attract more and diverse bacteria (Simoes *et al.*, 2009). Even without microbial detachment, pathogenic

or spoilage microorganisms can release toxic metabolites or spoilage heat-stable enzymes that affect the quality of the final product. This means that cross-contamination can happen without realization that may end up to the final product (Dogan and Boor, 2003). This means that biofilms formed in milk handling containers contribute to double burden or risks to the populations.

2.3.1 Major Microorganisms Responsible for Biofilms in Dairy Equipment

There is diverse colonization of food processing equipment by various gram-positive and gram-negative bacteria impacts the industry (Bhunja, 2018; Chmielewski and Frank 2003; Matthews *et al.*, 2017). Considering the cleaning and disinfection processes employed in various set ups, bacterial growth involves different behavioral characteristics. *Salmonella spp* which is one of the pathogenic microorganisms predominant in milk, has been shown to cause gastroenteritis or septicaemia with most deaths reported from this bacterium (Lamas *et al.*, 2018). Through production of proteinaceous extracellular fibres involved in host colonization, surface and cell contact, *Salmonella* can form biofilms (Cwiek *et al.*, 2019). *Staphylococcus aureus* on the other hand, which is a gram-positive, facultative anaerobe, non-motile and a non-spore former has been characterized by producing heat-stable enterotoxins at temperatures between 10-46 °C and is able to grow well in high salt, sugar and low water activity food products. It has also been associated with acute toxic shock regarded as a fatal condition, diarrhea, and vomiting (Giaouris *et al.*, 2015; Schelin *et al.*, 2017).

Bacillus cereus is an anaerobic facultative both vegetative and spore-former has a wide range of growth environments between 4-50 °C is also associated with diarrhea, abdominal pains and deaths. It has been shown to be resistant to chemicals and heat treatment (Houry *et al.*, 2010). *B. cereus* is commonly found in food products including dairy in equipment such as pipes, belts and storage tanks. Through secretion of enzymes such as lipases, proteases, bacteriocins, surfactants, and other metabolites, it has been found also to affect the sensory properties of food products (Grigore-Gurgu *et al.*, 2019).

Escherichia coli survives in natural environments and is a major microorganism forming biofilms in milk and reported to survive in acidic conditions like in fermented

milk products and also shown to cause diarrhea with blood, acute kidney injury and deaths (Nataro and Kaper, 1998). This microorganism has also been shown to cause infections even in very low doses, 50 colony forming units (CFU), thus low-grade biofilm contamination is a serious health problem (Galie *et al.*, 2018; Lee *et al.*, 2007). This bacterium has been shown to use membrane proteins and flagella to initiate surface attachments (Lim *et al.*, 2019). This therefore means biofilm detection through quantitative and qualitative methods, which sometimes may show weak biofilm formation should not be ignored.

Listeria monocytogenes, another common biofilm forming bacteria in the dairy industry, which is a gram-positive dangerous foodborne disease agent replicates in refrigeration temperatures causes gastroenteritis and listeriosis especially in infants, elderly, and pregnant women. In pregnant women it also causes abortion. This bacterium has also been shown to exhibit increased biofilm formation under refrigeration temperatures and is able to form both pure and multispecies biofilms (Chmielewski and Frank, 2003; Raheem, 2016). The presence of membrane proteins and flagella gives it a good adhesion ability to food contact surfaces (Lemon *et al.*, 2007).

Pseudomonas on the other hand is a facultative anaerobe heterotrophic, motile gram-negative rod-shaped, psychotropic spoilage bacteria found in low acid milk products with the extracellular appendages linked to attachment and other surface interactions (Chmielewski and Frank, 2003; Golovlev, 2002; Gonzalez-Rivas *et al.*, 2018). It is also known to produce huge amounts of extracellular polysaccharide (EPS) materials associated with attachments and multispecies formation of biofilms, which make them more resistant and stable (Chmielewski and Frank, 2003). Synergistic interactions between various bacterial species pose serious threat in control of biofilms in the food industry (Lee *et al.*, 2007).

Biofilms are therefore a serious food safety and quality concern in the dairy and food industry (Latorre *et al.*, 2010). This is aggravated by their tolerance to various environmental stress factors. Biofilms being a mix of various bacterial species existing in a community therefore determines their structure, action, and resistance to

environmental stress factors (Agarwal *et al.*, 2011). Complete elimination of biofilms, which is a mix with food-borne microorganisms on food contact surfaces remains difficult to accomplish hence forms a route for cross contamination (Ferreira *et al.*, 2010).

The process of biofilm formation is affected by various physiochemical factors like temperature, oxygen, light, and presence of compounds such as antibiotics (Alotaibi and Bukhari, 2021). Studies have shown that hydrophobic nature of some contact surfaces contribute to enhanced ability to form biofilm with different bacteria having varying optimum temperature. For instance, *Staphylococcus aureus* has been found to form biofilm better at higher temperatures from 20 to 37 °C whereas *P. fluorescens* forms biofilms best at low temperatures below 10 °C (Alotaibi and Bukhari, 2021; Cao *et al.*, 2021 Rossi *et al.*, 2016). Though extensive research has been done especially on biofilm formation in dairy milking and processing equipment, more studies are required on interactions of various microorganisms with plastic milk handling containers. This applies for both food-grade re-usable plastic handling containers and single-use plastic containers (Agarwal *et al.*, 2011). Food-contact surfaces have influence on the attachment of microbial cells. This also ultimately determines the effectiveness of cleaning after the microbial attachments (Frank, 2001).

2.3.2 Dairy Sector and Food Poisoning

Informal markets in Sub-Saharan Africa, Kenya included contribute to key outlets for major foods such as fruits and vegetables, milk, meat and other processed crop and animal sourced products. These market outlets are regarded as hotspots of food safety. Developing countries have reported infections caused by pathogenic microorganisms leading to morbidity and mortality (Bintsis, 2017; Elmonir *et al.*, 2018). It is estimated that in Africa, 135 million cases of foodborne diseases with 180,000 deaths occur on yearly basis (Pires *et al.*, 2021). Economic consequences include \$ 20 billion due to productivity losses and \$3.5 billion on cost of treating illnesses annually.

Microbial biofilms are resistant to various chemical and antimicrobial agents and are responsible for more than half of infectious diseases globally (Bjarnsholt, 2013; Jamal *et al.*, 2018; Yadav *et al.*, 2020). Food poisoning remains a public health concern in

Kenya (Jaffee *et al.*, 2018). Biofilms remain one of the channels through which high incidences of food poisoning has been reported in the recent past (Zhao *et al.*, 2017). Colonization by various gram-positive and gram-negative bacteria in the biofilms has been reported (Huang *et al.*, 2020). Biofilms formed from pathogenic microorganisms are thought to cause significant infections in humans (Jamal *et al.*, 2018).

Pastoral communities in Kajiado county, livestock sector remains their main source of livelihood making milk the most traded commodity (Kajiado County Integrated Development plan 2018-2022). Productivity of a population highly depends on their health status thus food safety remains key in ensuring consumers are protected against all forms of harm either caused advertently or inadvertently (Van Zon *et al.*, 2005). Despite the Government actively discouraging the selling of ‘hawked’ milk, the sector continues to grow with grave consequences to consumer health. Kenya government focus over years has been on non-communicable diseases with little research and resources into foodborne illnesses. High cases of diseases have been shown to contribute to low productivity, increased burden in health systems, reduced retention rates for school-going children, and sometimes fatalities are reported (Weil, 2007). Addressing this problem will contribute to reduced disease burden with more resources put into development projects thus relieving the health systems in the County and Country at large. Kenya’s informal markets for raw milk act as a route for rising cases of foodborne illnesses (Zavala and Revoredo-Giha, 2022). Foodborne diseases contribute to compromised health of population, reduces productivity of citizens and ultimately contributes to mortality (Jaffee *et al.*, 2018).

2.4 Biofilm Formation in Different Milk Handling and Processing Equipment Design Materials.

Dairy industry remains one of the highest sectors affected by microbial contamination through both spoilage and pathogenic microorganisms via microbial biofilm. Food processing and storage equipment forms suitable environment for development of biofilms with high heterogeneity multispecies biofilms, which depends on contact surface characteristics, food matrix components, environmental conditions, and bacterial cells (Govaert *et al.*, 2018; Lindsay and von Holy, 2006; Makovcova *et al.*, 2017; Tang *et al.*, 2011; Van Houdt and Michiels, 2010). Food components in milk

comprises of fats, proteins, carbohydrates, with minerals like calcium and magnesium in milk, lactose having been linked to enhanced biofilm development by different bacterial species (Duanis-Assaf *et al.*, 2016; Dutra *et al.*, 2018; Somerton *et al.*, 2015; Xue *et al.*, 2014).

Microorganisms have been reported to adhere to surfaces, which in turn result to formation of biofilms (Banda *et al.*, 2020). This has been reported to contribute to foodborne diseases. Milk handling containers, processing units, and transporting units are the main routes through which biofilms are formed. The attachment of bacterial cells into various surfaces poses a greater public health risk through consumption of contaminated finished products (Brooks and Flint, 2008; Brooks 2009). Detection of a bacterium in biofilms means presence of other organisms (Momba *et al.*, 2000). Biofilms adhere to solid surfaces producing a matrix of extracellular polysaccharides, proteins, and DNA material with a microbial community with diverse characteristics (Bjarnsholt *et al.*, 2009).

Studies on biofilm formation in various equipment and containers have been done. The methodology used under this study considered the duration of use of different design materials of the farm-level milk handling containers. This study thus further explored the proliferation of biofilms in farm-level plastic jerry cans and food-grade plastic mazzicans milk handling containers against various time durations of use. Studies have shown that biofilm formation is a complex and dynamic process affected by various factors such as texture or roughness of surface, flow velocity, pH, temperature, nutrient availability, hydrophobicity, presence of antimicrobial agents and extracellular appendages such as flagella (Alotaibi and Bukhari, 2021; Banerjee *et al.*, 2015; Cowle *et al.*, 2019; Gomes *et al.*, 2014). Incubation time has also been reported to be varying from few hours to days. Due to favorable environment provided by milk handling, transportation and processing equipment, biofilms pose a greater threat through colonization of the surfaces. Some of the triggers of rapid colonization of biofilms include nutrient availability and cellular stress. Biofilms being a colony of microbial cells in a network of water means ease in transport of nutrients, other metabolites, and waste products (Sauer *et al.*, 2007). This means that milk, which is a nutrient dense product provides favorable environment for biofilms to thrive.

Milk handling containers and processing equipment may undergo various changes in temperature, mechanical abrasion or chemical activity thus stressing the microbial cells that may react further into forming biofilms against the environmental stress. Other factors include pH, dead ends, cracks, flow conditions and adhesion forces. Therefore, the design material of milk processing and handling equipment plays a critical role in bacterial colonization and ultimately biofilm formation. Colonization of microbial cells in the biofilm causes predominant species thrive with competition reducing at different stages. This means a reduction in competition for nutrients with the dead cells providing more energy for the predominant colonies. This means favorable conditions can be maintained and be sustained for long (Bayles, 2007). Milk handling from milking to processing prior to dispatch to markets usually is a lengthy process taking numerous hours or days. Biofilm formation usually can take as short as minutes to hours depending on the favorable environmental conditions (Florjanic and Kristl, 2011). Factors that contribute to attachment and detachment of biofilms are diverse and not well understood (Chambless and Stewart, 2007). This means that different types of biofilms can be formed by either same microbial cells or different microbial cells depending on the environmental factors. This means that different milk handling environment, transport systems and processing environments have emerging risks that have not been understood completely. This applies also for plastic milk handling containers associated with varying handling, cleaning and sanitation procedures thus may pose environmental stress further aggravating formation of biofilms.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was done in Kajiado East Sub County, Kajiado County (Figure 1), $1^{\circ} 50' 31.463''$ S, $36^{\circ} 47' 30.696''$ E in Paka-Kenyewa ward in four different areas of Masimba, Sultan Hamud, Illamirror and Oltinka. This being a pastoral setup, prevalence of plastic container use in milk handling is high with adoption for mazzican container use also being high thus availability of sample for the study. The study was done during onset of short rains October-November-December 2023 because samples were readily available during this period. Survey questionnaire was administered to small-scale farmers in the same area where samples were collected after. Sample microbiological and biofilm analysis was done in Chuka University, Food technology laboratories.



Figure 1: Map of Kenya showing Kajiado District

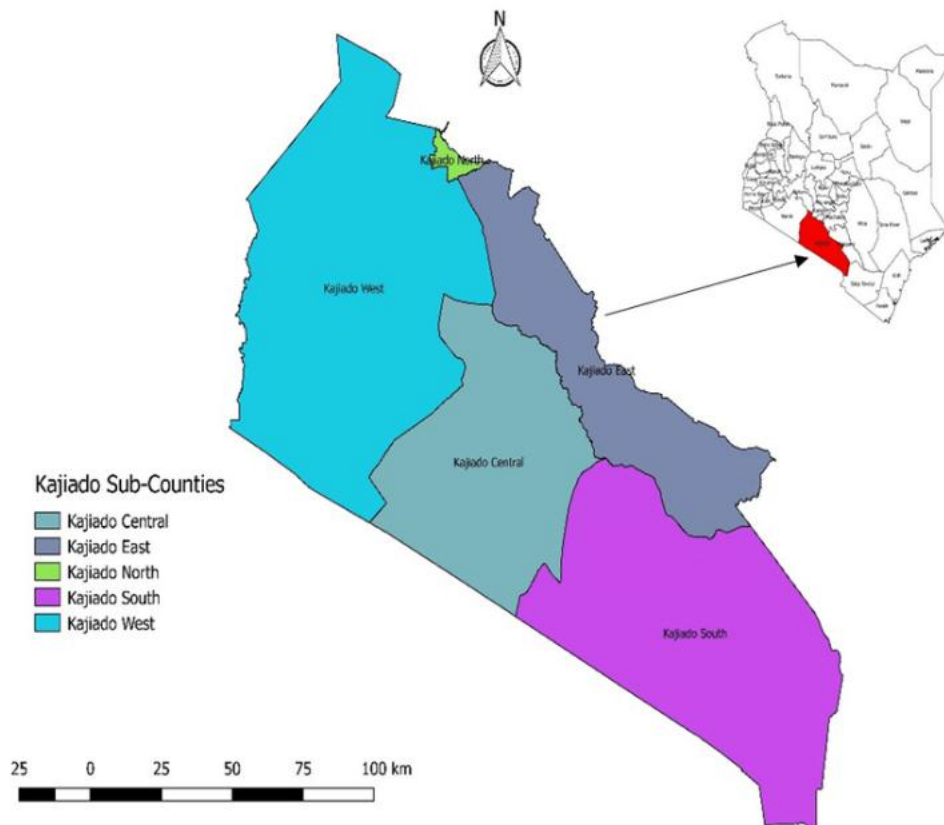


Figure 2: Map of Kajiado district showing Kajiado East, the study area (Source: Kenya National Bureau of Statistics, 2023).

3.2 Research Design

The completely randomized design (CRD) with a 2x2 factorial arrangement was used in this study. Qualitative and quantitative variables in the study are as per the figure 3 below. Independent variables in this study included: container design material, cleaning regime, water quality and personnel hygiene. The independent variable, container design material had two levels analyzed: marzipans and jerry cans. These are the primary containers used by small-scale farmers for milk storage. For the independent variable cleaning regime, survey identified the most common cleaning practices among farmers, and these were used to evaluate their effectiveness across container types. A total of 39 swabs were collected from 39 containers (14 marzipans and 25 jerry cans), with microbial analyses conducted in triplicate for precision. Quantitative variable was the microbial load from the container surfaces, personnel hands and water. Statistical analysis was conducted on these values to evaluate significant effects. Qualitative research methods focused on gathering data through a comprehensive survey of the

farmers. This provided insights into their cleaning practices, preferences for container materials, and the rationale behind their choices.

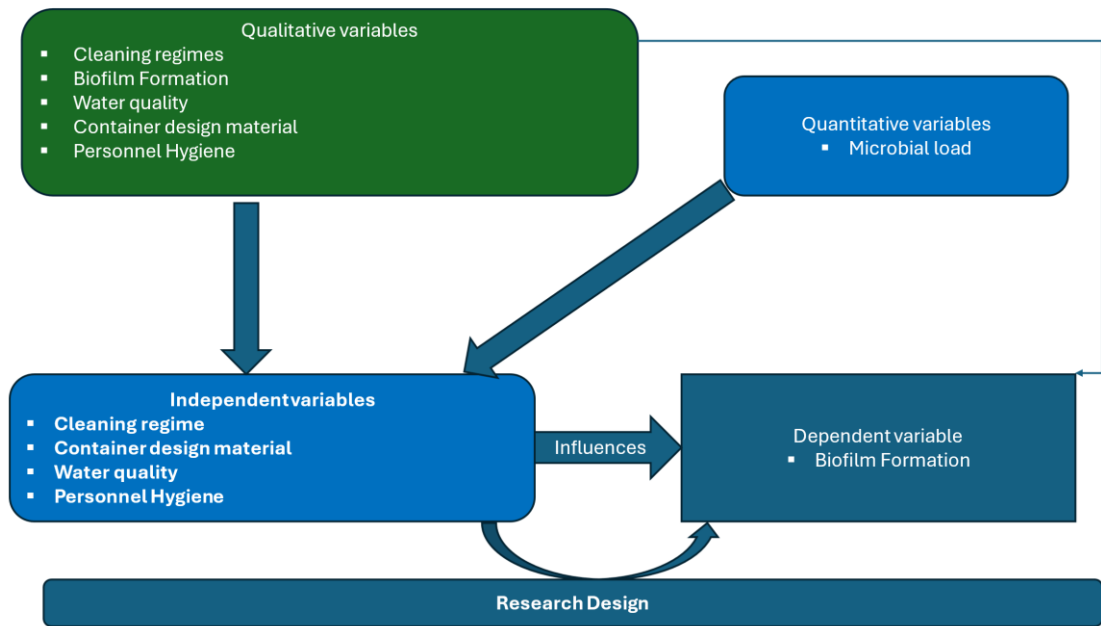


Figure 3: Schematic representation of multiple variables under study research design

3.3 The effect of cleaning regime on biofilm formation in farm level milk handling containers.

3.3.1 Sampling size determination and sampling procedure

3.3.1.1 Sample size determination

Formula for calculating sample size of small-scale farmers was as follows (Kish, 1965)

$$n = \frac{z^2 p(1-p)}{d^2}$$

where, n was the sample size, z was the z statistic at 95% confidence level (z=1.96), p was the estimated population proportion, taken as p=0.5 (maximum variability), e=the desired precision level of $\pm 5\%$ at 95% confidence level.

$$n = \frac{1.96 * 1.96 * 0.95(1 - 0.95)}{0.05 * 0.05}$$

$$n = 1.96 * 1.96 * 0.5(1-0.5) / 0.05 * 0.05$$

$$n = 84$$

A sample size of 101 farmers in Kajiado County pastoral community was a representative of 603 Samli dairy cooperative farmers. Table 1 below shows how representative samples of farmers were randomly selected from four clusters in Poka-Kenyewa ward, Kajiado County.

Table 1: Sampling frame.

Site/Cluster	Farmers' population	Sample	Targets
Masimba	260	39	36
Sultan Hamud	140	24	20
Oltinka	120	22	17
Illamirror	83	16	12
Total	603	101	85

3.3.1.2 Sampling procedure

This study was conducted in Kajiado County, Poka-Kenyewa ward. A random sample of 101 farmers handling milk was selected from a population of 603 farmers delivering milk to Samli dairy farmers cooperative society. To ensure a representative sample of farmers was selected and each had an equal chance of being selected, random sampling was employed. Farmers were randomly selected from four clusters in Masimba, Oltinka, Illamirror and Sultan Hamud. To avoid biasness, all farmers were given equal chance of being selected using a random number generated by random number calculator. All households from the four clusters were listed before using random number to select them randomly.

3.3.2 Questionnaire

Pretesting was done using semi-structured questionnaire administered to test ambiguity due to wording of questions, check on the length of time taken by respondents and test whether some questions were too sensitive based on cultural norms in the area. Pretesting was done in Osarai, a site with similar context as the study site. Ten farmers were selected from Osarai village in Kajiado East sub-County. Pre-testing established that the questionnaire met all requirements thus no further adjustment was done prior to data collection. Based on the outcome of the pretesting, no further reliability tests were conducted in this study. To establish the relationship between cleaning regime and biofilm formation, a questionnaire was administered to small scale farmers within Kajiado East Sub County, Poka-Kenyewa ward (Appendix 1). Multi-stage sampling was done to determine farmers to be interviewed. The sampling was done at village level and subsequently at households. Questionnaire was developed and uploaded in online open data kit (ODK). This helped in seamless administration of the tool, quality checks, cleaning of data and analysis of responses in a real-time basis.

Farmers were randomly selected and in cases where a farmer had more than one container, the various containers were treated as separate samples. Information that was sought through this exercise included: the duration of use of the plastic containers, cleaning methods, detergent used, cleaning duration, temperature of cleaning media and time taken before cleaning of containers after use. Farmers in Poka-Kenyewa ward had already been introduced to the food-grade mazzican plastic container.

3.4 Influence of microbiological water quality and personnel hygiene on biofilm formation in farm level milk handling containers

3.4.1 Sampling technique

Twelve 10 ml water samples were sourced from different water points where farmers drew, then preserved for analysis using ice cubes in a cool box maintained at a temperature of below 8^o C. Following randomization in selection of farmers, water samples collection was equally from a representative sample.

3.4.2 Swabbing procedure

Swabs were taken from personnel hands handling milk. Swabbing was carried out through sampling techniques from surfaces using contact plates and swabs (Griffiths, 2016). Swabbing was done on the hands of the milking personnel when ready and just before start of milking either between 6 and 8 am and/or evening between 6 and 7 pm.

3.4.3 Microbiological analysis

Microbial isolation and identification were done for both water samples and swabs from personnel hands. Serial dilutions for water samples were done up to 10⁻⁴ prior to enumeration and identification of target microorganisms of interest in this study. Gram staining was done using method described by Tripathi and Sapra (2020).

3.4.4 Biofilm formation assay

Identified microbial isolates from container design material, water and personnel hands were further subjected to qualitative analysis on their ability to form biofilm (Mohamed *et al.*, 2016).

3.5 Influence of container design material of farm level milk handling containers on biofilm formation.

3.5.1 Collection of Samples

Thirty-nine farm level milk handling containers (14 food-grade plastic mazzicans and 25 jerry cans) were sourced from small scale dairy farmers randomly selected from those involved in milk business. List of all farmers from four clusters was generated from which 39 random farmers were selected using a random number calculator. Swab samples of 39 were taken from containers after the cleaning and sanitation process and when just ready for collection or handling of the product. Raw milk food-grade mazzican plastic handling containers and jerry cans were collected from small scale dairy farmers selling milk to organized cooperative and middlemen in Poka-Kenyewa ward, Kajiado East Sub County, Kajiado County. Swabs were collected during normal milking time in the morning between 6 and 8 am and/or evening between 6 and 7 pm. Surface swabs were then preserved for further microbiological analysis using cool box maintained at temperatures below 8°C. The duration of use of the container before replacing varied from farmer to farmer. Swabs were taken after cleaning and sanitation. Swabbing was done using pre-wetted cotton buds at an area of 25 cm² in triplicates (Griffiths, 2016). Swabs were afterwards transferred into sterile test tubes containing freshly prepared 10 ml Ringer's solution and then stirred for about 25 seconds using a digital vortex mixer to disperse cells speed of approximately 300 revolutions per minute (rpm) and preserved in cool boxes maintained at temperature of below 8°C using ice cubes. All samples for microbiological testing were in triplicates.

3.6 Microbial Species in Biofilms

This study employed only phenotypic methods of culturing of microorganisms. Isolation and identification of various microbial cells is key in biofilm formation mechanisms. Differential and selective media were used for isolation and identification of major microorganisms of interest in current study. Gram staining followed up for morphological characterization of isolated colonies prior to confirmatory tests (Roberts and Greenhood, 2008; Spencer and de Spencer, 2008; Tripathi and Sapra, 2020).

3.6.1 *Staphylococcus aureus*

Culturing was done according to procedure described by Roberts and Greenhood (2008). A pure culture from trypticase soy agar was sub-cultured on a freshly prepared plate of mannitol salt agar. It was then incubated at a temperature of 37°C for 24 hours. Growth of white colonies surrounded by yellow zones indicated presence of *Staphylococcus aureus* and then proceeded by Gram staining.

3.6.2 *Salmonella typhi*

Samples were pre-enriched in buffered peptone water according to method described by Spencer and de Spencer (2008). After pre-enrichment, selenite broth was used to incubate the mixture for 24 hours at 37°C. Cultures obtained from this were then streaked on Xylose Lysine Desoxycholate agar (XLD) incubated for 48 hours at 37 °C. Purification then followed with MacConkey agar.

3.6.3 *Bacillus cereus*

The gram-positive *Bacillus cereus* were isolated using method described by Roberts and Greenhood (2008). Samples were incubated with Trypticase soy agar for 24 hours at 37°C before further culturing with blood agar (hemolytic colonies presumptive of *Bacillus cereus*).

3.6.4 *Pseudomonas aeruginosa*

The gram-negative *Pseudomonas aeruginosa* were isolated using method described by Roberts and Greenhood (2008). Cetrimide agar and blood agar were used for culturing. Samples were incubated with blood agar and cetrimide agar for 24 hours at 37°C. Beta hemolytic colonies on blood agar and appearance of yellow-green to blue-colored colonies on cetrimide agar were presumptive of *Pseudomonas aeruginosa*.

3.7 Biochemical Analysis

Following presumptive identification of pure colonies of *Staphylococcus aureus*, through culturing and gram staining, biochemical tests were carried out. Coagulase, citrate and catalase tests were conducted. Catalase was used to distinguish *Staphylococcus* from *Streptococcus* whereas coagulase producing *Staphylococcus aureus* was distinguished from other species of *Staphylococcus* through coagulase test.

A combination of these tests based on the biochemical diversity of microorganism of interest helped in confirmatory testing. Presumed *Salmonella typhi* pure colonies were subjected to two confirmatory biochemical tests. Triple sugar iron (TSI) agar to check production of hydrogen sulphide (H₂S) gas and alkalinity whereas urease test were conducted to check on degradation of urea in urea broth. Additionally, presumed *Bacillus cereus* and *Pseudomonas aeruginosa* isolates were confirmed by biochemical tests such as carbohydrate fermentation in TSI agar, catalase, oxidase test, indole production, hemolysis, citrate utilization, motility tests as described by Shoaib *et al.*, (2020).

3.8 Biofilm Formation Assay

Various methods for detection of biofilms are available with the complexity and heterogeneity of biofilms. Qualitative detections of biofilms were done using tube method as previously described by Mohamed *et al* (2016). The strength of the stain in the test tube was scored, with reference to a blank polystyrene 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 (Mohamed *et al.*, 2016).

3.9 Data Analysis

The data was analyzed using R Core Team software version 4.4.0 of 2024 with application of various statistical tests. The independent variables included container design material, cleaning regime, water quality and personnel hygiene whereas the dependent variable was the biofilm formation. Microbiological data were first transformed (\log_{10} cfu/ml) before analysis. Microbial counts continuous data were analyzed using non-classical ANOVA using Kruskal-Wallis test followed by Dunn's post hoc tests. Water quality and personnel hygiene under this study were treated as covariate that could affect the relationship between cleaning regime, container design material and biofilm formation. Chi square tests were used for confirming the association between cleaning regime and biofilm formation. Logistic regression tests were used to test effect of independent categorical variables (liquid detergent against bar soap) on the dependent categorical variable, biofilm formation. For the influence of microbiological water quality and personnel hygiene on biofilm formation, Fischer's

Exact test was used. Chi square and logistic regression was used to determine association and prediction respectively between container design material (mazzicans/jerry cans) and biofilm formation in R script statistical software at $\alpha < 0.05$. Additionally, robustness of data analyses was enhanced through permutation tests, trimmed mean in ANOVA, Fisher's Exact tests and bootstrapping methods.

3.10 Ethical Consideration

Local administration was consulted before administering questionnaires to farmers to give consent. This study involved sample collection from farmers who were selected on voluntary basis and informed on the purpose of the study. The identity of the participants in this study was not disclosed. National Commission for Science, technology and Innovation (NACOSTI) as in appendix III and Chuka University ethical considerations were also sought (Appendix II).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Cleaning Regime Effects on Biofilm Formation

This study was looking at the effect that cleaning regime has on biofilm formation on farm level milk handling containers. During this study farmers indicated that 45.4% of them used jerry cans for milk handling, 36.1% used mazzicans, 17.7% used aluminium and 04% used stainless steel (Figure 4). However, during collection of container samples for laboratory analysis, even those farmers who had reported use of aluminium containers did not have them. This could be explained that some farmers were aware that use of jerry cans was prohibited and thus gave misinformation about possessing aluminium cans. The rampant use of plastic cans could be because of their low costs thus more affordable than the aluminium cans. The nature of pastoral communities to move long distances to look for milk market could also be the reason for using jerry cans as more convenient container which is easy to carry and handle. Use of non-food grade containers can have effect on food safety and food losses due to presence of spoilage and pathogenic microorganisms such containers harbors. This agrees with a similar study in Kenya that most farmers used plastic containers for milking and transporting milk at 56% in Nakuru County (Mogotu *et al.*, 2022). In a similar study done in Kenya, it was reported also that 34.2% of farmers used plastic containers for milking and storage whereas 47.9% of farmers did not clean milk sheds and a further 83.6% did not isolate mastitis milk (Mogotu, 2021).

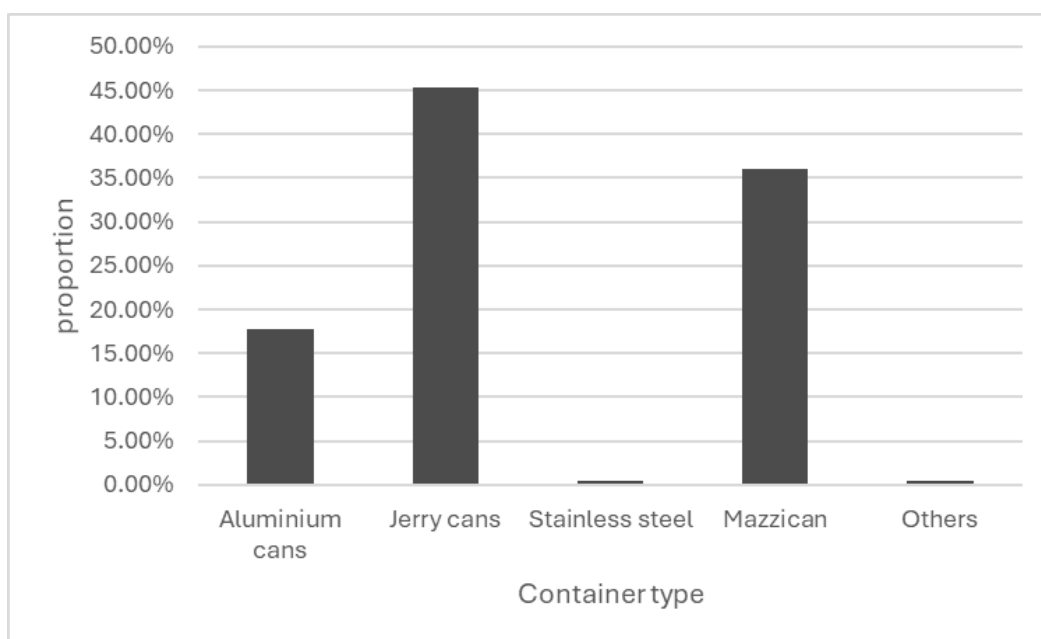


Figure 4: Milk handling containers used by pastoral communities in Kajiado County.

Cleaning regime employed by farmers showed that the use of liquid soap was at 83%, bar soap 16 % and shruvs 1% (Figure 5). The high use of liquid detergent compared to other methods could be because the liquid is able to penetrate to all parts of the container types commonly used unlike bar soap. The 100% of farmers using jerry cans could mean they prefer liquid detergent to conveniently clean their containers which have many dead ends.

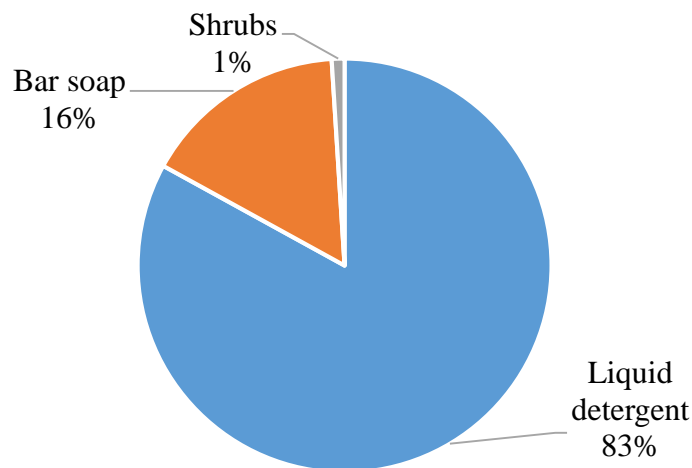


Figure 5: Graphical representation of detergents commonly used for cleaning milk handling containers in pastoral communities of Kajiado.

Ninety seven percent of farmers use mechanical abrasion methods during cleaning whereas only 2% farmers did not (Figure 6). High number of farmers who use abrasion methods could be because of the nature of the containers they commonly use to milk, handle and transport their milk. Since all farmers reported using plastic jerry cans, the nature of design of the jerry cans would require some mechanical abrasion for cleaning detergents to reach to all parts of the container.

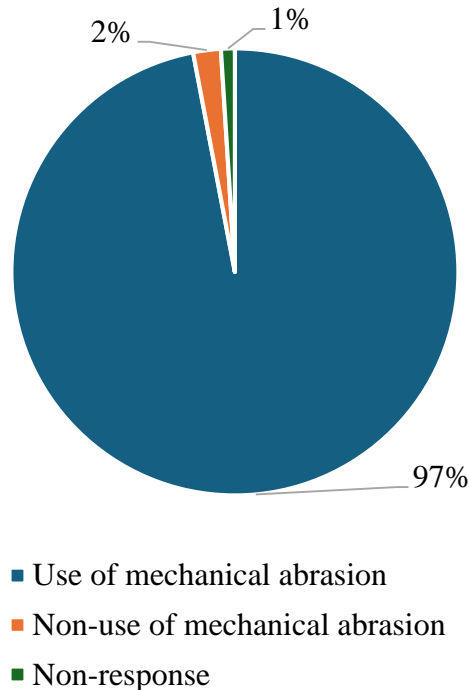


Figure 6: Proportion of farmers in Kajiado County using mechanical abrasion methods for cleaning milk handling containers.

This study showed that the use of bar soap compared to liquid detergent has a significant effect on biofilm formation ($P < 0.001$). This indicates an association between the cleaning regime and biofilm formation. This means the type of cleaning agent used (bar soap vs. liquid detergent) significantly influences biofilm formation on farm-level milk handling containers (Table 2). The results suggest that the cleaning regime applied differs in biofilm formation, reinforcing the need for improved cleaning protocols using more effective detergents.

Table 2: The logistic regression test with Odds Ratio of liquid detergent cleaning regime at 95% Confidence Interval for dependent variable biofilm formation

Characteristic	OR ¹	95% CI ¹	p-value
Cleaning Regime			
Bar soap at room temperature 16 to 25°C	—	—	
Liquid detergent at room temperature 16 to 25°C	0.01	0.00, 0.06	<0.001

¹OR = Odds Ratio, CI = Confidence Interval

Logistic regression test revealed that the odds ratio (OR) for liquid detergent was 0.01, with a 95% confidence interval (CI) ranging from 0.00 to 0.06 ($P = 0.001$). This indicates that using liquid detergent significantly reduces the likelihood of biofilm

formation compared to the bar soap (Table 3). This strongly suggest that liquid detergent is highly effective in preventing biofilm formation. The high likelihood of biofilm formation with bar soap suggests it is less effective at preventing microbial buildup compared to liquid detergent. The possible reason why liquid detergent may inhibit biofilm formation better than bar soap is because it has surfactant properties that are more effective in breaking down fats, proteins and other organic materials which are present in milk residues. On the other hand, bar soap has weak surfactant properties and may leave residues that promote microbial growth and subsequent biofilm formation. Liquid detergents also dissolve more readily in water and rinse completely than bar soap. The nature of hard water also used by the pastoral communities in Kajiado could mean bar soap reacting with calcium and magnesium ions forming scum which leaves residues creating a conducive environment for microbes to attach and form biofilm. To enhance hygiene in milk handling, recommended using liquid detergents, which are more effective at removing residues and preventing biofilm.

Table 3: Biofilm formation under different cleaning regimes for various bacterial species.

Cleaning Regime Type	Biofilm Formation (1- presence)	Microbial Species
Bar soap at room temperature	1	<i>S. aureus</i>
Bar soap at room temperature	1	<i>S. aureus</i>
Bar soap at room temperature	1	<i>S. aureus</i>
Liquid detergent at room temperature	1	<i>S. aureus</i>
Bar soap at room temperature	1	<i>S. aureus</i>
Bar soap at room temperature	1	<i>S. aureus</i>
Bar soap at room temperature	1	<i>S. aureus</i>
Liquid detergent at room temperature	1	Bacillus cereus
Bar soap at room temperature	1	Bacillus cereus
Bar soap at room temperature	1	Bacillus cereus
Bar soap at room temperature	1	Bacillus cereus
Bar soap at room temperature	1	Bacillus cereus
Bar soap at room temperature	1	Bacillus cereus
Bar soap at room temperature	1	Bacillus cereus
Bar soap at room temperature	1	<i>P. aeruginosa</i>
Bar soap at room temperature	1	<i>P. aeruginosa</i>
Bar soap at room temperature	1	<i>P. aeruginosa</i>
Bar soap at room temperature	1	<i>P. aeruginosa</i>
Liquid detergent at room temperature	1	<i>P. aeruginosa</i>

Similar studies have shown that contamination of milk and milk products is mainly caused by inadequate cleaning procedures which can lead to attachment of bacteria to surfaces (Marchand *et al.*, 2012, Srey *et al.*, 2013). Additionally, and in agreement with this study, Fink *et al.*, 2018 reported that some detergents used in cleaning contribute to cross-resistance thus aggravating the risk of biofilm formation. Also agreeing with this study, (Simoes *et al.*, 2010) reported that good hygiene and material science are the two key factors that contribute to biofilms in the food industry. *Bacillus cereus* has been reported to produce endospores complicating removal of biofilms by cleaning procedures (Auger *et al.*, 2009). However, in contrast with this study, it has been revealed that the mechanical washing of some surfaces with detergent and water can reduce contamination significantly (Gkana *et al.*, 2017).

Temperature of medium used by farmers could also determine formation and proliferation of biofilms. The cleaning media applied for cleaning containers and washing personnel hands was reported as warm temperatures 16-25 degrees centigrade. None of the farmers reported to boil water prior to applying it for cleaning and washing. This could be the reason why cases of biofilm formation were reported in personnel hands and container. These temperatures provided conducive environment for *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* to thrive. In agreement with this study, it has been reported that suboptimal temperatures and improper disinfection enhance biofilms (Slany *et al.*, 2017). However, in contrast, studies have observed no effect of heat on the microbes due to resistance (Bae *et al.*, 2009, Kim *et al.*, 2017, Rico-Munoz, 2017).

4.2 Influence of Microbiological Water Quality on Biofilm Formation

This study was looking at the influence that water quality has on biofilm formation on farm level milk handling containers. Ninety five percent of the farmers were found to use non-potable water as cleaning medium for the milk handling containers or washing hands prior to milking (bore holes 67%, water pans 20%, unprotected springs 8%) with only 5% using treated municipal water (Figure 7).

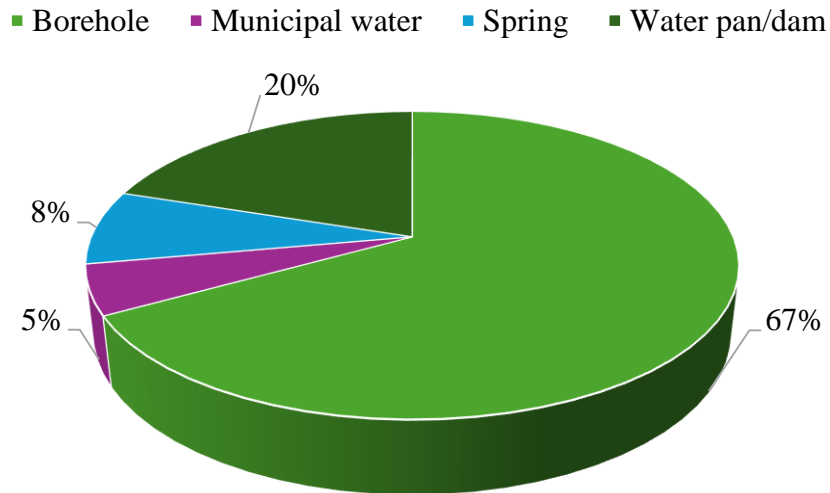


Figure 7: Sources of water where farmers in Kajiado County draw water for cleaning milk handling containers.

The twelve water samples drawn from different areas were from non-potable sources. The ability of microbial species to form biofilms in water samples was analyzed as per the table 4 below. Though *Bacillus cereus* and *Staphylococcus aureus* were detected in the water samples, *Pseudomonas aeruginosa* and *Salmonella typhi* were absent. Absence of *P. aeruginosa* could be due to microbial competition which happens as microorganisms compete for available nutrients, limiting its growth. However, the absence of *P. aeruginosa* in all the water samples analyzed doesn't guarantee food safety since it could mean contamination of water used might have existed in localized areas of water storage facilities which could fluctuate over time. *Salmonella typhi* absence also means that water sources used by pastoral communities in Kajiado is not highly polluted. *Salmonella typhi* presence is a serious public health issue since its presence in water is a strong indication of fecal contamination which could have resulted to waterborne disease outbreak in the area. However, in contrast with findings of this study, another study conducted in Paris (Perrin *et al.*, 2019), *P. aeruginosa* was present in 52.17% of water samples whereas 9% (Raposo *et al.*, 2016) and 25.2% (Wu *et al.*, 2016) of water samples have been reported also. The varying proportions reported could be due to several factors including temperature, nutrient and water flow differences. This emphasizes the need for regular water quality monitoring to mitigate public health risks.

Table 4: Microbial species present in water and their ability to form biofilms

Water Sample (W)	Transformed microbial data	Microbial species	Biofilm formation (S-strong, M-moderate, W-weak)
W	4.748188027	<i>S. aureus</i>	S
W	4.69019608	<i>S. aureus</i>	M
W	4.826074803	<i>S. aureus</i>	M
W	4.944482672	<i>S. aureus</i>	S
W	1	<i>S. aureus</i>	M
W	1.477121255	<i>Bacillus cereus</i>	M
W	6.477121255	<i>Bacillus cereus</i>	S

Chi-Square test of independence was done, robustness enhanced through further Fisher's Exact test, especially considering the relatively small sample size in the water category to examine the relationship between two covariates (water sample and personnel hand swabs) and biofilm formation. Fisher's Exact test revealed an association between water, personnel hands and biofilm formation ($P = 0.03792$). The logistic regression odds ratio of 0.203 suggests that the odds of biofilm formation in water samples are significantly lower than in personnel hand samples. Specifically, for every one unit of biofilm formation in personnel hand samples, there are approximately 0.203 units in water samples (Table 5). The possible reason why personnel hands had higher biofilm formation than water could be because bacteria on hands can easily adhere to skin, which is textured and uneven, providing surfaces for biofilms to anchor. The skin's natural oils and sweat can create microenvironments ideal for biofilm formation. Water, on the other hand, has fewer surfaces for bacteria to attach and initiate biofilm formation. Another study reported that surfaces that provide a more hydrophobic (low-wettability) properties encourages bacteria to adhere and form biofilms (Kokare *et al.*, 2009).

Table 5: The logistic regression test with odds ratio (OR) for biofilm formation in water samples compared to personnel hands with a 95% confidence interval (CI)

Characteristic	OR ¹	95% CI ¹	p-value
Sample			
Personnel Hand	—	—	
Water	0.20	0.03, 0.82	0.046

¹OR = Odds Ratio, CI = Confidence Interval

4.3 Influence of Personnel Hygiene on Biofilm Formation

This study was looking at the influence that personnel hand hygiene has on biofilm formation on farm level milk handling containers. Findings of this study has shown that personnel hands possess microorganisms possible of forming biofilms (Table 6). *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* were reported to form biofilms from hands of food handlers in Kajiado. This could be due to poor hand hygiene, frequent contact with contaminated surfaces, moisture from skin due to sweating and lack of use of disinfectants by personnel handling food. Hundred percent of farmers interviewed in Kajiado were found not to use disinfectants and used untreated water to wash their hands before milking. This implies risk of cross-contamination from hands to milk and milk handling containers. In agreement with this study, several studies have reported hands of food handlers are also involved in the transmission of various microorganisms to foods and are therefore significant vectors in the spread of foodborne diseases (Dorotíková *et al.*, 2022; Sasahara *et al.*, 2014; Yap *et al.*, 2019).

Table 6: Microbial species present in personnel hands and their ability to form biofilms

Personnel hand swabs (H)	Transformed microbial data	Microbial species	Biofilm formation (S-strong, M-moderate, W-weak)
H	4.041392685	<i>S. aureus</i>	M
H	2.29578694	<i>S. aureus</i>	S
H	2.401400541	<i>S. aureus</i>	S
H	2.025305865	<i>S. aureus</i>	S
H	2.139249218	<i>S. aureus</i>	M
H	2.012837225	<i>S. aureus</i>	W
H	2.636086515	<i>S. aureus</i>	W
H	2.012837225	<i>Bacillus cereus</i>	M
H	2.882068944	<i>Bacillus cereus</i>	M
H	2.06669855	<i>Bacillus cereus</i>	W
H	2.950608225	<i>Bacillus cereus</i>	M
H	2.749427099	<i>Bacillus cereus</i>	S
H	2.605089462	<i>Bacillus cereus</i>	S
H	2.910464316	<i>Bacillus cereus</i>	M
H	2.31386722	<i>Bacillus cereus</i>	M
H	2.57333584	<i>Bacillus cereus</i>	W
H	2.058426024	<i>P. aeruginosa</i>	M
H	2.772908195	<i>P. aeruginosa</i>	M
H	2.753199914	<i>P. aeruginosa</i>	S
H	2.887898488	<i>P. aeruginosa</i>	W

The possible reason why personnel hands have higher biofilm formation compared to water could be because human skin has a rough and uneven surface that provides a niche for microbial attachment. Dead cells as well provide microbial attachment in contrast with water which is usually stored in smooth surfaces which may not promote much microbial adhesion. Besides, water collected from Kajiado was stored in closed containers and could mean less exposure to contamination unlike personnel hands that are frequently exposed to contaminants which can adhere to the skin and form biofilms. The presence of *S. aureus*, *B. cereus* and *P. aeruginosa* in different hand swabs indicates a high health risk to consumers of milk products. This also suggests that animal handling during milking as well as cleaning and disinfection of food handlers' hands need to be improved. This agrees with a similar study that different factors are associated with biofilm formation (Avila-Novoa *et al.*, 2018; Gutiérrez *et al.*, 2012; Tang *et al.*, 2013)

4.4 Container Design Material Effect on Biofilm Formation

This study was looking at the influence that container design material has on biofilm formation on farm level milk handling containers. The duration of use of the containers before replacing by farmers was varying between 1 to over 12 months for jerry cans and mazzicans. Of the 90 mazzicans, 36 had been used for a period between 4-9 months with 54 of them reported to have been used for over one year. Of the 113 jerry cans, majority, 104 were reported to have been used for a period between 4-9 months whereas only 8 were reported to have been used for a period between 1-3 months (Figure 8).

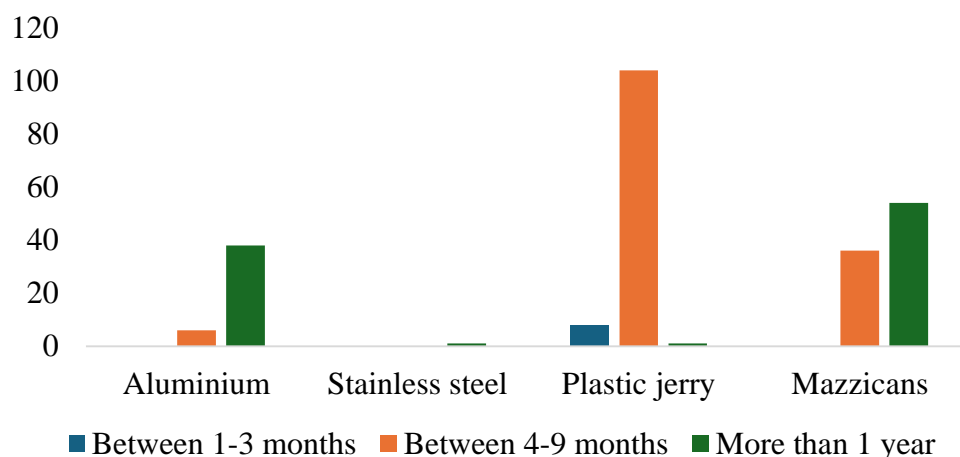


Figure 8: Duration of use for different milk handling container types used by pastoral communities in Kajiado County.

Mazzicans and jerry cans both reported abilities to form biofilms (Table 7). Bacterial multi-species were reported from this study comprising of *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa*.

Table 7: Container design material with multi-species bacteria ability to form biofilms

Container Design (M-Mazzicans, J-Jerry cans)	Biofilm Formation(1-Presence)	Microbial Species
J	1	<i>S. aureus</i>
J	1	<i>S. aureus</i>
J	1	<i>S. aureus</i>
J	1	<i>S. aureus</i>
J	1	<i>S. aureus</i>
J	1	<i>S. aureus</i>
M	1	<i>S. aureus</i>
J	1	<i>Bacillus cereus</i>
J	1	<i>Bacillus cereus</i>
J	1	<i>Bacillus cereus</i>
J	1	<i>Bacillus cereus</i>
J	1	<i>Bacillus cereus</i>
M	1	<i>Bacillus cereus</i>
M	1	<i>Bacillus cereus</i>
J	1	<i>Bacillus cereus</i>
J	1	<i>Bacillus cereus</i>
J	1	<i>P. aeruginosa</i>
M	1	<i>P. aeruginosa</i>
J	1	<i>P. aeruginosa</i>
M	1	<i>P. aeruginosa</i>
M	1	<i>P. aeruginosa</i>

The influence of container design material of farm level milk handling containers on biofilm formation was explored in this study using Chi-square test, subsequent Fischer's Exact test followed by logistic regression to enhance the robustness of the analysis (Table 8). Logistic regression model also showed that for the mazzicans container design material there was no significant effect of container design material on the likelihood of biofilm formation ($P = 0.768$). The intercept also suggests that the overall model does not significantly predict biofilm formation ($P = 0.198$). The estimated odds ratio of 0.839 (Table 8) suggests that the odds of biofilm formation in mazzican were slightly lower than in jerry can design material, but no significant effect ($P = 0.8$).

Table 8: The logistic regression test with odds ratio (OR) for biofilm formation in different container design materials with a 95% confidence interval (CI)

Characteristic	OR ¹	95% CI ¹	p-value
Container.Design Materials			
Jerry cans	—	—	
Mazzican	0.84	0.24, 2.70	0.8

¹OR = Odds Ratio, CI = Confidence Interval

Mazzicans (food-grade) and jerry cans (non-food-grade) from this study have similar abilities to form biofilms despite their difference in design material could be because biofilms are strongly influenced by surface roughness. Both mazzicans and jerry cans could have similar surface characteristics that can serve as sites for bacterial attachment and biofilm formation. Mazzicans had longer period of use than jerry cans suggesting that they could have developed microscopic scratches attributable to mechanical abrasion thus wearing out over time from time which increases bacterial adhesion making them equally susceptible to biofilm formation just like jerry cans. The frequent exposure of both jerry cans and mazzicans to similar contaminants such as raw milk and environment dust which is high in Kajiado could also mean that either of the containers design material if not cleaned properly or is reused without adequate cleaning, bacterial population would increase regardless of the material's food-grade nature. This could suggest that the longer the use of food-grade or non-food grade milk handling containers, the higher likelihood of those containers acting as inoculum for microorganisms and subsequently formation of biofilms (Myszka and Czaczyk, 2011). Therefore, there is need of proper container choice, thorough cleaning protocols and adherence to of food safety standards to avert risks of foodborne diseases and food losses. This agrees with a similar study that showed biofilms contribute to bacterial colonization in most surfaces (Parul *et al.*, 2019). Similarly, it has been reported that microbes colonize, multiply and yield resistance to high temperatures once they are converted into biofilms (Felipe *et al.*, 2017). Prolonged contact time in addition to other factors like nutrients and moisture similarly have been reported to contribute to biofilm formation (Myszka and Czaczyk, 2011). Food contact materials that are frequently used and with long contact times form better biofilm communities especially those with 'dead zones' (Giaouris and Samoes, 2018). Single use containers have also been shown to reduce microbial contamination (Fink *et al.*, 2017). However, in contrast with this study, it has been reported that attachment requires large number of cells and not all

cells get into contact with surface at the same time (Kimkes and Heinemann, 2020). A study contrasting these findings also showed that the length of bacterial survival in the environment depends on multiple factors and not only time among them the bacterial species and overall bioburden (Neely, 2000).

4.5 Microbial Multi-species of Biofilms in Containers, Water and Personnel Hands

The four microorganisms of interest in this study: *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Salmonella typhi* were identified using phenotypic methods through gram staining and confirmed through biochemical methods (Appendix V). The tests were conducted for container, water, and personnel hand swab samples. Of the seventy-six samples collected and analyzed for the four biofilm-forming microorganisms, fourteen were from mazzicans, twenty-five jerry cans, twenty-five personnel handling milk hand swabs, and twelve water samples (Table 9). Microbial counts for *Staphylococcus aureus*, *Bacillus cereus*, and *Pseudomonas aeruginosa* were high in all the samples. However, of all the samples analyzed no cases of *Salmonella typhi* were recorded. *Salmonella spp* presence could have meant a serious public health issue since its presence is a strong indication of fecal contamination which could have resulted to foodborne disease outbreak in the area. Microorganisms detected in this study included: *Staphylococcus aureus* (71.0%), *Bacillus cereus* (60.5%) and *Pseudomonas aeruginosa* (27.6 %) as per table 9 below (P = 0.7162).

Table 9: Prevalence of microorganisms in different samples.

Sample type and numbers	Positive Microorganism by type			
	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella typhi</i>
Mazzicans (14)	6(42%)	7(50%)	5(35.7%)	0
Jerry cans (25)	18(72%)	14(56%)	7(28%)	0
Hand swabs (25)	21(84%)	20(80%)	9(36%)	0
Water (12)	9(75%)	5(42%)	0	0
Total	54(71.0%)	46(60.5%)	21(27.6%)	0

The possible reason why the three microorganisms were identified could be because of the unhygienic conditions reported in personnel, water quality and container design material. This means these microorganisms could co-exist in the same environment without over-competing due to favorable environment for all. This signals a high risk of contamination, infection and resistance against cleaning regimes employed thus

require efforts to safeguard public health. This agrees with a similar study conducted in Greece that the most prevalent species identified was *Staphylococcus aureus* which was also dependent on seasonality gaps (Lianou *et al.*, 2023). *Bacillus cereus* varied widely from 2%-65.3% in dairy products and plants as reported in another study, majorly contributed by the level of unhygienic conditions, like findings of this study (Tirloni *et al.*, 2022). This agrees with another study, which established that this microorganism exists in diverse environments including water, air and soil and found to interact with *Staphylococcus aureus* (Li *et al.*, 2023) Besides, *P. aeruginosa* was reported in 52.17% of water samples in another study in China (Zhou *et al.*, 2023).

Staphylococcus aureus detected in hand swabs was 84%, water at 75%, jerry cans at 72% whereas mazzican was at 42%. *Bacillus cereus* detected in hand swabs was at 80%, jerry cans at 56%, mazzicans at 50% and water at 42%. *Pseudomonas aeruginosa* detected on hand swabs was at 36%, mazzicans at 35.7%, jerry cans at 28% with no water samples testing positive. The possible reason for having these three microorganisms in all the samples could be because of unhygienic handling practices employed by pastoral communities. This means that cross-contamination could occur, and microorganisms could co-exist for mutual benefits, resource partitioning and protection from environmental stress. This agrees with other studies that formation of multispecies bacterial biofilms is a common occurrence characterized by presence of *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* among other bacterial species (Durango *et al.*, 2004; Kandhai *et al.*, 2010). Similarly, Yao *et al.*, 2022 found out that microorganisms interact and communicate in a multispecies biofilm with varying survival mechanisms.

Microbial counts for *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* from data obtained from laboratory in this study were further analyzed using non-parametric methods following violations of parametric data. Kruskal-Wallis test showed a statistically significant difference in microbial counts among the container design material, personnel hand swabs, and water samples ($P < 0.001$). This suggests that personnel hygiene practices are likely to influence the microbial contamination levels in farm-level milk handling containers. Dunn's post-hoc test was performed following the significant Kruskal-Wallis test to explore differences in microbial counts

among the container, hand swab, and water samples. The results indicated no significant difference between the container and hand swab samples ($P = 1.000$). This could be because in pastoral communities, milk handlers often directly touch the containers (e.g., jerry cans, mazzicans) with their bare hands while milking or transporting milk. If hands are contaminated, they can easily transfer bacteria like to the containers, resulting in similar contamination levels. Besides, milk handling containers are reused multiple times without thorough cleaning, allowing bacteria to persist on the surfaces. When handlers touch these contaminated surfaces, they transfer bacteria back to their hands, creating a cycle of cross-contamination. However, significant differences were found between the container and water samples ($P < 0.001$) and between the hand swab and water samples ($P < 0.001$). This suggests that water samples have significantly higher microbial load compared to both containers and hand swabs. The trimmed mean ANOVA produced a test statistic of $F = 825.5742$ with a p-value of < 0.001 , indicating highly significant differences between groups. The effect size was substantial at 0.81, with a bootstrap confidence interval of (0.78; 0.86). Additionally, the median comparison has a test statistic of $F = 164.9672$ and a p-value < 0.001 , further confirming significant differences in medians across types. The bootstrap trimmed means test also showed a consistent test statistic of 825.5742 and a p-value < 0.001 , with 65.8% of the variance explained and an effect size of 0.811. Further robustness of data analysis was enhanced through the Monte Carlo Permutation Procedure (MCPB20) test which showed that the container design materials did not significantly differ from the personnel hand swabs ($P = 0.808$), while both the container design materials and personnel hand swabs showed significant differences from the water sample ($P < 0.001$). These results collectively suggest that water is significantly distinct from the other two sample types, while the container and hand swab are similar in their microbial counts. The possible reason for this could be because 97% of water used in the pastoral community of Kajiado were not treated before use and drawn from non-potable sources that were not protected. The exposure to the harsh environmental conditions in Kajiado County could be a key contributor of high bacterial load compared to that of containers and hand swabs. Besides, bacterial load in hand swabs compared to container design material was varying. This could mean that hand swabs were both contaminated from both water and the other environmental factors whereas containers were a bit enclosed from external environment.

The three biofilm producing microorganisms in this study: *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* were prevalent in the water, jerry cans, mazzicans and personnel hand swab samples were analyzed on their ability to form biofilms. The results of analysis showed that different samples had different prevalence rates for the four microorganisms. Of the 54 samples positive to *Staphylococcus aureus*, 37.0% had ability to form biofilms. Prevalence of biofilm-producing *Staphylococcus aureus* was highest in water samples at 55.6% followed by jerry cans at 38.8%, personnel hand swabs at 33.3% with the least being mazzicans at 16.6% (Table 10).

Table 10: Prevalence of biofilms for three microorganisms of interest

Sample type and numbers	Positive Biofilms by microbial mix type		
	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Pseudomonas aeruginosa</i>
Mazzicans	1 of 6(16.6%)	2 of 7(28.5%)	2 Of 5(40%)
Jerry cans	7 of 18(38.8%)	7 of 14(38.8%)	4 of 7(57.1%)
Hand swabs	7 of 21(33.3%)	9 of 20(33.3%)	4 of 9(44.4%)
Water	5 of 9(55.6%)	2 of 5(55.6%)	0

These findings showed that water quality and personnel hygiene are some of the confounding factors (covariates) that determine formation of biofilms in different container design materials. Of the 46 samples positive to *Bacillus cereus*, 43.5% had the ability to form biofilms. Prevalence of biofilm-forming *Bacillus cereus* in jerry cans was at 50%, personnel hand swabs at 45%, water samples at 40% and mazzicans at 28.5% (P= 0.878). Of the 21 samples positive to *Pseudomonas aeruginosa*, 46.6% had the ability to form biofilms. Prevalence of biofilm-forming *Pseudomonas aeruginosa* in jerry cans was at 57.1%, personnel hand swabs at 44.4%, mazzicans at 40% with water samples showing no biofilm-producing *Pseudomonas aeruginosa* (P = 0.186).

Comparing the three microorganisms identified, it shows that *Pseudomonas aeruginosa* has ability to form biofilms at 46.6%, *Bacillus cereus* at 43.5% and *Staphylococcus aureus* at 37.0% (P = 0.540). Unhygienic conditions in handling milk in pastoral community of Kajiado could be the reason why all the three microorganisms were present. Conducive environment for the three microorganisms to thrive and co-exist together resulted from the unhygienic practices employed by farmers. This means that hygienic conditions should be observed regardless of the microbial types since

microbial mix in biofilms manifest in a similar fashion. In agreement with this study that mixed microbial community exists, complexity of biofilm formation is based on varying factors including environmental conditions, roughness of surfaces, hydrophobicity, surface charge, temperature, and presence of substrates, several other studies have reported similar findings (Anand *et al.*, 2014; Makovcova *et al.*, 2017; Tang *et al.*, 2011; Yuan *et al.*, 2018; Yuan *et al.*, 2020). Additionally, it has been reported that mixed biofilms result into enhanced growth and resistance to disinfectants (Habimana *et al.*, 2010). Similarly, and in agreement with this study, *Bacillus cereus* biofilms have been reported to associate with other microorganisms (Majed *et al.*, 2016).

Biofilms were further categorized into weak, moderate and strong based on their ability to stick to polystyrene tubes with visible marks after washing with dye (Appendix V). Even when weak biofilms are detected by agar plating methods, stringent control measures need to be taken to mitigate since this may be a serious public health concern. It has been shown also that some bacteria exhibit viable but non-culturable phenomenon and therefore weak biofilms may not necessarily mean that they cannot contribute to aggravation of spoilage, pathogenesis and dispersion of mature biofilms (Zhao *et al.*, 2017; Yan *et al.*, 2021). From the findings, it is evident that biofilms are highly heterogeneous and complex, since variations by type, microbial counts and species was recorded. This agrees with past similar study (Fernández-Gómez *et al.*, 2021).

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

Dairy industry has faced several challenges in ensuring food safety. In this study, focus was on milk-handling practices at the farm level in rural Kenya. It is estimated that 65-80% of all infections are biofilm related and that most microbes exist in form of biofilms. Biofilm-forming bacteria are major sources of both pathogenic and spoilage microorganisms in the dairy industry. These include pathogenic microorganisms namely *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica enteritidis* and Typhimurium serotypes whereas spoilage microorganisms include *Pseudomonas aeruginosa*. Biofilms affect the safety and quality of milk and milk products. Despite the stringent hygiene protocols in dairy production, biofilm formation in milk handling containers remains a significant challenge. Biofilms, which are microbial communities attached to surfaces, pose serious risks as they can harbor spoilage and pathogenic bacteria. Biofilms are difficult to remove once formed leading to contamination of milk products thus compromising on food safety and public health.

Several factors: design material, cleaning regimes, quality of water and hygiene practices of personnel handling the containers can influence biofilm formation. The influence of these factors on biofilm formation in farm level milk handling containers is not fully known. This research sought to investigate the influence of container design material, cleaning procedures, water quality and personnel hygiene on biofilm formation in farm level milk handling containers. The objectives of this study included: to assess the effect of cleaning regime on biofilm formation in farm level milk handling containers, to determine the influence of microbiological water quality on biofilm formation in farm level milk handling containers, to determine the influence of personnel hygiene on biofilm formation in farm level milk handling containers, and to determine the influence of container design material of farm level milk handling containers on biofilm formation.

The study was done in Kajiado East Sub County, Kajiado County in Poka-Kenyewa ward in four different areas of Masimba, Sultan Hamud, Illamirror and Oltinka. Research design employed completely randomized design (CRD) with a 2x2 factorial

arrangement was used in this study. Independent variables in this study included: container design material, cleaning regime, water quality and personnel hygiene. The independent variable, container design material had two levels analysed: mazzicans and jerry cans. For the independent variable cleaning regime, survey identified the most common cleaning practices among farmers, and these were used to evaluate their effectiveness across container types. A total of 39 swabs were collected from 39 containers (14 mazzicans and 25 jerry cans), with microbial analyses conducted in triplicate for precision.

The data was analyzed using R Core Team software version 4.4.0 of 2024 with application of various statistical tests. Microbiological data were first transformed (\log_{10} cfu/ml) before analysis. Microbial counts continuous data were analyzed using non-classical ANOVA using Kruskal-Wallis test followed by Dunn's post hoc tests. Logistic regression and Chi square test of independence tests were also employed. Robustness of the data was enhanced by conducting Fisher's Exact text, permutation and bootstrapping methods.

Findings of this study revealed that biofilm formation in farm-level milk handling containers is determined by the cleaning regime, among other factors. Liquid detergent was found to be a contributor to less biofilm formation as compared with bar soap. There was also reported association between water quality and biofilm formation. This means that the microbiological water quality of water in Kajiado is a contributor of biofilm formation. Findings of this study also shown that personnel hands' hygiene is associated with bacterial biofilm formation. Bacterial multispecies comprising of *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* were reported to co-exist in biofilms formed. Mazzicans and jerry cans both reported abilities to form biofilms. The study also reported that mazzicans (food-grade) and jerry cans (non-food-grade) have similar abilities to form biofilms despite their difference in design material.

5.2 Conclusion

From the above findings, biofilm formation is contributed by multiple factors ranging from poor hand hygiene, frequent contact with contaminated surfaces, and lack of use of disinfectants by personnel handling food. This therefore means that biofilm is a

potential source of spoilage of milk products in Kajiado County thus a contributor of food waste and loss leading to economic losses. Additionally, biofilm formation contributes to public health issues through foodborne illnesses caused by lack of adherence to food safety standards in milk handling in Kajiado.

5.3 Recommendation of Study

- i. It is recommended that cleaning regime for farm-level milk handling containers used by smallholder farmers to be standardized since it's a contributing factor to proliferation of biofilms.
- ii. Water quality and personnel hygiene are also risk factors towards biofilm formation thus need to observe good hygienic practices when handling milk.
- iii. Need to observe period of use for containers regardless of the container design material. Long use of containers may be a contributing factor to biofilm formation.
- iv. An advisory opinion is also given to evaluate the existing policy and regulatory frameworks governing milk production and handling in Kajiado county to identify gaps and suggest improvements to ensure stringent adherence to hygiene standards.

5.4 Suggestions for Further Studies

- i. It is suggested that future long-term studies to observe the changes in biofilm formation in different container types over different seasons and weather conditions under advanced cleaning technologies. This will help understand how external factors such as temperature and humidity impact biofilm formation.
- ii. Further studies need to be conducted to understand other factors beyond this study that lead to biofilm formation in milk handling equipment since biofilms are complex.

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APPENDICES

Appendix I: Smallholder farmers survey questionnaire

HOUSEHOLD QUESTIONNAIRE FOR SMALL SCALE MILK PRODUCERS IN KAJIADO COUNTY.

Introduction

Am a student of Chuka University pursuing MSC in Food Science and Technology. Am carrying out research on “Influence of container design material, cleaning regime, water quality and personnel hygiene on biofilm formation on farm-level milk handling containers in Kajiado County, Kenya”. The content of the respondent is confidential and will only be used for academic purposes.

Consent-Yes/No

Part 1: Socio-demographic characteristics

Date	
Name of Interviewer	
ID of Questionnaire	
County	
Sub-County	
Village	
Name of group	
Name of interviewee/Unique identifier (optional)	
Sex- Male or Female (M, F)	

GPRS Coordinates

Part 2: Social economic status

2.1. What is your highest level of education

a, Preschool, b, primary school, c, secondary school, d, tertiary college/University

2.2. What is your marital status i, Married, ii, Single, iii, Divorced iv, Widow(er)

Do you own livestock

(Make observations also in the farm)

a) Yes b) No

2.3. How many animals are in lactation in the current season

a) Five or more b) Three or more c) more than one d) None

2.4. Are your dairy cows housed? YES/NO.

If YES, which type of house

	Yes/No
Brick walled	
Poles with roofs	
Poles with no roof	
Open field	
Others, specify	

2.5. What type of breeds do you mainly rear i, Indigenous, ii, cross-breeds iii, Exotic breeds, iv, Others, specify.

Part 3: Knowledge, Attitude and practices assessment

Which milk handling container(s) do you mainly use for milking, milk storage and transportation?

	Container	Yes	No	Number of containers	Duration of use?	How often do you clean container after use?
3.1	Aluminum					
3.2	Stainless steel					
3.3	Jerry cans					
3.4	Mazzicans					
3.5	Gourds					
3.6	Others, specify					

a) Do you have a milking shed? YES/NO

b) How regular do you clean your milking shed, i, Daily, ii, weekly, iii, Bi-weekly iv, Monthly v, others—specify

5.a) Do you clean hands before milking your herd? i, YES, ii, NO iii, Sometime.

If YES/Sometimes, what to you use to clean your hands i, Cold water only, ii, warm water only, iii, water and soap, iv, water soap and milking cloth v, disinfectant vi, Others, specify.

b) Do you use pre-milking products YES/NO. If Yes which ones i, milking jerry ii, home-made lubricants, iii, Others, specify.

c) Do you use post-milking products YES/NO. If Yes which ones i, milking jerry ii, home-made lubricants, iii, Others, specify.

d) Do you use a cloth for cleaning udder before milking YES/NO. If yes how long do you use without changing to a new one i, 1 week, ii, 2 weeks, iii, 1 month, iv, others—specify

e) How do you ensure maintain hygiene of the cloth i, cleaning with cold water only, ii, cleaning with water and soap iii, cleaning with water and disinfecting iv, others—specify

f) How long do you take before washing milking equipment/containers/cloths after emptying, i, Immediately, ii, within 1 hour, iii, 2-3 hours iv, 4-6 hours, v, Others, specify

6.a) How do you detect infected udder with mastitis i, have testing kit ii, pour milk on black surface iii, observe for possible clots iv, others, specify

b) Do you segregate spoilt milk with mastitis or other diseases. YES/NO
If NO, how do you segregate i, feeding young ones, ii, blending with good quality milk
ii, others, specify

c) In the last one month has any of your milking herd been infected with mastitis, YES/NO

7.a) What is the common mode of transporting your milk for marketing?

1=use motor bikes 2=use public means of transport/matatu, 3=trekking,
4=others, specify)

a) 1 b) 2 c) 3 d) 4

b) Does the milk transportation system observe refrigeration- YES/NO,
If YES, state the refrigeration method

8.a) Milk storage containers: How long do you store evening fresh raw milk before consuming or selling? i, between 4-6 hours, ii, 6-8 hours iii, over 12 hours

b) For the above, do you mix with morning milk? YES/NO.

If YES, do you empty container for cleaning prior to mixing? YES/NO

9. How can you recognize milk handling container that is not adequately cleaned?

(Leave the question open for the farmer to answer. Then tick the box with the given answer or note down the given answer).

- a) Presence of milk traces
- b) Presence of foreign material
- c) Presence of smell/odour
- d) Presence of cleaning detergents
- e) Don't know
- f) Other:

10. What are the detergents/disinfectants you commonly use to clean/sanitize the above containers?

- a) Herbs
- b) bar soap
- c) Liquid detergents
- d) No detergents/only water
- e) Other:

Specify _____

11. What are the temperatures of the medium used in cleaning for each of the types of containers mentioned above?

(Leave the question open for the farmer to answer. Then tick the box with the given answer or note down the given answer).

Container type	Temperatures against each of the container types
Aluminium	
Stainless steel	
Plastic jerry cans	
Mazzicans	
Gourds	
Others, specify	

- a) Room temperature (16-25⁰C)
 - b) cold temperatures (1-15⁰C)
 - c) Lukewarm temperatures (30-40⁰C)
 - d) Hot temperatures (above 40⁰C)
 - e) Don't know
 - f) Other:
-

12. Do you normally use mechanical scourer to clean your containers?

- a) Yes (If yes which ones)
- b) No

Container type	Time for scouring (for each of the container types)	
Aluminium	Less than 60 seconds	
Stainless steel	1-2 minutes	
Plastic jerry cans	3-5 minutes	
Mazzicans	5-10 minutes	
Gourds	Others, specify	
Others, specify		

13. How long do you normally use the container before replacing?

- a) 1 week
- b) 2 weeks
- c) 1 month
- d) 2 months
- e) 3 months
- f) 4 months
- g) Others, specify-

Container type	Duration of use for each of the container types
Aluminium	
Stainless steel	
Plastic jerry cans	
Mazzicans	
Gourds	
Others, specify	

14. Where do you normally source water for cleaning your containers?

- a) Rainwater
- b) Borehole
- c) municipal water
- d) Water pan/dam
- e) Spring
- f) Other: _____

15. What is the approximate distance between your home to the nearest milk marketing point?

16. What time do you take to market product from the nearest market point?

Information sharing

17.a) Where do you regularly get information on dairy farming, clean milk production, hygiene and milk quality?

i, radio spots, ii, agricultural extension officers, iii, local NGOs, iv, peer farmer groups, v, Chief barazas vi, others, specify

b) How often do you receive the above information?

i, daily, ii, weekly, iii, Fortnight iv, Monthly v, others, specify-----

c) In the last one month, have you accessed any information about the above topics?

Yes/No

General observations and remarks from the interviewer-----

End.

Do you have any questions?-----

Thank the interviewee

Appendix II: Data Analysis

Data Analysis

Influence of container design material, cleaning regime, water quality and personnel hygiene on biofilm formation in farm-level milk containers in Kajiado County, Kenya
Setting the working directory

```
setwd("E:/Phineas/Data")
```

Objective 1. To assess the effect of cleaning regime on biofilm formation in farm level milk handling containers

The Chi-Square test is specifically designed for categorical data (e.g., cleaning regime and biofilm formation). Both the cleaning regime and biofilm formation are categorical variables (eg "yes = 1" or "no = 0" for biofilm formation).

Objective is assessing independence - whether there is a significant association between two categorical variables. So to to determine if there is an association between the cleaning regime and the presence of biofilm, is best suited for a Chi-Square test.

Loading libraries

```
library(dplyr)
```

```
##
```

```
## Attaching package: 'dplyr'
```

```
## The following objects are masked from 'package:stats':
```

```
##
```

```
## filter, lag
```

```
## The following objects are masked from 'package:base':
```

```
##
```

```
## intersect, setdiff, setequal, union
```

```
library(ggplot2)
```

```
library(gmodels)
```

```
library(car)
```

```
## Loading required package: carData
```

```
##
```

```
## Attaching package: 'car'
```

```
## The following object is masked from 'package:dplyr':
```

```
##
```

```
## recode
```

```
library(emmeans)
```

Reading the data

```
# Loading the data from CSV
```

```
dataphineas <- read.csv("CleaningRegimeData.csv")
```

```
# Viewing the first six rows of the data
```

```
head(dataphineas)
```

```
##           Cleaning.Regime Biofilm.Formation Species
```

```
## 1 Bar soap at room temp 16 to 25 deg.C      1 Staphy aureus
```

```
## 2 Bar soap at room temp 16 to 25 deg.C 1 Staphy aureus
## 3 Liquid detergent at room temp 16 to 25 deg.C 0 Staphy aureus
## 4 Bar soap at room temp 16 to 25 deg.C 1 Staphy aureus
## 5 Liquid detergent at room temp 16 to 25 deg.C 0 Staphy aureus
## 6 Bar soap at room temp 16 to 25 deg.C 0 Staphy aureus
```

Data exploration

Checking the column names

```
colnames(dataphineas)
```

```
## [1] "Cleaning.Regime" "Biofilm.Formation" "Species"
```

Cleaning and summary stats

```
# Cleaning any spaces or special characters
```

```
dataphineas <- dataphineas %>%
```

```
  rename(
```

```
    Cleaning_Regime = Cleaning.Regime,
```

```
    Biofilm_Formation = Biofilm.Formation
```

```
)
```

```
# Summary stats
```

```
summary(dataphineas)
```

```
## Cleaning_Regime Biofilm_Formation Species
```

```
## Length:54 Min. :0.0000 Length:54
```

```
## Class :character 1st Qu.:0.0000 Class :character
```

```
## Mode :character Median :0.0000 Mode :character
```

```
## Mean :0.3704
```

```
## 3rd Qu.:1.0000
```

```
## Max. :1.0000
```

Checking missingness

```
# Checking for missing values
```

```
sum(is.na(dataphineas))
```

```
## [1] 0
```

Frequency table

```
# Freq table for cleaning regime vs biofilm formation
```

```
(table_cleaning_biofilm <- table(dataphineas$Cleaning_Regime,
```

```
dataphineas$Biofilm_Formation))
```

```
##
```

```
##           0 1
```

```
## Bar soap at room temp 16 to 25 deg.C 2 17
```

```
## Liquid detergent at room temp 16 to 25 deg.C 3 2
```

Containers cleaned with bar soap at room temperature (16 to 25°C) showed a higher incidence of biofilm formation (17 occurrences) compared to those cleaned with liquid detergent at the same temperature (3 occurrences). Specifically, 17 out of 19 samples cleaned with bar soap had biofilm formation, whereas only 3 out of 35 samples cleaned with liquid detergent exhibited biofilm.

The higher biofilm formation with bar soap suggests it is less effective at preventing microbial buildup compared to liquid detergent. To enhance hygiene in milk handling, recommended using liquid detergents, which are more effective at removing residues and preventing biofilm

Chi-Square test

```
# Chi-Square test of independence for cleaning regime and biofilm formation
```

```
(chisq.test(table_cleaning_biofilm))
```

```
##
```

```
## Pearson's Chi-squared test with Yates' continuity correction
```

```
##
```

```
## data: table_cleaning_biofilm
```

```
## X-squared = 31.182, df = 1, p-value = 2.349e-08
```

The test results show a Pearson's Chi-squared statistic of 31.182 with 1 degree of freedom, and a p-value of <0.001.

The extremely low p-value indicates a highly significant association between the cleaning regime and biofilm formation. This means the type of cleaning agent used (bar soap vs. liquid detergent) significantly affects the level of biofilm formation on milk handling containers. The results suggest that the cleaning effectiveness is not equal across different cleaning regimes, reinforcing the need for improved cleaning protocols using more effective detergents. Think of the policies to enforce e.

```
screenreg(logistic_phineas, digits = 2, title = "Regression Results", caption = "Source: retail dataset")
```

```
##
```

```
##
```

```
=====
```

```
##                               Model 1
## -----
## (Intercept)                    2.14 **
##                               (0.75)
## Cleaning_RegimeLiquid detergent at room temp 16 to 25 deg.C -4.51 ***
##                               (0.96)
## -----
## AIC                             37.26
## BIC                             41.24
## Log Likelihood                   -16.63
## Deviance                         33.26
## Num. obs.                         54
##
```

```
=====
```

```
## *** p < 0.001; ** p < 0.01; * p < 0.05
```

```
library(gtsummary)
```

```
## Warning: package 'gtsummary' was built under R version 4.4.1
phineasmod1 <- glm(Biofilm_Formation ~ Cleaning_Regime, data = dataphineas, family =
binomial)
```

```
(t1 <- tbl_regression(phineasmod1, exponentiate = TRUE))
## Table printed with {flextable}, not {gt}. Learn why at
## https://www.danieldsjoberg.com/gtsummary/articles/rmarkdown.html
## To suppress this message, include `message = FALSE` in the code chunk header.
```

Characteristic	OR ¹	95% CI ¹	p-value
Cleaning_Regime			
Bar soap at room temp 16 to 25 deg.C	—	—	
Liquid detergent at room temp 16 to 25 deg.C	0.01	0.00, 0.06	<0.001

¹OR = Odds Ratio, CI = Confidence Interval

The odds ratio (OR) for Liquid detergent is 0.01, with a 95% confidence interval (CI) ranging from 0.00 to 0.06, and a p-value less than 0.001. This indicates that using liquid detergent significantly reduces the likelihood of biofilm formation compared to the bar soap regime. The very low odds ratio and the confidence interval not including 1, combined with the extremely low p-value, strongly suggest that liquid detergent is highly effective in preventing biofilm formation.

Predicted probabilities - assessing model fit

```
dataphineas$predicted_prob <- predict(logistic_phineas, type = "response")
```

```
head(dataphineas$predicted_prob)
```

```
## [1] 0.89473684 0.89473684 0.08571429 0.89473684 0.08571429 0.89473684
```

Post hoc analysis

```
emmeansphineas <- emmeans(logistic_phineas, pairwise ~ Cleaning_Regime, type =
"response")
```

```
print(emmeansphineas)
```

```
## $emmeans
```

```
## Cleaning_Regime          prob    SE df asymp.LCL
```

```
## Bar soap at room temp 16 to 25 deg.C    0.8947 0.0704 Inf    0.6626
```

```
## Liquid detergent at room temp 16 to 25 deg.C 0.0857 0.0473 Inf    0.0279
```

```
## asymp.UCL
```

```
##    0.974
```

```
##    0.234
```

```
##
```

```
## Confidence level used: 0.95
```

```
## Intervals are back-transformed from the logit scale
```

```
##
```

```
## $contrasts
```

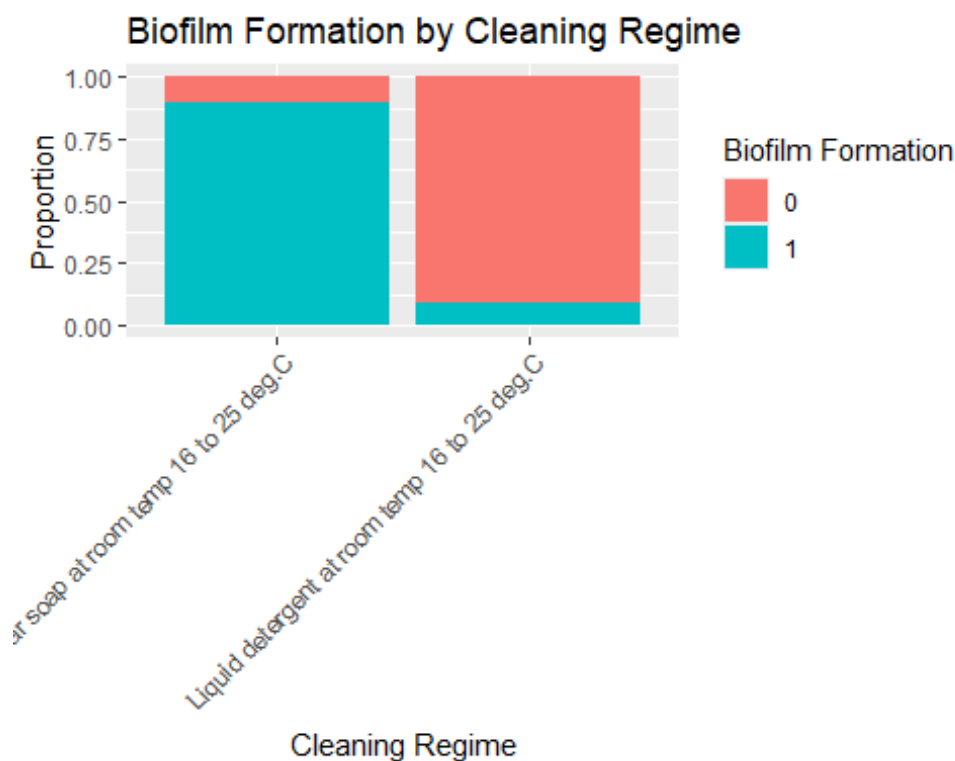
```
## contrast
```

```
## Bar soap at room temp 16 to 25 deg.C / Liquid detergent at room temp 16 to 25
deg.C
```

```
## odds.ratio SE df null z.ratio p.value
## 90.7 87.1 Inf 1 4.690 <.0001
##
```

Tests are performed on the log odds ratio scale

The probability of biofilm formation is significantly higher when using Bar soap at room temperature (16 to 25 deg.C), with a predicted probability of 0.8947 (SE = 0.0704), compared to only 0.0857 (SE = 0.0473) for liquid detergent at room temperature (16 to 25 deg.C). The odds ratio of 90.7 suggests that the odds of biofilm formation are approximately 90 times higher when using Bar soap compared to liquid detergent, with a highly significant p-value of <0.0001. This strong contrast reinforces the conclusion that the choice of cleaning regime has a profound impact on the likelihood of biofilm formation, highlighting the effectiveness of liquid detergent in reducing biofilm risk.



Objective 2. To determine the influence of microbiological water quality on the biofilm formation in farm level milk handling containers

Libraries, recalling directory, and loading data

```
# Loading libs
library(dplyr)
library(ggplot2)
library(emmeans)
library(car)
```

```
# Setting working directory
setwd("E:/Phineas/Data")
```

```

# Loading data
dataphineas2 <- read.csv("MicrobialWQ.csv")

# Viewing the first 6 recs
head(dataphineas2)
## Sample Species Biofilm.Formation
## 1 W Staph aureus 0
## 2 W Staph aureus 0
## 3 W Staph aureus 1
## 4 W Staph aureus 0
## 5 W Staph aureus 0
## 6 W Staph aureus 0
Preparing the data
# Cleaning the data - handling missing values
dataphineas2 <- dataphineas2 %>%
  filter(!is.na(Biofilm.Formation))

# Renaming columns for easier access
dataphineas2 <- dataphineas2 %>%
  rename(
    Biofilm_Formation = Biofilm.Formation
  )

# Converting biofilm formation to a binary factor
dataphineas2$Biofilm_Formation <- as.factor(dataphineas2$Biofilm_Formation)
Chi-Square test of independence for sample and biofilm formation
(table_sample_biofilm <- table(dataphineas2$Sample, dataphineas2$Biofilm_Formation))
##
## 0 1
## H 27 17
## W 16 2
(chisq_test_sample_biofilm <- chisq.test(table_sample_biofilm))
##
## Pearson's Chi-squared test with Yates' continuity correction
##
## data: table_sample_biofilm
## X-squared = 3.3506, df = 1, p-value = 0.06718
A Chi-Square test of independence was done to examine the relationship between
sample type (H and W) and biofilm formation. The analysis has a Chi-Squared stat of
3.3506 with 1 degree of freedom and a p-value of 0.06718, indicating no statistically
significant association at the 0.05 level, although the result is close to this threshold.

```

The results imply that while the association between sample type and biofilm formation is not statistically significant (or rather loosely, is marginally significant), the observed values suggest a possible link, particularly with a higher proportion of biofilm formation in the H samples compared to the W samples. Given that the p-value is near the significance level, it may be beneficial to conduct additional analyses, such as Fisher's Exact Test, especially considering the relatively small sample size in the W category.

A p-value of 0.03792 indicates a statistically significant association at the 0.05 level. The estimated odds ratio is 0.203, suggesting that biofilm formation is less likely in W samples compared to H samples. The 95% confidence interval for the odds ratio ranges from 0.0202 to 1.0422.

The significant p-value indicates that there is a meaningful association between sample type and biofilm formation. The odds ratio of 0.203 suggests that the odds of biofilm formation in W samples are significantly lower than in H samples. Specifically, for every one unit of biofilm formation in H samples, there are approximately 0.203 units in W samples. The significant p-value (0.03792) confirms a meaningful association, indicating that (maybe) environmental factors related to H samples may promote increased biofilm development

Logistic regression model

```
logistic_phineas2 <- glm(Biofilm_Formation ~ Sample, data = dataphineas2, family =
binomial)
summary(logistic_phineas2)
##
## Call:
## glm(formula = Biofilm_Formation ~ Sample, family = binomial,
## data = dataphineas2)
##
## Coefficients:
## Estimate Std. Error z value Pr(>|z|)
## (Intercept) -0.4626 0.3096 -1.494 0.1351
## SampleW -1.6168 0.8114 -1.993 0.0463 *
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
## Null deviance: 76.413 on 61 degrees of freedom
## Residual deviance: 71.262 on 60 degrees of freedom
## AIC: 75.262
```

```
##
```

```
## Number of Fisher Scoring iterations: 4
```

The coefficient for the Sample W is -1.6168, with a standard error of 0.8114 and a z-value of -1.993, resulting in a p-value of 0.0463. This indicates that sample type W is significantly associated with lower odds of biofilm formation compared to sample type H at the 0.05 significance level. The intercept coefficient is -0.4626, but it is not statistically significant ($p = 0.1351$).

The negative coefficient for Sample W suggests that being in the W group decreases the log-odds of biofilm formation compared to the H group. Specifically, the odds of biofilm formation are significantly lower in W samples, aligning with the Fisher's Exact Test.

```
(t1 <- tbl_regression(phineasmod2, exponentiate = TRUE))
```

```
## Table printed with {flextable}, not {gt}. Learn why at
```

```
## https://www.danielsjoberg.com/gtsummary/articles/rmarkdown.html
```

```
## To suppress this message, include `message = FALSE` in the code chunk header.
```

Characteristic	OR ¹	95% CI ¹	p-value
Sample			
H	—	—	
W	0.20	0.03, 0.82	0.046

¹OR = Odds Ratio, CI = Confidence Interval

The odds ratio (OR) for biofilm formation in W compared to H is 0.20, with a 95% confidence interval (CI) of 0.03 to 0.82. The p-value associated with this odds ratio is 0.046, statistically significant association at the 0.05 level. An odds ratio of 0.20 implies that the odds of biofilm formation in W are significantly lower than in H samples; - indicates that W samples have only 20% of the odds of forming biofilm compared to H samples.

Objective 3. To determine the influence of personnel hygiene on the biofilm formation in farm level milk handling containers

Libs

```
if(!require(car))install.packages("car")
if(!require(ggplot2))install.packages("ggplot2")
if(!require(reshape))install.packages("reshape")
if(!require(granova))install.packages("granova")
if(!require(pastecs))install.packages("pastecs")
if(!require(multcomp))install.packages("multcomp")
if(!require(compute.es))install.packages("compute.es")
if(!require(WRS2))install.packages("WRS2")
if(!require(multcompView))install.packages("multcompView")
if(!require(fastDummies))install.packages("fastDummies")
if(!require(dplyr))install.packages("dplyr")
if(!require(flextable))install.packages("flextable")
```

Data and summary

```

### Loading the data
data <- read.csv("MicrobialCountData.csv")

# Viewing the first 6 rows of the dataset
head(data)
## Sample Type Microbial.Count Species
## 1 J1 Container 2.600973 Staph aureus
## 2 J2 Container 2.171434 Staph aureus
## 3 J3 Container 2.674402 Staph aureus
## 4 J4 Container 2.139249 Staph aureus
## 5 J5 Container 2.689131 Staph aureus
## 6 J6 Container 2.210158 Staph aureus
# Summary stats
summary(data)
## Sample Type Microbial.Count Species
## Length:115 Length:115 Min. :1.000 Length:115
## Class :character Class :character 1st Qu.:2.247 Class :character
## Mode :character Mode :character Median :2.557 Mode :character
## Mean :2.681
## 3rd Qu.:2.777
## Max. :6.477
# Checking for missing values
sapply(data, function(x) sum(is.na(x)))
## Sample Type Microbial.Count Species
## 0 0 0 0
Descriptive stats
library(reshape2)
##
## Attaching package: 'reshape2'
## The following objects are masked from 'package:reshape':
##
## colsplit, melt, recast
dataphineas3_melted <- melt(dataphineas3,
id.vars = c("Sample",
"Type",
"Species"))
library(pastecs)
descriptive_stats <- by(dataphineas3_melted$value,
dataphineas3_melted$Type, stat.desc)

print(descriptive_stats)
## dataphineas3_melted$Type: Container
## nbr.val nbr.null nbr.na min max range

```

```

## 56.00000000 0.00000000 0.00000000 2.01283723 2.99930457 0.98646735
##      sum      median      mean    SE.mean CI.mean.0.95      var
## 139.17428987 2.50335279 2.48525518 0.03733256 0.07481612 0.07804832
##   std.dev   coef.var
## 0.27937130 0.11241152
## -----
## dataphineas3_melted$Type: Hand Swab
##   nbr.val  nbr.null  nbr.na    min     max    range
## 49.00000000 0.00000000 0.00000000 2.01283723 3.32221930 1.30938207
##      sum      median      mean    SE.mean CI.mean.0.95      var
## 123.07009574 2.55690527 2.51163461 0.04419458 0.08885916 0.09570488
##   std.dev   coef.var
## 0.30936206 0.12317160
## -----
## dataphineas3_melted$Type: Water Sample
##   nbr.val  nbr.null  nbr.na    min     max    range
## 10.00000000 0.00000000 0.00000000 4.39794001 4.94939001 0.55145000
##      sum      median      mean    SE.mean CI.mean.0.95      var
## 46.73858880 4.64541354 4.67385888 0.04772621 0.10796419 0.02277791
##   std.dev   coef.var
## 0.15092353 0.03229099

```

The Container group has a mean microbial count of 2.485, with a standard dev of 0.279, - moderate variability, while the Hand Swab group shows a slightly higher mean of 2.512 and greater variability (sd of 0.309). In contrast, the Water Sample group exhibits the highest mean count at 4.674, but a lower std dev of 0.151, suggesting less variability among its values.

```

# Levene's test for homogeneity of variance
(levene_test <- leveneTest(Microbial.Count ~ Type,
  data = dataphineas3))
## Warning in leveneTest.default(y = y, group = group, ...): group coerced to
## factor.
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 2 3.5272 0.03269 *
##      112
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

Both assumptions are violated. So we transform the data, test again, and should they be violated, run a non parametric version of analysis of variance.

Log transformation

```

# Adding a small constant (0.001) to avoid log(0)
dataphineas3_melted$log_value <- log(dataphineas3_melted$value
  + 0.001, base = 10)

```

```

# Removing rows with zero or negative values (if needed)
dataphineas3_melted <- dataphineas3_melted[dataphineas3_melted$value > 0, ]

head(dataphineas3_melted)
## Sample   Type   Species   variable   value log_value
## 1    J1 Container Staph aureus Microbial.Count 2.600973 0.4153028
## 2    J2 Container Staph aureus Microbial.Count 2.171434 0.3369466
## 3    J3 Container Staph aureus Microbial.Count 2.674402 0.4273890
## 4    J4 Container Staph aureus Microbial.Count 2.139249 0.3304643
## 5    J5 Container Staph aureus Microbial.Count 2.689131 0.4297735
## 6    J6 Container Staph aureus Microbial.Count 2.210158 0.3446198
Exploring assumptions after log transformation
# Shapiro-Wilk test for normality
(shapiro_test_log <- shapiro.test(dataphineas3_melted$log_value))
##
## Shapiro-Wilk normality test
##
## data: dataphineas3_melted$log_value
## W = 0.82446, p-value = 2.209e-10
# Levene's test for homogeneity of variance after log transformation
(levene_test_log <- leveneTest(log_value ~ Type,
                               data = dataphineas3_melted))
## Warning in leveneTest.default(y = y, group = group, ...): group coerced to
## factor.
## Levene's Test for Homogeneity of Variance (center = median)
##   Df F value Pr(>F)
## group  2  6.3351 0.002474 **
##   112
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Before transformations, the assumptions of normality and homogeneity of variance
were violated, as indicated by the Shapiro-Wilk test ( $W = 0.71797$ ,  $p < 0.001$ )
showing significant non-normality in microbial counts, and Levene's test ( $F = 3.5272$ ,
 $p = 0.03269$ ) showing significant differences in variances across container types.
After applying log transformation, normality remained an issue ( $W = 0.82446$ ,  $p <
0.001$ ), and Levene's test continued to show significant variance differences ( $F =
6.3351$ ,  $p = 0.002474$ ). Although the Box-Cox transformation aimed to stabilize
variance, it still failed to achieve normality ( $W = 0.62419$ ,  $p < 0.001$ ) while indicating
homogeneity of variance ( $F = 0.7553$ ,  $p = 0.4722$ ) post-transformation.
Assumptions still violated after transformations.
Decision: We will not explore the classical ANOVA, but rather non-parametric tests,
and robust versions
### Kruskal-Wallis test

```

```

kruskal_test <- kruskal.test(value ~ Type,
                             data = dataphineas3_melted)

print(kruskal_test)
##
## Kruskal-Wallis rank sum test
##
## data: value by Type
## Kruskal-Wallis chi-squared = 27.273, df = 2, p-value = 1.196e-06
Kruskal-Wallis chi-squared value of 27.273 with 2 df and a p-value of <0.001 - a
statistically significant difference in microbial counts among the container, hand swab,
and water samples; so personnel hygiene practices likely influence the microbial
contamination levels in farm-level milk handling containers.
Post hoc for Kruskal
library(dunn.test)

# Performing Dunn's test for post-hoc analysis
dunn_phineas <- dunn.test(dataphineas3_melted$value,
                          dataphineas3_melted$Type,
                          method = "bonferroni") # Bonf' correction
## Kruskal-Wallis rank sum test
##
## data: x and group
## Kruskal-Wallis chi-squared = 27.2733, df = 2, p-value = 0
##
##
## Comparison of x by group
## (Bonferroni)
## Col Mean-|
## Row Mean | Containe Hand Swa
## -----+-----
## Hand Swa | -0.337378
## | 1.0000
## |
## Water Sa | -5.113564 -4.868913
## | 0.0000* 0.0000*
##
## alpha = 0.05
## Reject Ho if p <= alpha/2
print(dunn_phineas)
## $chi2
## [1] 27.27328
##

```

```
## $Z
## [1] -0.3373785 -5.1135640 -4.8689130
##
## $P
## [1] 3.679158e-01 1.580683e-07 5.610689e-07
##
## $P.adjusted
## [1] 1.000000e+00 4.742048e-07 1.683207e-06
##
## $comparisons
## [1] "Container - Hand Swab" "Container - Water Sample"
## [3] "Hand Swab - Water Sample"
```

Dunn's post-hoc test was performed following the significant Kruskal-Wallis test to explore differences in microbial counts among the container, hand swab, and water samples. The results indicated no significant difference between the container and hand swab samples ($p = 1.000$). However, significant differences were found between the container and water samples ($p < 0.001$) and between the hand swab and water samples ($p < 0.001$). This suggests that water samples have significantly higher microbial contamination compared to both containers and hand swabs.

Robust methods - selected

Trimmed mean

```
# Trimmed means ANOVA
```

```
trimmed_phineas <- t1way(value ~ Type,
                        data = dataphineas3_melted,
                        tr = 0.2)
```

```
print(trimmed_phineas)
```

```
## Call:
```

```
## t1way(formula = value ~ Type, data = dataphineas3_melted, tr = 0.2)
```

```
##
```

```
## Test statistic: F = 825.5742
```

```
## Degrees of freedom 1: 2
```

```
## Degrees of freedom 2: 28.14
```

```
## p-value: 0
```

```
##
```

```
## Explanatory measure of effect size: 0.81
```

```
## Bootstrap CI: [0.78; 0.85]
```

```
# This function computes a one-way ANOVA for the medians.
```

```
# Homoscedasticity assumption not required. There shouldn't be too many ties.
```

Comparing medians

```
# Comparing medians
```

```
median_phineas <- med1way(value ~ Type,
                        data = dataphineas3_melted)
```

```
print(median_phineas)
```

```

## Call:
## med1way(formula = value ~ Type, data = dataphineas3_melted)
##
## Test statistic F: 164.9672
## Critical value: 2.4446
## p-value: 0
Bootstrap
# Performing bootstrap trimmed means test
bootstrap_phineas <- t1waybt(value ~ Type,
                             data = dataphineas3_melted,
                             nboot = 599)
print(bootstrap_phineas)
## Call:
## t1waybt(formula = value ~ Type, data = dataphineas3_melted, nboot = 599)
##
## Effective number of bootstrap samples was 565.
##
## Test statistic: 825.5742
## p-value: 0
## Variance explained: 0.658
## Effect size: 0.811
MCPPB20 test
mcppb_phineas <- mcppb20(value ~ Type,
                         data = dataphineas3_melted,
                         tr = 0.2, nboot = 599)
print(mcppb_phineas)
## Call:
## mcppb20(formula = value ~ Type, data = dataphineas3_melted, tr = 0.2,
##         nboot = 599)
##
##           psihat ci.lower ci.upper p-value
## Container vs. Hand Swab  -0.00931 -0.16688  0.15323 0.80801
## Container vs. Water Sample -2.15852 -2.33301 -2.00816 0.00000
## Hand Swab vs. Water Sample -2.14921 -2.31495 -1.98609 0.00000

```

The trimmed mean ANOVA produced a test statistic of $F = 825.5742$ with a p-value of < 0.001 , indicating highly significant differences btwn groups. The effect size was substantial at 0.81, with a bootstrap confidence interval of (0.78; 0.86). Additionally, the median comparison has a test statistic of $F = 164.9672$ and a p-value < 0.001 , further confirming significant differences in medians across types.

The bootstrap trimmed means test also shows a consistent test statistic of 825.5742 and a p-value < 0.001 , with 65.8% of the variance explained and an effect size of 0.811. Finally, the MCPPB20 test highhlights specific comparisons: the Container group did not significantly differ from the Hand Swab group ($p = 0.808$), while both

the Container and Hand Swab groups showed significant differences from the Water Sample group ($p < 0.001$). These results collectively suggest that the Water Sample group is significantly distinct from the other two sample types, while the container and hand swab groups are similar in their microb counts.

Post Hoc tests

```
# Post-Hoc Bonferroni test
```

```
bonferroni_phinea <- pairwise.t.test(dataphineas3_melted$value,  
                                   dataphineas3_melted$type,  
                                   p.adjust.method = "bonferroni")
```

```
print(bonferroni_phinea)
```

```
##
```

```
## Pairwise comparisons using t tests with pooled SD
```

```
##
```

```
## data: dataphineas3_melted$value and dataphineas3_melted$type
```

```
##
```

```
##      Container Hand Swab
```

```
## Hand Swab 1 -
```

```
## Water Sample <2e-16 <2e-16
```

```
##
```

```
## P value adjustment method: bonferroni
```

```
# Post-Hoc Holm test
```

```
holm_phineas <- pairwise.t.test(dataphineas3_melted$value,  
                                dataphineas3_melted$type,  
                                p.adjust.method = "holm")
```

```
print(holm_phineas)
```

```
##
```

```
## Pairwise comparisons using t tests with pooled SD
```

```
##
```

```
## data: dataphineas3_melted$value and dataphineas3_melted$type
```

```
##
```

```
##      Container Hand Swab
```

```
## Hand Swab 0.64 -
```

```
## Water Sample <2e-16 <2e-16
```

```
##
```

```
## P value adjustment method: holm
```

```
# Post-Hoc BH Test (Benjamini-Hochberg)
```

```
bh_phine <- pairwise.t.test(dataphineas3_melted$value,  
                             dataphineas3_melted$type,  
                             p.adjust.method = "BH")
```

```
print(bh_phine)
```

```
##
```

```
## Pairwise comparisons using t tests with pooled SD
```

```
##
```

```
## data: dataphineas3_melted$value and dataphineas3_melted$Type
```

```
##
```

```
## Container Hand Swab
```

```
## Hand Swab 0.64 -
```

```
## Water Sample <2e-16 <2e-16
```

```
##
```

```
## P value adjustment method: BH
```

The Container and Hand Swab groups are not significantly different from each other, while both differed significantly from the Water Sample group. Subsequent trimmed mean ANOVA and median comparisons reinforce these findings with highly significant results ($p < 0.001$), and the bootstrap trimmed means test supports the consistent effect size of approximately 0.81. Post-hoc tests using Bonferroni, Holm, and Benjamini-Hochberg adjustments further confirm that while the Container and Hand Swab groups were statistically similar ($p = 0.64$), both exhibited significant differences from the Water Sample group ($p < 0.001$).

Objective 4. To determine the influence of container design material in biofilm formation on farm level milk handling containers

Libraries and data

```
# Loading libraries
```

```
library(dplyr)
```

```
library(ggplot2)
```

```
library(gmodels)
```

```
library(car)
```

```
library(emmeans)
```

```
library(stargazer)
```

```
library(texreg)
```

```
library(gtsummary)
```

```
# Loading the data
```

```
ContainerData <- read.csv("ContainerData.csv")
```

```
*Frequency table for container design vs biofilm formation**
```

```
(table_container_biofilm <- table(ContainerData$Container.Design,  
ContainerData$Biofilm.Formation))
```

```
##
```

```
## 0 1
```

```
## J 23 15
```

```
## M 11 6
```

Chi-Square test of independence for container design & biofilm formation

```
(chisq_test_container_biofilm <- chisq.test(table_container_biofilm))
```

```
##
```

```
## Pearson's Chi-squared test with Yates' continuity correction
```

```
##
## data: table_container_biofilm
## X-squared = 2.5586e-31, df = 1, p-value = 1
The Chi-Square stat is extremely small (essentially zero), and the p-value is 1; - there
is no evidence of a relationship between container design (J or M) and the presence of
biofilm (0 or 1).
Fisher's Exact Test for container design and biofilm formation
if (min(table_container_biofilm) > 0) {
  fisher_test_container_biofilm <- fisher.test(table_container_biofilm)
  print(fisher_test_container_biofilm)
} else {
  print("Fisher's Exact Test cannot be performed due to zero counts.")
}
##
## Fisher's Exact Test for Count Data
##
## data: table_container_biofilm
## p-value = 1
## alternative hypothesis: true odds ratio is not equal to 1
## 95 percent confidence interval:
## 0.2075772 3.1463236
## sample estimates:
## odds ratio
## 0.8390656
The Fisher's Exact Test has a p-value of 1, indicating no significant association
between the biofilm formation and container type; - the odds of biofilm formation are
not significantly different between the two container designs (J and M). The estimated
odds ratio of 0.839 suggests that the odds of biofilm formation in containers of design
M are slightly lower than in design J, but the confidence interval (0.2076 to 3.1463)
includes 1, reinforcing the conclusion that there is no statistically significant
difference.
Logistic regression model
logistic_container <- glm(Biofilm.Formation ~ Container.Design, data = ContainerData,
family = binomial)
summary(logistic_container)
##
## Call:
## glm(formula = Biofilm.Formation ~ Container.Design, family = binomial,
## data = ContainerData)
##
## Coefficients:
## Estimate Std. Error z value Pr(>|z|)
## (Intercept) -0.4274 0.3319 -1.288 0.198
```

```
## Container.DesignM -0.1787 0.6064 -0.295 0.768
```

```
##
```

```
## (Dispersion parameter for binomial family taken to be 1)
```

```
##
```

```
## Null deviance: 73.144 on 54 degrees of freedom
```

```
## Residual deviance: 73.057 on 53 degrees of freedom
```

```
## AIC: 77.057
```

```
##
```

```
## Number of Fisher Scoring iterations: 4
```

The coefficient for the container design M was -0.1787 with a p-value of 0.768, indicating that there is no significant effect of container design on the likelihood of biofilm formation. The intercept's p-value of 0.198 also suggests that the overall model does not significantly predict biofilm formation. The null and residual deviance values are similar, indicating that the model does not explain much more variability than a null model - no difference.

Creating nice, publishable tables

```
stargazer(logistic_container, report = "vc*stp", type = "text", out =  
"./container_results.txt")
```

```
##
```

```
## =====
```

```
##           Dependent variable:
```

```
##           -----
```

```
##           Biofilm.Formation
```

```
## -----
```

```
## Container.DesignM      -0.179
```

```
##           (0.606)
```

```
##           t = -0.295
```

```
##           p = 0.769
```

```
##
```

```
## Constant              -0.427
```

```
##           (0.332)
```

```
##           t = -1.288
```

```
##           p = 0.198
```

```
##
```

```
## -----
```

```
## Observations           55
```

```
## Log Likelihood         -36.528
```

```
## Akaike Inf. Crit.     77.057
```

```
## =====
```

```
## Note:          *p<0.1; **p<0.05; ***p<0.01
```

```
screenreg(logistic_container, digits = 2, title = "Regression Results", caption = "Source:  
Container dataset")
```

```
##
## =====
##           Model 1
## -----
## (Intercept)   -0.43
##              (0.33)
## Container.DesignM -0.18
##              (0.61)
## -----
## AIC           77.06
## BIC           81.07
## Log Likelihood -36.53
## Deviance      73.06
## Num. obs.     55
## =====
## *** p < 0.001; ** p < 0.01; * p < 0.05
# gtsummary table
container_mod <- glm(Biofilm.Formation ~ Container.Design, data = ContainerData, family
= binomial)
(t1 <- tbl_regression(container_mod, exponentiate = TRUE))
## Table printed with {flextable}, not {gt}. Learn why at
## https://www.danielsjoberg.com/gtsummary/articles/rmarkdown.html
## To suppress this message, include `message = FALSE` in the code chunk header.
```

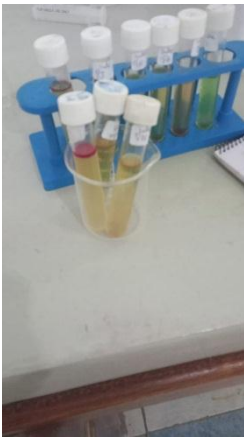
Characteristic	OR ¹	95% CI ¹	p-value
Container.Design			
J	—	—	
M	0.84	0.24, 2.70	0.8

¹OR = Odds Ratio, CI = Confidence Interval

Appendix III: Laboratory test sampled photos



Tube method of detecting biofilms a, samples under incubation, b, results after staining showing weak lining (weak biofilms)



Indole positive *B.cereus*

Appendix IV: Institutional Ethics Review committee

CHUKA



UNIVERSITY

Knowledge is Wealth (*Sapientia divitia est*) Akili ni Mali

CHUKA UNIVERSITY INSTITUTIONAL ETHICS REVIEW COMMITTEE

Telephones: 020-2310512/18

Direct Line: 0772894438

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P. O. Box 109-60400, Chuka

Website: www.chuka.ac.ke

21st June, 2023

REF: CUIERC/ NACOSTI/399
TO: Phineas Muthaura Gikunda

RE: Effect of Container Design, Cleaning Regime, Water Quality and Personnel Hygiene on Biofilm formation on Farm-Level Milk Handling Containers in Kajiado County, Kenya
This is to inform you that *Chuka University IERC* has reviewed and approved your above research proposal. Your application approval number is *NACOSTI/NBC/AC-0812*. The approval period is 21st June, 2023 – 21st June, 2024.

This approval is subject to compliance with the following requirements;

- i. Only approved documents including (informed consents, study instruments, MTA) will be used
- ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *Chuka University IERC*.
- iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *Chuka University IERC* within 72 hours of notification
- iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to *Chuka University IERC* within 72 hours
- v. Clearance for export of biological specimens must be obtained from relevant institutions.
- vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
- vii. Submission of an executive summary report within 90 days upon completion of the study to *Chuka University IERC*.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) <https://oris.nacosti.go.ke> and also obtain other clearances needed.

Yours sincerely

Dr. Benjamin Kanga
SECRETARY

