PREVALENCE, SUSCEPTIBILITY PATTERNS AND RISK FACTORS ASSOCIATED WITH *Staphylococcus aureus* PRESENCE IN MARKETED MILK AND MILK PRODUCTS WITHIN NAIROBI CITY COUNTY, KENYA

BY

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree or any other award in any other University.

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DEDICATION

First, I thank God for the grace He gave me all through my work to the end. Secondly, I dedicate my work to my dear wife Mary Macharia, my parents Mr & Mrs Andrew Macharia and Mr & Mrs Joseph Kinuthia, my brothers Peter Maina and James Mwangi and my sisters Teresia Wambui, Serah Waithera, Hildah Muthira and Evelyn Wangari for their constant assistance whenever I needed and prayers that made me emerge strong and successful at the end. I love you all and may God bless, reward and prosper you.

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ABBREVIATIONS AND ACRONYMS

ANOVA : Analysis of Variance

ANT : Amino-glycoside adenyl-trasnferase

CA-MRSA : Community associated Methicillin-resistant *S. aureus*

CMR : Centre for microbiology research

CLSI : Clinical and Laboratory Standards Institute

ESL : Extended Shelf Life

FWM : Fresh whole milk

GISA : Glycopeptide intermediate *S. aureus*

HCCP : Hazard Critical Control Point System

HTST : High temperature short time

IC : Ice cream

ISO : International Standards Organization

KEBS : Kenya Bureau of Standards

KEMRI : Kenya Medical Research Institute

MDR : Multi-drug resistant

MIC : Minimum inhibiting concentration

MMP : Milk and milk products

MRSA : Methicillin-resistant *S. aureus*

NCCLS : National Committee for Clinical Laboratory Standards

PBP : Penicillin binding protein

PVL : Panton-valentine leukocidin

RNA : Ribonucleic acid

ROK : Republic Of Kenya

SCC : Staphylococcal Cassette Chromosome mec

SDP : Smallholder Development Programme

SE : Staphylococcal enterotoxins

SSSS : Staphylococcal Scalded Skin Syndrome

S. aureus : *Staphylococcus aureus*

TBC : Total Bacteria Count

TSB : Tryptone soya broth

UHT : Ultra high temperature

UK : United Kingdom

US : United States

USDA : United States Development Agency

VISA : Vancomycin-intermediate *S. aureus*

VRSA : Vancomycin-resistant *S. aureus*

WHO : World Health Organization

YM : Fermented milk

ABSTRACT

Staphylococcus aureus is a major food-borne pathogen that poses a serious threat to public health. In Kenya, with the continuous water shortage, proper sanitary conditions are not sufficiently met and hence pre-disposing the community to *S. aureus* infections. One of the difficulties of controlling *S. aureus* food poisoning is that food can contain a very high population of the bacteria without being noticeably identified. It has been suggested that food-borne diseases represent one of the most widespread and overwhelming public health problems in poor resource settings. The increasing rate of multidrug resistant *S. aureus* has continued to pose a challenge to the pharmaceutical firms and patients management. The aim of this study was therefore to determine the presence of *S. aureus* in milk and milk products, antimicrobial susceptibility patterns and factors associated with food contamination. A total of 334 samples were collected for analysis in the laboratories. A loop-full of each sample was streaked directly on MacConkey agar and Blood agar. Suspected isolates were subcultured in Mannitol salt agar which was used as an indicator media. Sensitivity test was accomplished using Muller-Hinton agar. Biochemical tests; Catalase test and Coagulase test were used as confirmatory tests for *S. aureus*. To determine antimicrobial susceptibility, panels of selected antibiotics commonly used in empirical treatment of *S aureus* infections were obtained from different classes. The antibiotics were Penicillin G, Erythromycin, Vancomycin, Chloramphenicol, Tetracycline, Gentamycin, Methicillin and Ciprofloxacin. From all the 54 samples of raw milk analyzed, 35 (64.81%) samples were contaminated by *S aureus*. In pasteurized milk, out of 112 samples, 23 (20.54%) samples were contaminated while in yoghurt, out of 112 samples, 12 (10.71%) samples were contaminated. In ice cream, out of 56 samples, 2 (3.57%) were contaminated. All isolates were found to be 100 % sensitive to Tetracycline, Ciprofloxacin, Erythromycin and Methicillin. Resistance to Penicillin G was occasionally observed across the different sources of milk and milk products. From the milk outlets selling raw milk within the study area, regular opening of the containers to sell milk pre-disposed the milk to hand contamination and consequently greater risks of contamination by environmental contaminants. Out of 120 respondents interviewed, an average of 84 (70%) claimed to be aware of the health risks associated with milk. Of the respondents, 28 (23%) claimed to be aware of diseases associated with consumption of contaminated milk. Most of them claimed to have encountered stomach disorders and diarrhea while others claimed to have experienced body rashes, severe headache and vomiting. On average, 8 (9%) of the respondents claimed to have contracted a disease as a result of drinking contaminated milk within the last one year. It was established that 118 (98%) of food handlers did not receive any formal training regarding food hygiene. Information generated from the study provides a basis upon which formulation of better policies regarding raw milk and milk products can be based on. In addition, given the imminent risks of infection and resistance involved, the relevant authorities should adopt severe inspection measures in order to regulate or prohibit the informal sale of milk.

CHAPTER ONE

INTRODUCTION

1.1 Background

Staphylococcus aureus (*S. aureus*) is Gram positive and spherical cocci of about (0.8-1.0 micron) in diameter appearing in grape like clusters. *S. aureus* are aerobes and facultative anaerobes that thrive at an optimum temperature of 37º C while the temperature at which they are active ranges between 12- 44º C. They have an optimum pH of 7.5. They have been found to grow well on ordinary lab media forming a golden yellow color in agar (William *et al.*, 2005).

Pathogenic microorganisms are introduced in milk products when raw milk used to process them is contaminated or by cross-contamination (Muriuki *et al.,* 2011). In Kenya, 86 % of milk is marketed raw and only 14 % is processed (SDP, 2004). Raw milk can be 20-50 % cheaper than the formal and pasteurized milk on the market (SDP, 2004). Unprocessed milk is also sold in desired quantities which give the low income earners access since they can buy as little as they can afford (SDP, 2004). Milk handling equipment is one of the most significant sources of microbial contamination in milk (Muriuki *et al.,* 2011). If equipment is inadequately cleaned and milk residues are left on wet surfaces it will result in microbial growth which could contaminate the milk (Muriuki *et al.,* 2011).

S. aureus produce enterotoxins that are virulent factors responsible for food poisoning in humans. In essence, according to Julia *et al*. (1995) enterotoxins are basic proteins which

are resistant to heat, acid and digestive enzymes. There are four immunologically distinct enterotoxins that are designated as enterotoxins A, B, C and D (Julia *et al.,* 1995). The enterotoxins produced cannot be destroyed by exposure to 100˚C for 30 minutes, therefore posing a serious challenge to their elimination from the contaminated food and other edibles (William *et al.*, 2005).

Enterotoxins consumed incubate under favorable conditions and after 4-6 hours, symptoms of infection appear such as nausea, dizziness, severe abdominal pains and cramps, loss of appetite, diarrhea and vomiting up to 24 hours. This period of incubation is of great significance since it differentiates food poisoning caused by *S. aureus* to that caused by *Salmonell*a infection which appear 24-48 hours after eating contaminated food. *S. aureus* is associated with most wound infections in man and animals and may localize in boils, tonsillitis, cancerous cells, infections in the mammary glands and infection of sinuses. It was previously thought that contaminated water was the only source of diarrhea, however, it is now understood that food contaminated with *S. aureus* equally causes food poisoning. Risk factors include: eating food that was prepared by a person with a skin infection, eating food kept at room temperature, eating improperly prepared food, sharing the same food with someone who has symptoms of infections. *S. aureus* is a formidable pathogen of humans and livestock (William *et al.*, 2005).

The genetic plasticity of *S. aureus* has facilitated the emergence of persistent and multidrug-resistant strains that have a major impact on human and animal health (Foster, 2004). There has been a high risk factor of about 60 % prevalence rate of *S. aureus*

infections as a result of their high resistance to antibiotics used and 39.5 % of the infections have their source from milk and milk products (Tacconeli *et al*., 2008).

Figures from the health information system in the Ministry of Health reveals that between 1997 and 1999, there were 6833 cases and 566 deaths were reported from food contamination (ROK, 2000). Foods that are most commonly involved in food-borne disease are meat and meat products, poultry, eggs, milk and milk products, sweetmeats and rice preparations (Tambekar *et al.*, 2004).

1.2 Statement of the problem

Staphylococcus aureus are able to grow at low temperatures and therefore posing a serious threat. With the current high risk factor of about 32 % prevalence rate of *S. aureus* infections, resistance to the commonly used antibiotics is also on the rise. In Kenya, there exists limited information on prevalence of *S. aureus* in raw milk while none has been documented on milk products. Susceptibility patterns of *S. aureus* isolated from milk and milk products has also not been well documented. Currently, most of the milk sold by vendors and intermediaries within Nairobi County is not widely monitored in informal markets. Milk and milk products in some outlets are put in shelves and crates where ambient conditions can favor microbial growth. In other shop outlets, milk and milk products are stored without any form of refrigeration. Veterinary and public health services historically have worked independently and lack of cooperation has contributed to inadequate attention to effective control of zoonoses. In addition to institutional constraints, there are capacity training and technical constraints (Schelling *et al*., 2007). This study attempted to fill this gaps by generating information that allows risk

assessments to be conducted in informal markets and suggest possible drugs of choice in empirical treatment of *S. aureus* infections.

1.3 Justification

Consumers may consume contaminated milk and milk products sold from the outlets which may predispose them to infections. Although milk is a very nutritious food, it can be associated with health risks such as presence of zoonotic pathogens and antimicrobial drug residues. There is therefore need for information that can allow risk based approaches to be conducted in milk and milk products` outlets. The genetic plasticity of *S. aureus* facilitates the emergence of persistent and multidrug-resistant strains that have a major impact on human and animal health currently. More research and information on susceptibility patterns of *S. aureus* isolated from milk and milk products in Kenya should be done, in order to document effective antibiotics in treatment of *S. aureus*.

1.4 Research questions

i. Is marketed milk and milk products within Nairobi County contaminated with *S. aureus*?
ii. What are the levels of susceptibility to the various antimicrobials by *S. aureus?*
iii. Is risk assessment on milk food safety hazard conducted in the outlets?

1.5 Research hypothesis

Marketed milk and milk products within Nairobi County are not contaminated with *S. aureus.*

1.6 Objectives of the study

1.6.1 General objective

To determine the prevalence, susceptibility patterns and risk factors associated with *S. aureus* presence in milk and milk products supplied for consumption within Nairobi County.

1.6.2 Specific objectives

i. To determine the presence of *S. aureus* in marketed milk and milk products within Nairobi County.
ii. To determine antimicrobial susceptibility patterns of *S. aureus* isolates.
iii. To determine factors associated with milk food safety hazard.

1.7 Significance of the study

S. aureus has become resistant to many commonly used antibiotics. This study was necessary since its findings indicate the sensitivity patterns of *S. aureus*, as well as determine risk factors associated with milk food. The findings from the study suggest the possible antibiotics of choice to be used in empirical treatment of *S. aureus* infections since lack of information on susceptibility patterns of *S. aureus* strains implicated in infections leads to poor management, resulting in treatment failures. In addition, information generated from this research provides a basis upon which formulation of better policies regarding processed milk and milk products can be based on by policy makers using the generated information that allows risk assessments be conducted in informal markets. Finally, the findings of the study are an addition of information to the existing literature on food contamination and the challenge faced by the pharmaceutical

industry due to drug resistance by microorganisms and therefore gives more information to the general public.

CHAPTER TWO
LITERATURE REVIEW

2.1 Biology and Characteristics of *S. aureus*

S. aureus is Gram positive and spherical cocci appearing in grape like clusters. They are aerobes and facultative anaerobes that thrive at an optimum temperature of 37º C and the temperature at which they are also active range between 12- 44º C. They have an optimum pH of 7.5. They have been found to grow well on ordinary lab media forming a golden yellow color in agar (William *et al.*, 2005). *Staphylococcus aureus* is capable of using mannitol as a food source and produce acidic products of fermentation that lowers the pH of the media. The acidity of the media causes the pH indicator, phenol red to turn yellow. *S. aureus* ferments mannitol while *S. epidermidis* does not (William *et al.*, 2005). Pathogenic *S. aureus* are both coagulase positive and catalase positive.

Access to good quality, safe and nutritious milk is considered a basic right of the people. Contaminated food leads to food-borne diseases, which cause considerable morbidity and mortality (Tambekar *et al.*, 2006). *Staphylococcus aureus* are ubiquitous and responsible for a wide range of clinically different diseases which range from the relatively harmless skin pimples through abscesses, impetigo, food poisoning, osteomyelitis, mastitis, primary pneumonia, Staphylococcal Scalded Skin Syndrome (SSSS), to Septicemia (Acco *et al*., 2003).

Staphylococcus aureus strains were once resistant to semi-synthetic penicillinase-resistant β-lactams such as methicillin and oxacillin, the most commonly used class of antibiotics for skin infection. These strains were termed ‘methicillin resistant *S. aureus*,

or MRSA," a term that implied cross-resistance to all β-lactams including all penicillins and cephalosporins. Staphylococci are normal inhabitants of the skin and mucous membranes of animals and humans. Pathogenic strains are usually coagulase-positive and have been found to cause disease in their hosts throughout the world (Murray *et al.*, 2006).

Results of antibiotic resistance clearly suggest a possibility of potential public health threat of *S. aureus* resulting from contamination of milk and milk products with pathogenic bacteria (Thaker *et al.*, 2013). *Staphylococcus aureus* was the first bacterium in which penicillin resistance was found in 1947, just four years after the drug started being mass-produced. Methicillin was then the antibiotic of choice, but has since been replaced by oxacillin due to significant kidney toxicity (Panlilio *et al.*, 1992). Methicillin-resistant *S. aureus* (MRSA) was first detected in Britain in 1961 and is now quite common in hospitals. Isolates of *S. aureus* were found to be highly resistant to Penicillin G (Thaker *et al.*, 2013). In UK, only 2 % of all *S. aureus* isolates are sensitive to penicillin with a similar picture in the rest of the world. Strains with intermediate (4-8 ug/ml) levels of resistance, termed GISA (glycopeptide intermediate *S. aureus*) or VISA (vancomycin intermediate *S. aureus*), began appearing in the late 1990s (Borgen *et al.*, 2002).

It has been found that the widespread use of antibiotics plays a significant role in the emergence of resistant strains of *S. aureus* (Thaker *et al.*, 2013). Resistant bacteria have been reported with increased frequency world-wide in human medicine in recent years

and have now reached such a level that most old antibiotics are worthless and only a few, if any can be used for treatment (Bauer *et al*., 1966; Richard *et al*., 2014). In some countries, antibiotics are sold over the counter without a prescription which compounds the problem. According to Acco *et al*. (2003) non-therapeutic use of antimicrobials corresponds to resistance rates. In 1997 in a Taiwan hospital, five patients underwent open heart surgery and developed surgical wound infections and mediastinitis caused by MRSA (Wang *et al*., 2001). Surg *et al*. (1974) pointed out that *S. aureus* in community acquired infections increased resistance to penicillin antibiotics. It therefore clearly shows that the complexity of treating *S. aureus* is greatly fuelled and generated by its ability to resist antibiotics (Hierholzer *et al.*, 1995).

In Japanese hospitals, strains of *S. aureus* were heterogeneous and resistant to Vancomycin antibiotics (Lancet *et al.,* 1997). Therefore, increase in the resistance to this antimicrobial agent, coupled with its increasing prevalence as a nosocomial pathogen is of a major concern (Lancet *et al.,* 1997). Brun-Buisson *et al*. (1998) noted that Methicillin resistance in nosocomial *S. aureus* isolates, has been increasing dramatically and is also associated with resistance to other useful anti-staphylococcal compounds. He further noted that the possible ways to decrease the incidence of nosocomial *S. aureus* infections include instituting more effective infection control, decreasing nasal colonization, developing vaccines and finally developing new improved antimicrobials **(**Brun-Buisson *et al*., 1998).

There is therefore, a need for an urgent solution to curb staphylococcal infection which if measures are not put in place in the near future, could be disastrous to the community within the area with a pathogenic outbreak due to its resistance to antibiotics **(**Brun-Buisson *et al*., 1998). *Staphylococcus aureus* golden pigment impairs neutrophil by killing and promotes virulence through antioxidants activity (Bastian *et al.*, 2005). In chemo-organoleptic bacteria, recent research has shown that in addition to the well known C40 carotenoids, C30 and C50 can also be found. C30 also called diapocarotenoid was first described in a strain of *S. aureus*. Unpigmented variants were found to be highly resistant to therapeutic antibiotics and their selection due to antibiotic treatment may be the cause of failure of such chemotherapeutic treatments (Abdrezzak *et al*., 2008). Antimicrobial susceptibility-profile of the staphylococcal strains revealed a high incidence of *S. aureus* to penicillin G. In addition, it presented considerable resistance to the oxacillin, erythromycin and lincomycin (Abdrezzak *et al*., 2008).

2.2 The mechanisms of antibiotic resistance

Staphylococcal resistance to penicillin is mediated by penicillinase (a form of ß-lactamase) production: an enzyme which breaks down the β-lactam ring of the penicillin molecule. Penicillinase-resistant penicillins such as methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin and flucloxacillin are able to resist degradation by staphylococcal penicillinase (Brun-Buisson *et al*., 1998). Resistance to methicillin is mediated via the mec operon, part of the staphylococcal cassette chromosome mec (SCCmec). Resistance is conferred by the mecA gene, which codes for an altered penicillin-binding-protein (PBP2a or PBP2') that has a lower affinity for binding β-lactams (penicillins, cephalosporins and carbapenems). These antibiotics including

carbapenems have a β-lactam ring in their molecular structures. This allows for resistance to all β-lactam antibiotics and obviates their clinical use during MRSA infections. As such the glycopeptide, vancomycin, is often deployed against MRSA (Miller *et al.*, 2007).

Amino-glycosides such as Kanamycin, Gentamicin, and Streptomycin were once effective against staphylococcal infections until the organism evolved mechanisms to destroy the amino-glycosides action. This action occurs via protonated amine and hydroxyl interactions with the ribosomal RNA of the bacterial 30S ribosome. There are two main mechanisms of amino-glycoside resistance studied so far and a genetic disorder: Amino-glycoside modifying enzymes, active efflux of the drug out of the bacteria and ribosomal mutations (Miller *et al*., 2007).

Today, *S. aureus* has become resistant to many commonly used antibiotics. In UK, only 2 % of all *S. aureus* isolates are sensitive to penicillin with a similar picture in the rest of the world, due to a penicillinase (a form of ß-lactamase). The β-lactamase-resistant penicillins (methicillin, oxacillin, cloxacillin and flucloxacillin) were developed to treat penicillin-resistant *S. aureus* and are still used as first-line treatment. Methicillin was the first antibiotic in this class to be used and it was introduced in 1959. Two years later, the first case of methicillin-resistant *S. aureus* (MRSA) was reported in England (Miller *et al.*, 2007).

Vancomycin resistant *S. aureus* (VRSA) is a strain of *S. aureus* that has become resistant to the glycopeptides. The first case of vancomycin-intermediate *S. aureus* (VISA) was

reported in Japan in 1996 but the first case of *S. aureus* truly resistant to glycopeptide antibiotics was only reported in 2002. Three cases of VRSA infection have been reported in the United States (Borgen *et al.*, 2001). However, antimicrobial resistance pattern of *S. aureus* vary greatly among different countries which may reflect differences in infection control policies and other factors (Kumar *et al*., 2010). Vancomycin and teicoplanin are also used as last-line treatment for serious invasive infections. The problem with these antibiotics is in the need to monitor drug levels regularly by blood tests, toxicity and the need for intravenous administration since there is no oral preparation available (Blot *et al.,* 2002). Resistance to Vancomycin can only occur if a strain of *S. aureus* becomes resistant to glycopeptides (Menichetti *et al*., 2005).

2.3 Food contamination by *S. aureus*

The quality of milk may be lowered by factors such as milk adulteration, contamination during and after milking, presence of udder infection, poor modes of food packaging and compromised sterilization and storage by refrigeration. It has been documented that pasteurization does not completely free milk from bacteria (Karel *et al.,* 2004). Food-borne diseases represent one of the most widespread and overwhelming Public Health problems of the modern world. According to the latest edition of the World Health Statistics Quarterly, food-borne diseases may be 300-350 times more frequent than reported cases tend to indicate (WHO, 2002).

Since *S. aureus* is normally resident in humans, the high level of *S. aureus* present in cow's milk may have resulted from transmission between the two species, emphasizing the need to improve sanitary conditions in the milking environment. The presence of *S.*

aureus in raw milk generally comes from cows with mastitis, from handlers or from deficient hygiene (Lilian *et al*., 2011). When found in milk, high levels of contamination can be reached quickly under favorable conditions. Its presence in milk and milk products can be a great risk to human health, causing a public health problem, as these bacteria produce toxins that can cause deadly infections to the final consumers of the products (Lilian *et al*., 2011). *Staphylococcus aureus* has been reported to be generally heat labile and counts above 10^3CFU/ml in milk increase the risk of staphylococcal toxin production making it more resistant to the heat processes of pasteurization (Lilian *et al*., 2011).

It was previously thought that contaminated water supply was the main source of diarrhea (WHO, 2002). However, it is now understood and known that food contaminated with *S. aureus* equally cause diarrhea cases (WHO, 2002). Since staphylococcal enterotoxins (SEs) are more stable than *S. aureus* bacteria, it is possible to test a food product and obtain negative *S. aureus* culture results and positive SE tests. The presence of *S. aureus* shows up unsanitary conditions in the manner in which milk and milk products are handled right from management of the cows` health, the milking process to processing and finally in distribution of the dairy products to the final consumers (Lilian *et al*., 2011).

Hazard to public health is particularly linked to the ability of (50 %) of these strains to produce thermo-stable enterotoxins associated with food poisoning (Balaban *et al.,* 2000). Milk and milk products are common vehicles for staphylococcal food poisoning (Chiang *et al.,* 2008). They have frequently been implicated in food poisoning and often

contaminated raw milk is involved (Raimundo *et al*., 1999). These products are highly susceptible to a variety of microorganisms because of their high nutritive value and complex chemical composition. Biological changes produced by these organisms can be either desirable or undesirable (Chiang *et al*., 2008). High prevalence rate of enterotoxigenic *S. aureus* in raw milk continues to be a potential problem in Tehran (Iran) (Tang *et al*., 2000). Reports indicate that the prevalence of enterotoxin genes differs noticeably among the countries (Moon *et al.*, 2007). Depending on the country, prevalence of the common enterotoxin gene ranges from *sea, seb, sec* to *sed* (Moon *et al.*, 2007).

The high counts of *S. aureus* in raw milk are thought to be of unknown origin. One would presume there is no care for the health of the herd, cases of subclinical mastitis are not detected or herds are inadequately treated (Ribeiro *et al*., 2009). Leite *et al*. (2002) did not detect *S. aureus* in the samples analyzed of pasteurized milk in Salvador. Whereas for organic milk from the interior of São Paulo, Ribeiro *et al*. (2009) found that 25.7 % of the samples were contaminated with *S. aureus* out of 148 samples. In most cases, contamination by *S. aureus* in milk is due to animals with mastitis. Studies conducted at the experimental station in Nova Odesa, São Paulo, by Zafolon *et al*. (2008) showed that the prevalence of *S. aureus* was higher by up to 54.4 % in rainy periods, and these data can help producers take preventive measures in their handling during these periods. In 208 samples of milk from cows with mastitis, Fagundes *et al*. (2010) isolated 6.7 % of *S. aureus* and 14.3 % were enterotoxin producers. In Palestine, 48 36.9 % of samples were positive for *S. aureus* from a total of 130 samples (Farhan *et al*., 2007).

Farhan *et al*. (2007) found counts of *S. aureus* varying between 7.1 x 10^5 to 12.6 x 10^5 CFU/ml in raw milk sold in the region of Lahore, Pakistan, which are significant numbers likely to lead to production of enterotoxin. In Turkey, Ekici *et al*. (2004) found 18.18 % of samples contaminated from the total of 66 samples. In the north of Morocco, Bendahon *et al.* (2008) isolated 40 % of *S. aureus* in raw milk from 27 samples, and in India, 61.7 % positivity was detected in 60 samples of raw milk researched (Lingathurai *et al.* (2011). Park *et al*. (2007) analyzed 30,019 samples of raw milk in Korea and detected 104 (0.35 %) samples contaminated with *S. aureus*. Mammary gland is the principal place of infection of *S. aureus* when the animal has mastitis. Skins of shanks are significant reservoirs of *S. aureus* due to their anatomical position. Other places include the muzzle, groin and wounds reinforcing the care that needs to be taken during milking so as not to disseminate the bacteria to other animals, compromising the quality of the milk according to Capurro *et al*. (2010). There is an average of 32 % prevalence of *S. aureus* in raw milk (Bendahon *et al*., 2008; Tacconeli *et al*., 2008; Shah *et al.,* 2015)

2.4 Milk and milk products handling practices

The most important factors in the prevalence of milk and milk products contamination include the lack of appropriate skills and knowledge or due to negligence by milk and milk products handlers and consumers, in safe handling (WHO, 2002). Survey of infection from contamination have shown that most such cases occur as a result of an error in handling the dairy products during industrial processing, in homes, shopping centers, the military or in schools (WHO, 2002).

A major risk in milk and milk products contamination may arise from handlers depending on the degree of conscientiousness in handling them and the training they acquired on handling them during industrial processing and storage. Klein *et al.* (1999) reported that safety of food handling is determined by the degree of consciousness, the amount of training received by handlers and the degree of supervision applied in each particular establishment. Uses of contaminated raw milk to process milk products and cross-contamination are ways of introducing the pathogenic microorganisms in the products. World Health Organization (2002) reported that the raw ingredient, use of contaminated equipment and contamination by infected persons were recognized as the major sources of food-borne disease outbreak in Israel. Milk and milk products production and processing are marketed through informal channels on which little information is available (Broutin *et al.*, 2005).

In Senegal, it was revealed that the main constrains for improving the quality of milk is due to inadequate equipment for milk conservation and processing (Broutin *et al.*, 2005). The presence of *S. aureus* shows up unsanitary conditions in the cattle herd and counts above 10^3CFU/ml in milk increase the risk of staphylococcal toxin production more resistant to the heat processes of pasteurization (Lilian *et al*., 2011). Normally the production of enterotoxin is found at temperatures of 40ºC to 45°C, although Smith *et al.* (1982) detected production of toxins at temperatures of 10ºC to 46ºC. Various conditions favor the growth of *S. aureus* and the production of enterotoxins such as the temperature, water activity, concentrations of salts and pH, and even the competitiveness of the micro-flora (Jorgensen *et al.*, 2005).

Milk can be contaminated by *S. aureus* when there is infection of the mammary gland or by bad hygienic habits such as coughing, sneezing and not washing hands when handling milk storage equipment during or after milking. In this case, man is responsible for contamination. *Staphylococcus aureus* colonizes the nasal pathways in human beings (Lilian *et al*., 2011). Microorganisms found on the hands and on the uniform of food handlers especially of milk, are a reflection of hygiene habits as the most important factor in contamination of milk. As food contamination by human hands cannot be completely controlled, suitable refrigeration at temperatures below 5ºC is one of the ways of preventing *S. aureus* contamination and consequently the formation of staphylococcal toxin (Tortora *et al*., 2005).

There is a dire need for knowledge on proper milk and milk products handling, right from production, industrial processing, storage and in the market place, where major towns and institutions such as universities colleges and schools carrying very high populations are involved in consumption. Development of improved milk and milk products production is largely dependent on the emergence of processing sectors. In the north of Morocco, 40 % of *S. aureus* were isolated in raw milk from 27 samples (Bendahon *et al.,* 2008). Dirty hands of worker, poor quality of milk, unhygienic conditions of manufacture unit, inferior quality of material used and water supplied for washing the utensils could be the source of the bacterial contamination of milk products (Tambekar *et al*., 2004).

2.5 Milk pasteurization as a preservation technique

Pasteurization is typically associated with milk. High temperature short time (HTST) pasteurized milk typically has a refrigerated shelf life of two to three weeks, whereas

ultra pasteurized milk can last much longer when refrigerated, sometimes two to three months (Ranieri *et al.,* 2009). When UHT treatment is combined with sterile handling and container technology such as aseptic packaging it can even be stored unrefrigerated for 3–4 months (Sieber *et al*., 1994). Ultra high temperature (UHT) is also used for milk treatment.

In HTST process, milk is forced between metal plates or through pipes heated on the outside by hot water, and is heated to 71.7 °C (161 °F) for 15–20 seconds. Ultra High Temperature processing holds the milk at a temperature of 138 °C (280 °F) for a fraction of a second. Extended Shelf Life (ESL) milk has a microbial filtration step and lower temperatures than HTST. Milk simply labeled "pasteurized" is usually treated with the HTST method, whereas milk labeled "ultra-pasteurized" or simply "UHT" has been treated with the UHT method (Lilian *et al.,* 2011). Pasteurized milk may also be predisposed to toxin production since some shop owners switch off the chillers at night to save electricity leaving the products exposed to varying temperatures (Lilian *et al.*, 2011). It has in addition been documented that pasteurization does not completely free milk from bacteria (Karel *et al.,* 2004).

Pasteurization methods are usually standardized and controlled by national food safety agencies such as the USDA in the United States and the Food Standards Agency in the United Kingdom. High temperature short time (HTST) pasteurization standard was designed to achieve a 5-log reduction, killing (99.99 %) of the number of viable micro-organisms in milk. This is considered adequate for destroying almost all yeasts, mold,

common spoilage bacteria and also to ensure adequate destruction of common pathogenic heat-resistant microorganisms. High temperature short time (HTST) pasteurization processes must be designed so that the milk is heated evenly, and no part of the milk is subjected to a shorter time or a lower temperature (Ranieri *et al.,* 2009).

Different investigators have reported that *Staphylococcus* species isolated from dairy products of bovine are able to produce high levels of enterotoxins. Smith *et al*. (2007) reported 54 % of bovine mastitic milk isolates to be enterotoxigenic while Salandra *et al.* (2008) reported 55.9 % to be enterotoxin producing *Staphylococcus* isolates from dairy products in Italy. The different rates of enterotoxin production found in this report could be explained by the different techniques used in these studies, differences in the origin of the isolates or by geographical differences. Plastic containers have characteristics that make them unsuitable for milk handling. They have been noted to scratch easily and provide hiding places for bacteria during cleaning and sanitization. They are also poor conductors of heat and hence hinder effective sterilization by heat (Soomro *et al*., 2003).

2.6 Milk and milk products safety control measures

There exists the Food Drugs and Chemical Substances Act Cap 254 laws of Kenya (Food hygiene) in which hygienic requirements are clearly spelt out. It has further been emphasized by the Public Health Act Cap 242 which points out the sanitary measures to be observed in food premises (WHO, 2002). The International Standards Organization (ISO) 9000 of 1998 deals mainly with quality assurance of manufactured foods (Hoyle *et al*., 1998). There are volunteer programmes that promote good agriculture and manufacturing practices and the application of modern methods of food safety assurance

such as the Hazard Critical Control Point System (HCCP) (Brayan *et al*., 1992). It is essential for the public health authorities to take the necessary steps in strictly enforcing the hygienic concept. This will prevent pathogenic contamination at various stages of processing, storage, handling and transportation of milk and milk products (Tambekar *et al.,* 2006). The present study suggests the need for more strict preventive and control measures used to avoid pre and post process contamination in milk food products. The importance of microorganisms in milk means that their microbial contamination index can be used to judge the quality of milk, the sanitary conditions during production and also possible infection of the herd (Guerreiro *et al*., 2005).

Microbiological analyses of foods are important to keep the population informed as to the sanitary level, principally of milk. As food contamination by human hands cannot be completely controlled, suitable refrigeration at temperatures below 5ºC is one of the ways of preventing *S. aureus* contamination and consequently the formation of staphylococcal toxin (Tortora *et al*., 2005). The standard plate count per milliliter for raw reconstituted milk or pasteurized milk at the plant shall not exceed 3.0×10^4 CFU/ml (EAS, 2007). This study therefore attempted to fill gaps that will allow risk assessments be conducted in both formal and informal markets and susceptibility patterns of *S. aureus* isolated from milk and products.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was carried out in Nairobi County (Figure 3.1). It covers an approximate area of 696 km^2 and encompasses the Nairobi City and its metropolis. The city is surrounded by expanding villa suburbs. It was ranked 14^{th} largest city in Africa by the year 2009 including the population of its suburbs. It is in addition a cosmopolitan and a multicultural city (KCBS, 2009). According to the (2009) Census, Nairobi County was established to have a population size of 3,375,000 which translated to a density of 4,850/km^2 in all 985,016 households. The County is bordered by Kiambu County, Machakos County and Kajiado County. Nairobi County hosts the country`s largest industrial centre which accounts for 20 % of the gross domestic product with the areas around it being prime agricultural lands (KCBS, 2009). The principal products include: processed foods, construction materials, soaps and chemicals while agricultural products include: livestock products, poultry products and horticulture. The county is characterized by a 22 % poverty level (NSE, 2007).

The study area covered was the consumer outlets within the county: Supermarkets and shops. Supermarkets were designated as A, B, C and D to avoid conflict of interest. Pasteurized milk, fermented milk and ice-cream were obtained from supermarkets. Raw milk was obtained from selected shops in Roysambu, Kasarani, Dagoreti, Lang'ata, Embakasi and Westlands (Figure 3.2) that are characterized by many raw milk outlets. Raw milk was not sold in the supermarkets. Laboratory work and analysis was carried

out at Kenyatta University as well as the Centre for Microbiology Research-KEMRI based in Kenyatta National Hospital.

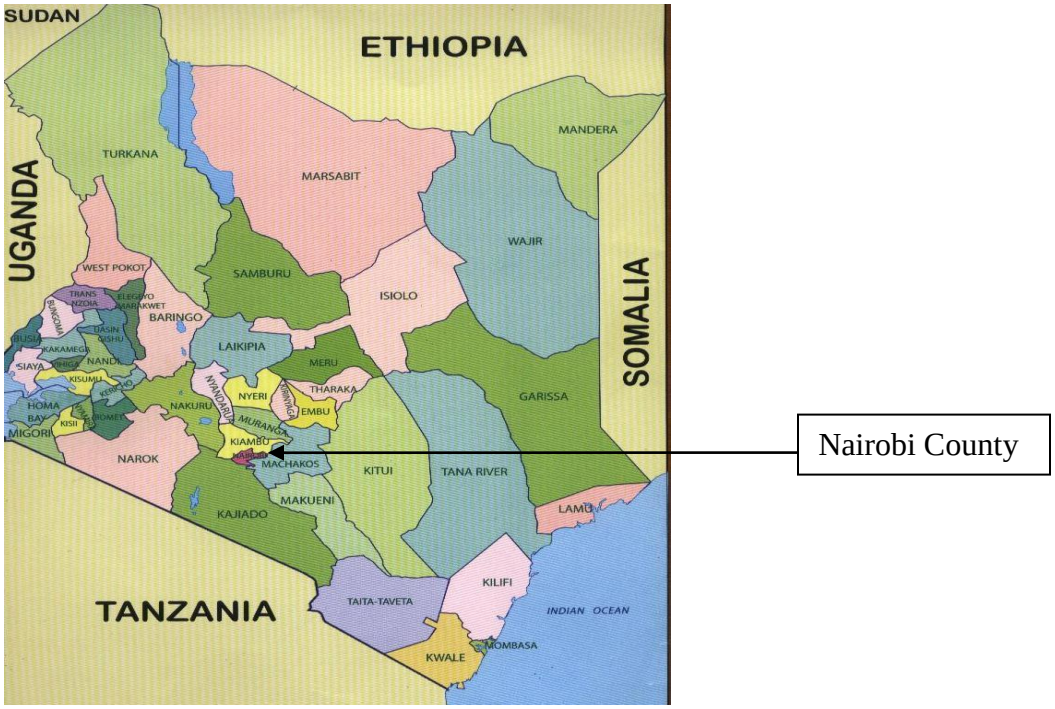


Figure 3.1: Different Counties in Kenya. Nairobi County is represented with a purple color (KCBS, 2009)

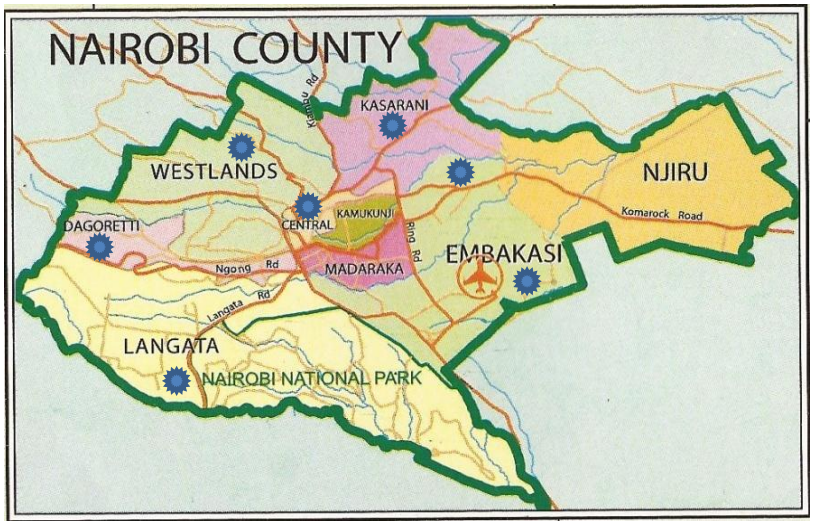


Figure 3.2: Nairobi County (KCBS, 2009**),** where milk and milk products were collected from selected outlets.
- Sites where sampling was done.

3.2 Raw milk and milk products

Fifty four samples of raw milk were analyzed. Milk products were pasteurized milk (fresh whole milk A, 56 samples and fresh whole milk B, 56 samples), fermented milk (yoghurt milk A, 56 samples and yoghurt milk B, 56 samples) and ice-cream (IC), 56 samples.

3.3 Sample size

Sample size was determined using the Fisher formula as defined by Fisher *et al*. (1993), as given below.

$$N = \frac{Z^2 p q}{D^2}$$

$$N = \frac{1.96^2 \, x \, 0.32 \, x 0.68}{(0.05)^2} = 334$$

Where: **N-** Minimum number of the required samples, **p**- Proportion of the population estimated to have a problem (32 %), **q-** Proportion of the population without a problem (68 %), **Z-** Standard normal deviate (1.96) set at 95% confidence level and **D**- Degree of accuracy set at (0.05).

3.4 Sampling design

Cross sectional study design was employed applying the combination of Hazard analysis and Critical Control Point (HACCP) and *Codex Alimentarius Commission*

microbiological risk assessment. Purposive sampling design was employed in making the choice of supermarket. In particular, the criteria used was homogeneous sampling and four supermarkets were arrived at in the Nairobi central business district, based on the availability of all the required target samples in their storage facilities. Milk products from the supermarkets were randomly picked from the storage facilities taking into consideration the expiry date as indicated in the packet. Those with a shelf life of two days and below to expiry were not considered fit for bacteriological isolation as they were considered to be too old, due to proliferation of microorganisms initially present in milk. Normal pasteurized milk has a shelf life of four days as the top limit while long life milk has a maximum top limit of three months. Fermented milk has a maximum top limit of two months while ice-cream has a shelf life of up to six months if put under refrigeration. Raw milk is fit for microbiological isolation within six hours after milking if not boiled while up to fifteen hours if boiled. Fourteen samples of each product were collected (Table 3.1). Each sample was labeled depending on the supermarket it was collected from.

Raw milk was randomly obtained from shops in the selected points of distribution between 6.00 am and 7.00 am in the morning to acquire milk directly from farmers. This timing was necessary to acquire milk directly from farmers delivering milk to the milk shops. The samples were put inside an insulated cool box with ice-packs and transported to the laboratory. This exercise was repeated fortnightly up to the 9^{th} week to evaluate and establish the pattern of possible contamination. Nine samples of raw milk were collected from each of the six regions identified for analysis by the end of the intended

period. A total of three hundred and thirty four samples of milk and milk products were obtained from supermarkets and shops. This value was determined by Fisher formula (Fisher *et al*., 1993). One hundred and twelve samples of pasteurized milk were randomly picked from the supermarket, 112 samples of fermented and 56 samples of ice-cream respectively. Fifty four samples of raw milk were obtained directly from farmers and milk shops. Participatory epidemiological approach was focused on two groups: Milk shop owners (20 shop owners from each of the six regions) and Consumers/customers (20 consumers from each of the six regions). Information was obtained through a survey by means of face to face focus group discussions with consumers using a checklist to obtain qualitative information on their perception regarding safety of milk. At the milk shop level, interviews were conducted using well-structured questionnaires while no interview was conducted in the supermarkets. A total of 120 shop owners were interviewed within the study area (Appendix I and Appendix II).

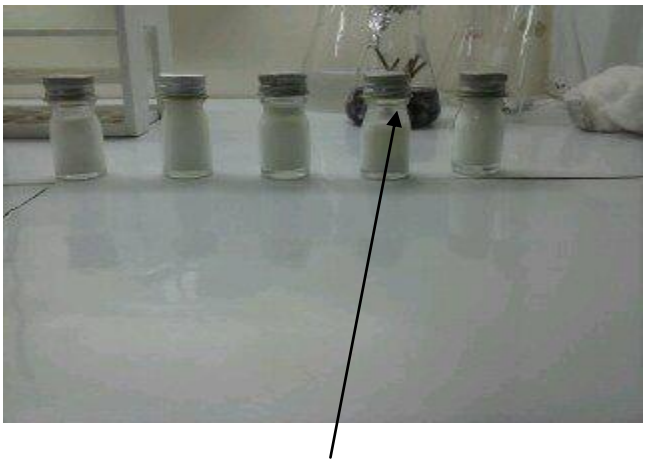
Table 3.1: Number of milk products collected and labeled from each supermarket

	SUPERMARKET OUTLETS			
Milk Product	**Sp A**	**Sp B**	**Sp C**	**Sp D**
FWM A	14	14	14	14
FWM B	14	14	14	14
YM A	14	14	14	14
YMB	14	14	14	14
IC	14	14	14	14

Sp – supermarket, FWM – fresh whole milk (pasteurized milk), YM – yoghurt (fermented milk), IC – ice cream.

3.5 Processing of samples

The working bench was first disinfected on arrival to the laboratory using formaldehyde (5 %), due to its broad-spectrum biocidal activity and is both effective for surface and space decontamination. The samples of pasteurized milk and fermented milk in 250 ml packaging were aliquoted 5 ml each into universal bottles aseptically and incubated at 37°C for 24 hours to activate and enhance microbial growth. Samples of raw milk were also aseptically aliquoted 5 ml into universal bottles and incubated at 37^{o}C for 24 hours. Ice-cream while still in containers were thawed and aliquoted 5 ml each into universal bottles aseptically and also incubated at 37^{o}C for 24 hours. After incubation, the contents of the universal bottles in all cases were thoroughly mixed by shaking. Plating on culture media was finally done for isolation of *S. aureus* (Mueena *et al*., 2015).



universal bottle containing milk

Plate 3.1: Milk sample aliquoted into universal bottle before incubation

3.6 Microbiological isolation in Blood agar and MacConkey agar

In isolation of microorganisms, loop-full of the samples were aseptically streaked directly on Blood agar and MacConkey agar and incubated at 37°C for 24 hours. MacConkey agar without crystal violet was preferred since it permits growth of Gram- positive bacteria. The media used were prepared as indicated in (Appendix III). Gram staining was done and microscopy done on the selected colonies. Presence of *S .aureus* was determined from milk and milk product samples using standard confirmatory methods for identification of *S. aureus*. *Staphylococcus* sp was denoted by pale pink, opaque color on the agar plate as shown in (Table 4.2). The other microorganisms were identified using standard methods for microbiological isolation and identification.

3.7 Subculture of suspected isolates in Mannitol salt agar

Mannitol salt agar was used as an indicator media for *S. aureus*. Isolates of *Staphylococcus* origin were sub-cultured in Mannitol Salt Agar (MSA) plates and incubated at 37° C and examined after 24-48 hrs for growth and change in the color of the medium. Organisms capable of using mannitol as a food source produce acidic products of fermentation that lowers the pH of the media. The acidity of the media causes the pH indicator, phenol red to turn yellow. *S. aureus* ferments mannitol while *S. epidermidis* does not. After incubation at 37° C, golden yellow halo colonies that were big, spherical, raised and smooth appearing in clusters were observed to grow fermenting Mannitol salt agar and hence the characteristic yellow color caused by *S. aureus*.

3.8 Isolation of *Candida* in Sabouraud`s dextrose agar

On Sabouraud`s dextrose agar, Candida appears as smooth creamy golden colonies. Germ tube test was done as a confirmatory test for *Candida albicans.* It was done by aliquoting 0.5 ml of sheep serum in a test tube and then inoculating it with the suspected yeast colonies (1-2 large colonies). The test tube containing the inoculum was then incubated for 2-3 hours at 37^{o} C. Using a Pasteur pipette, a drop of the suspension was placed on a slide and covered with a coverslip. The wet mount was examined microscopically for germ tubes. A positive result was indicated by a short hyphal extension arising laterally from a yeast cell with no constriction at the point of origin. A negative result had no hyphal extension arising from a yeast cell.

3.9 Biochemical tests

S. aureus identification was done by colony morphology, Gram staining and biochemical tests.

3.9.1 Identification of *S. aureus* by catalase test

For Gram-positive cocci, catalase test was performed to distinguish catalase-negative *Streptococcus* sp from catalase-positive *Staphylococcus* sp. Using a microscope slide, 3 % of hydrogen peroxide was added to a small sample of the isolate on the slide. Isolates that are catalase positive bubbles vigorously within 30 seconds forming bubbles on the slides while those that are catalase negative do not. Isolates identified as catalase-positive were further identified by coagulase test. Pathogenic *S. aureus* are both catalase and coagulase positive.

3.9.2 Identification of *S. aureus* by coagulase test

Isolates were inoculated into test tubes containing 0.5 ml of rabbit plasma. After mixing by gentle rotation, the tubes were incubated at 37°C along with a negative control tube containing a mixture of 0.5 ml of sterile tryptone soya broth (TSB) and 0.5 ml of rabbit plasma. Clotting was evaluated at 30 min intervals for the first 4 hours of the test and then after 24 hours of incubation. They were then allowed to stand undisturbed (Talaro *et al.*, 2005). Coagulation was verified after incubation, taking into consideration the following criteria:

1 Plasma does not form clumps at all and flows easily on tilting the test tube.
2 Small clumps are formed and the plasma flows gently on tilting the tube.
3 Large and sticky clumps are formed but the plasma flows slightly on tilting the tube.
4 The plasma completely clots and sticks on one end of the tube and cannot flow.

The isolates were considered coagulase positive if they exhibited either group 3 or 4 characteristic level of coagulation. The capacity of *S. aureus* to coagulate plasma is the principal characteristic of pathogenic *S. aureus* and is highly correlated to the capacity to produce enterotoxins harmful to the tissues of the infected host (Murray *et al.*, 2006). *Staphylococcus epidermidis* was differentiated from *Staphylococcus saprophyticus* by novobiocin susceptibility test. *Staphylococcus epidermidis* is sensitive while *Staphylococcus saprophyticus* is resistant to novobiocin antibiotic. The test was done by using a sterile swab in spreading the test suspension of pure cultures in tryptic soy broth

on the entire surface of Muller Hinton agar. Aseptically, 5 ug novobiocin disk was applied on the inoculated agar and incubated at 37^0 C for 24 hours. Zone diameter of inhibition was measured. Zone of inhibition <12 mm was interpreted as resistant while ≥16 mm was interpreted as sensitive. *Escherichia coli* were differentiated by IMViC tests. They were both Indole and Methyl red positive.

3.9.3 Microbiological identification by IMViC tests

Indole test was performed by growing pure cultures on sterile Tryptophan broth for 24-48 hours. After incubation, 5 drops of Kavoc`s reagent were added to the culture broth. A positive result was indicated by a red layer at the top of the tube and a negative layer was indicated by lack of color change at the top of the tube. Methyl red test and Voges-Proskauer test were both done in Methyl red-Voges-Proskauer broth. A positive Methyl red test was identified by development of a red color after addition of methyl red reagent. A negative result was indicated by no color change. In Voges-Proskauer test, a positive result was indicated by development of a red-brown color after addition of Barritt`s A and Barritt`s B reagent. Citrate utilizantion test was performed on Simmons citrate agar. A positive citrate result was indicated by growth and a blue color change. A negative Citrate utilization test was indicated by lack of growth and color change in the tube.

3.10 Antimicrobial sensitivity test

To establish antimicrobial sensitivity testing, the correctly identified *S. aureus* was thawed at room temperature and the sample used for subculture. Panels of selected antibiotics commonly used in empirical treatment of *S aureus* infections informed the

choice of antibiotics used. Antibiotics used were: Penicillin G (10µg) a penicillin, Erythromycin (15µg) a macrolide, Vancomycin (5µg) a glycopeptide, Chloramphenicol (30µg), Tetracycline (30µg) a tetracycline, Gentamycin (30µg) an (aminoglycoside), Methicillin (10µg) a penicillin and Ciprofloxacin (5µg) a fluoroquinolone.

Their effects on growth of *S. aureus* were evaluated by harvesting young and overnight pure cultures after 24 hours of incubation using a sterile inoculating loop to touch four or five isolated colonies of the pathogen growing on agar and then used to inoculate a tube of sterile culture broth. The culture was then incubated at 37ºC until it became slightly turbid. It was then diluted with normal saline to match McFarland`s turbidity standard of 0.5 which is an approximate cell count density of 1.5×10^8 cells. A sterile cotton swab was dipped into the standardized bacterial test suspension and used to evenly spread the entire surface of Mueller-Hinton agar plate. After the agar surface dried for about 5 minutes, the appropriate antibiotics were placed at equidistant on it using sterile forceps.

Filter papers containing the antibiotics of choice were gently pressed down to ensure maximum contact with the surface of Mueller-Hinton agar. The test plates were then incubated at 37° C for 24 hours and then removed and inspected for satisfactory growth of *S. aureus.* The diameter of the zones of inhibition for each antibiotic was read as recommended by the Clinical and Laboratory Standards Institute (CLSI) standard (CLSI, 2009). Antimicrobial sensitivity was determined by the disk diffusion assay as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2009). Resistance and sensitivity by pathogenic *S. aureus* was categorized as sensitive (S),

intermediate (I) or resistant (R) using the zone of inhibition interpretation guidelines defined by the Clinical and Laboratory Standards Institute. The reference strain used for quality control was *S. aureus* ATCC$^{@}$ 25923 to check and compare sensitivity of *S. aureus* isolates to the different antibiotics they were exposed to (CLSI, 2009).

3.11 Determination of products with the highest level of contamination

All the products investigated for contamination with *S aureus* were marked. Total bacteria counts from the contaminated samples were determined using a colony counter to establish the levels of contamination. A percentage (%) conversion was obtained from the product(s) contaminated with *S. aureus*.

3.12 Data analysis

The data collected was subjected to descriptive statistics, frequencies and percentages for the prevalence of the bacteria in the milk and milk products. Analysis of variance (ANOVA) was used to compare zones of inhibition by the antimicrobial agents on the growth of the bacteria (Appendix IV). Chi-square test was used to determine any significant association between variables in the level of bacterial contamination and in risk factors assessment. These analyses were carried out using Statistical package of social science (SPSS) computer software at a test level of $P \leq 0.05$ levels.

CHAPTER FOUR

RESULTS

4.1 Microbiological isolation from marketed milk and milk products

Milk and milk product samples were determined to have *Staphylococcus* sp. This led to further tests to determine the presence of pathogenic *S. aureus* in the samples.

Table 4.1: Microbiological isolation in Blood agar and MacConkey agar

Identification	Colony on MacConkey agar without crystal violet	Colony on Blood agar	Catalase Test	Indole test	Coagulase test	Methyl red test	Voges-Proskauer test	Citrate utilization test
Escherichia coli	Pink to red, Lactose-ve, flat, dry	Non-hemolytic	+ve	+ve	-	+ve	-ve	-ve
Staphylococcus aureus	Pale pink, Opaque	Hemolytic, opaque, whit-yellow	+ve	-	+ve	-	-	-
***Bacillus* sp**	Forms dull, rough, wrinkled colonies	Hemolytic, wrinkled colonies	-	-	-	-	-	-
Candida	-	Moist, opaque, creamy colonies.	-	-	-	-	-	-

KEY: +ve - positive, -ve - negative

4.1.1 Subculture of suspected isolates in Blood agar and Mannitol salt agar

Pathogenic *S. aureus* showed β-hemolysis in blood agar after incubation while *S. epidermidis* exhibited γ-hemolysis, Plate 4.1.

Plate 4.1: Plates showing β- Hemolysis a characteristic of *S. aureus* in Blood agar

In Mannitol salt agar, presence of growth and change of pH in the media (red to yellow) was regarded as confirmative identification of the salt tolerant Staphylococci. (Plate 4.2a and 4.2b). The negative control was Plate 4.2a while the positive result was Plate 4.2b.

Plate 4.2a: A plate showing streaked isolates of suspected *S. aureus* on Mannitol salt agar (MSA) before incubation. Notice the red color of phenol red indicator

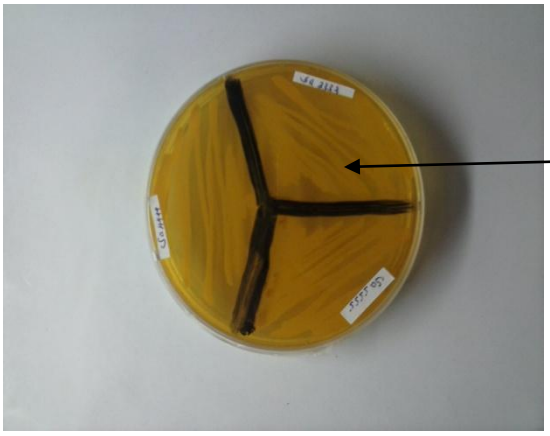


Plate 4.2b: A plate showing complete fermentation of Mannitol salt agar (MSA) by suspected *S. aureus* isolates after incubation. Notice the plate is turned yellow.

4.1.2 Gram staining properties of suspected colonies

All suspected cultures of *Staphylococcus* species were subjected to Gram's stain and observed under a light microscope for Gram's reaction, size, shape and cell arrangements. The Gram-stained smears from typical colonies that showed Gram-positive cocci

occurring in bunches, grapelike irregular clusters were taken as presumptive *Staphylococcus* species (Plate 4.3a and Plate 4.3b). The colonies were isolated and sub-cultured awaiting further confirmatory test. However, Gram positive rods were also found and identified as Gram +ve *Bacilli* sp based on their Gram's reaction, size, shape and cell arrangements. Gram +ve *Bacillus* sp appeared rod-shaped when observed under a microscope. They formed spores when they were left to overgrow in the culture media.

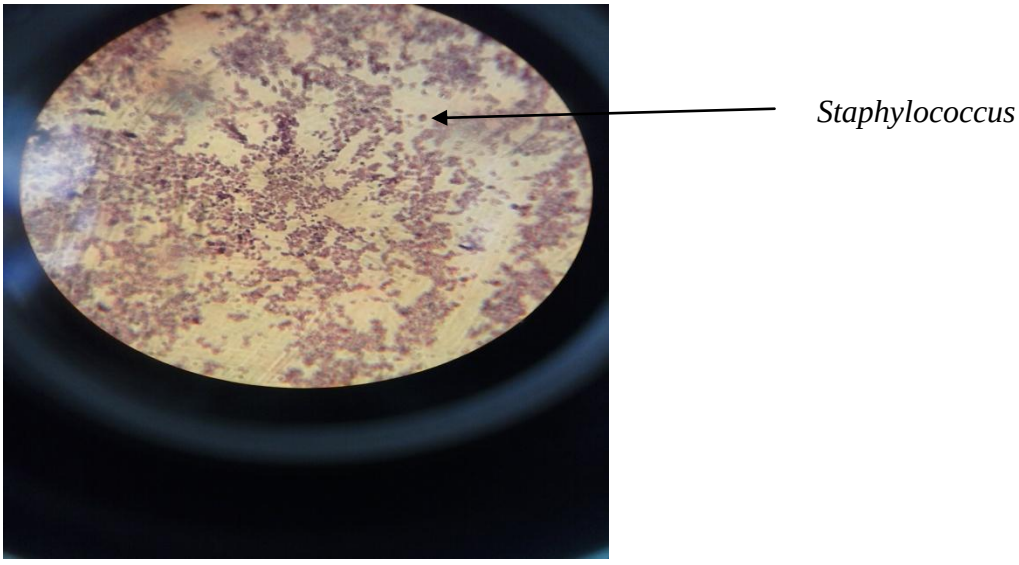


Plate 4.3a: Notice the Gram +ve dark spherical shaped cell in purple. The cell is *Staphylococcus*

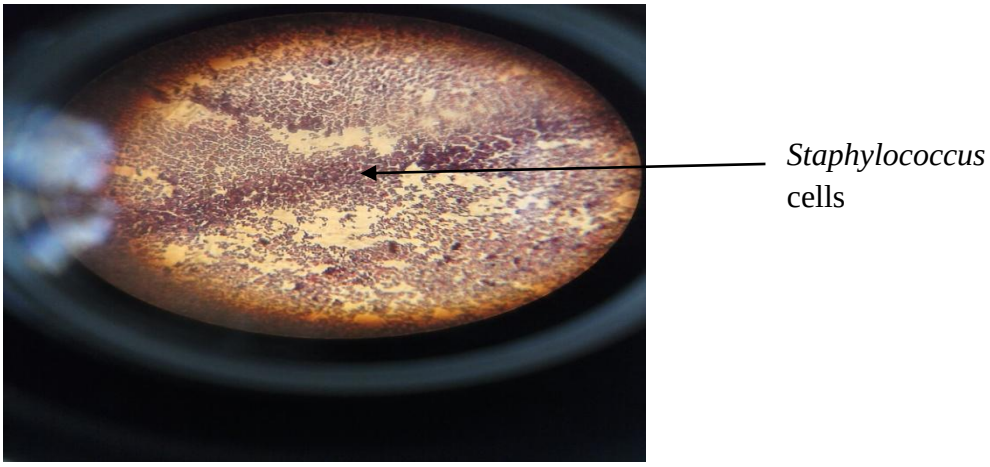


Plate 4.3b: Notice the many dark spherical shaped cells in purple

4.2 Microbial contamination of milk and milk products

Besides the targeted *S. aureus*, other bacteria and a fungus were isolated from milk and milk products in this study. These were *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Bacillus* sp and *E. coli* bacteria while the fungus was *Candida* as shown in (Table 4.2). Novobiocin susceptibility test was done to differentiate *S. epidermidis* from *S. saprophyticus* where *S. epidermidis* is sensitive. To establish if there was any significant difference in contamination of specific milk and milk products, Chi-square test was used and a contigency table was set up to establish which of them had the highest microbial contamination. The chi-square statistic was 58.8055, H = 4.17, df = 3, P = 0.00001. There was a significant difference in microbial contamination set at $P<0.05$.

Table 4.2: Microbiological contamination of milk and milk products

Organism	Number of contaminated samples in milk			
	Pasteurized milk (n =112)	**Fermented milk (n = 112)**	**Ice-cream (n= 56)**	**Raw milk (n = 54)**
Staphylococcus epidermidis	62 (55.4 %)	28 (25 %)	18 (32.1 %)	49 (87.5 %)
Staphylococcus aureus	23 (20.5 %)	12 (10.7 %)	2 (3.6 %)	35 (64.81 %)
Staphylococcus saprophyticus	31 (27.7 %)	18 (16.7 %)	7 (12.5 %)	15 (26.8 %)
Bacillus sp	70 (62.5 %)	68 (60.7 %)	11 (19.6 %)	18 (32.1 %)
Candida	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	2 (3.6 %)
E. coli	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	45 (80.4 %)
	H = 4.17	df = 3	P = 0.00001	

4.3 Biochemical tests on *Staphylococcus* species isolated

Biochemical tests of microorganisms found to be of *Staphylococcus* origin were done and the results showed that *Staphylococcus aureus* coagulated rabbit plasma forming sticky clots in the test tubes. They were also catalase positive in 3 % hydrogen peroxide as opposed to the other *Staphylococcus* sp (Appendix V).

4.3.1 Coagulase test results

Plates illustrated in (4.5a, 4.5b, and 4.5c) below shows level 4 degree of coagulation as indicated in (Section 3.8.2). Blood plasma completely clots and sticks on one end of the tube and cannot flow.

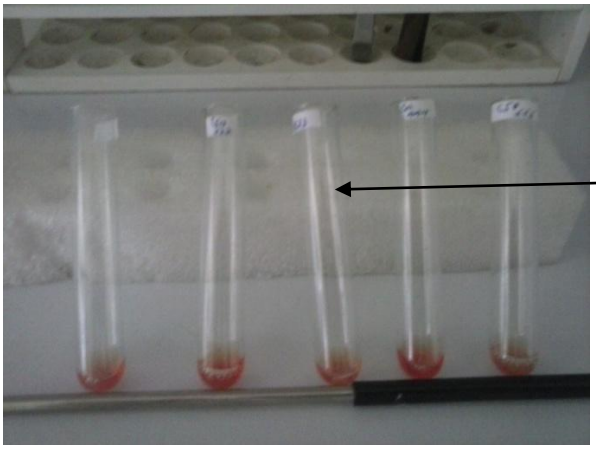


Plate 4.4: Notice test tubes containing blood plasma inoculated with suspected *S. aureus* isolates at the onset of observation.

Plate 4.5a: A test tube inverted at horizontal showing a positive coagulase test

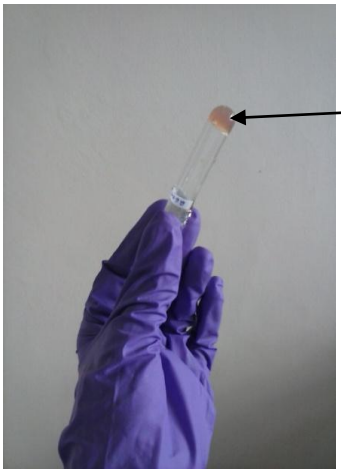


Plate 4.5b: Diagonally inverted test tube showing a positive coagulase test

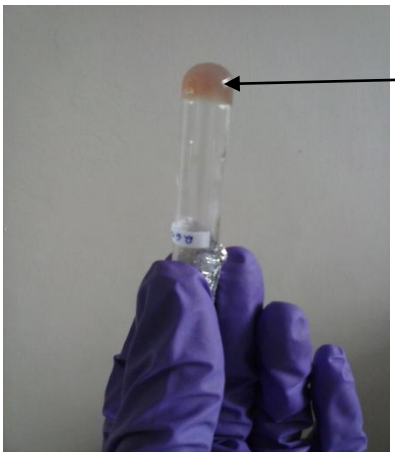


Plate 4.5c: Notice the test tube of coagulated plasma completely (vertically) inverted showing a positive coagulase test. Solidified plasma sticks on one end of the tube

4.4 Levels of contamination by pathogenic *S. aureus*

The levels of total bacteria counts recorded in milk and milk product samples were compared to WHO and KEBS recommended safety levels of 3.0×10^4 and 2.0×10^6 colony forming units (CFU/ml) in milk. In all the samples, the total bacteria count (TBC) values of contaminated samples were higher than WHO recommended safety levels

showing that the samples were of unacceptable levels of contamination and therefore not safe for consumption (Table 4.3). There was a significant difference in contamination compared with the acceptable level by WHO. Chi-square statistic = 354543.98, df = 4, p = 0.0001.

Table 4.3: Total Bacterial Counts in milk and milk products

Source	**Average TBC Values (CFU/ml)**
Raw milk (Direct from farmers)	3.2 a × 10^6
Raw milk (From shop outlets)	3.8 a × 10^6
Pasteurized milk	4.2 a × 10^7
Fermented milk	4.8 a × 10^7
Ice-cream	3.3 a × 10^7
WHO acceptable level of contamination	3.0 b × 10^4
Kenya Bureau of Standards maximum limits	2.0 c × 10^6

Values indicated with similar letters are not significantly different (p> 0.05). Values indicated with different letters are significantly different (p< 0.05).
TBC- total bacteria count, CFU- colony forming unit

A total of 334 samples of milk and milk products were randomly collected from the outlets identified. One hundred and twelve samples of pasteurized milk were collected accounting for 33.5 % of the total samples. Out of this, 23 (20.54 %) of the samples were found contaminated with *S. aureus.* Twelve of the yoghurt samples were contaminated by *S. aureus*, accounting for 10.71 % of the yoghurt samples, two of the ice-cream samples were contaminated, accounting for 3.6 % of the ice-cream samples and thirty five samples of raw milk, accounting for 64.81 % of the raw milk samples were contaminated with *S. aureus* (Table 4.4).

Table 4.4: Samples of milk and milk products contaminated with *S. aureus*

	Samples contaminated by pathogenic *S. aureus*	
Samples	**n**	**n (%)**
Pasteurized milk	112	23 (20. 54 %)
Yoghurt	112	12 (10. 71 %)
Ice – cream	56	2 (3. 57 %)
Raw milk	54	35 (64. 81 %)
Total	334	72 (21.56 %)

KEY: n - total number of samples, n (%) - samples contaminated in percentage

From all the six regions investigated for isolation of *S. aureus* in raw milk, Langata had the highest number of contaminated samples while Embakasi had the lowest (Figure 4.1).

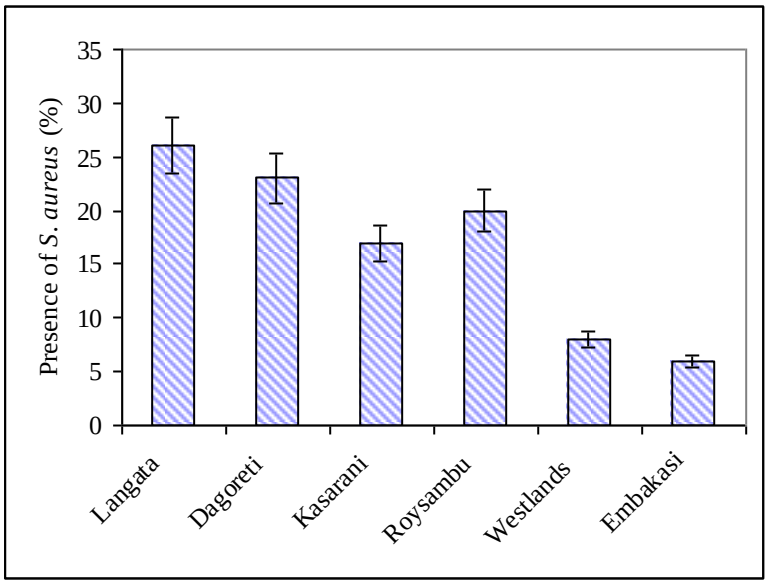


Figure 4.1: Presence of *S. aureus* isolates in raw milk from different settlements within Nairobi County.

4.5 Distribution of *S. aureus* isolated within 9 weeks of investigation

Distributions of *S. aureus* isolates in the various products were as indicated in Figure 4.2 and Appendix V.

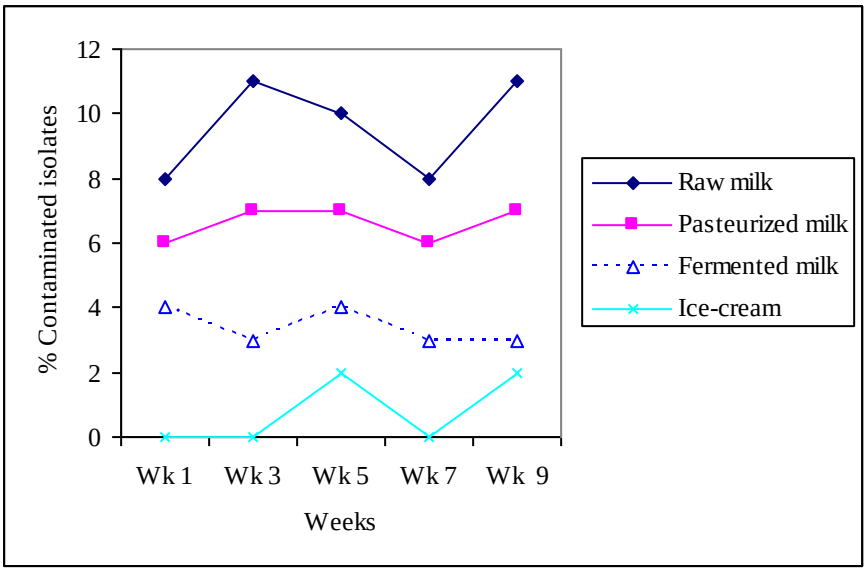
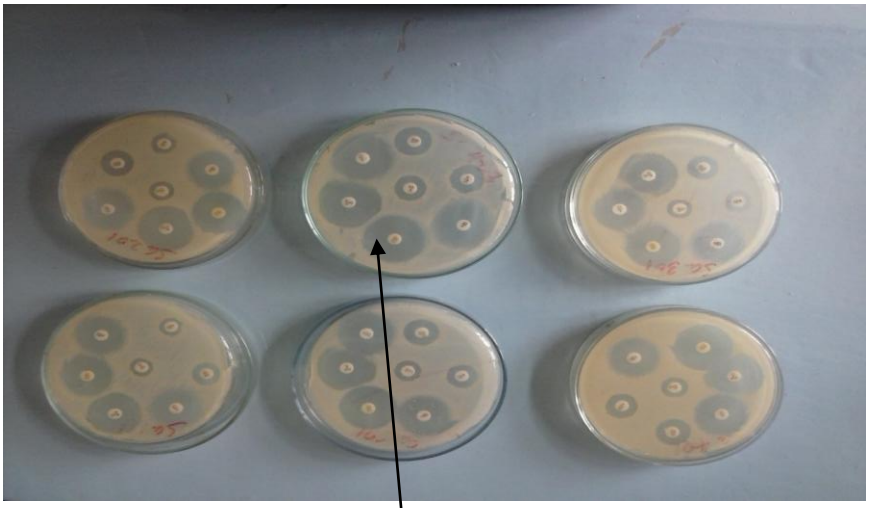


Figure 4.2: Percentage contamination of milk and milk product by *S. aureus* within 9 weeks of investigation

4.6 Antimicrobial susceptibility patterns of *S. aureus* isolated from milk and milk products

4.6.1 Susceptibility patterns of *S. aureus* isolates in the samples

The clear zones in Plate 4.6 are a demonstration of the susceptibility patterns of *Staphylococcus aureus*.



zone of inhibition measured in mm

Plate 4.6: Zones of inhibition showing sensitive strains

4.6.2 Sensitivity profiles obtained from samples of milk and milk products

4.6.2.1 Isolates from pasteurized milk in packets

Zones of inhibition of the growth of *S. aureus* in pasteurized milk when exposed to 8 different types of antibiotics showed that, there was significantly higher inhibition by Gentamycin (mean inhibition zone 26.9 mm), Erythromycin (mean 26.7 mm) and Penicillin (mean 26.4 mm) than the other antibiotics. Out of the 23 *S. aureus* isolates, 5 isolates were sensitive to Gentamycin and 1 isolate was sensitive to Penicillin. All the 19 isolates were sensitive to Tetracycline, Ciprofloxacin, Erythromycin and Methicillin. 13 of the *S. aureus* isolates were resistant to Penicillin and none of the isolates was resistant to the other seven antibiotics, f = 421.45, df = 7, p= 0.0001 (Table 4.5 and Table 4.6).

Table 4.5: Mean zones of inhibition of *S. aureus* by antimicrobial agents in pasteurized milk

***S. aureus* Isolate**	**Chl (Mm)**	**Gen (Mm)**	**Van (Mm)**	**Pen (Mm)**	**Tet (Mm)**	**Cip (Mm)**	**Ery (Mm)**	**Met (Mm)**
Mean	25.4c	26.9d	14.3a	26.4d	20.0b	13.5a	26.7d	13.3a
SE	0.35	0.31	0.23	0.37	0.31	0.25	0.21	0.38

Values indicated with similar letters are not significantly different ($p > 0.05$). Values indicated with different letters are significantly different ($p < 0.05$).

Chloramphenicol (Chl), Gentamycin (Gen), Vancomycin (Van), Penicillin G (Pen), Tetracycline (Tet), Ciprofloxacin (Cip), Erythromycin (Ery), Methicillin (Met)

Table 4.6: Sensitivity profiles of *S. aureus* obtained from pasteurized milk

***S. aureus* (S. a) Isolate**	**Chl Zone diameter (Mm)**	**profile**	**Gen Zone diameter (Mm)**	**profile**	**Van Zone diameter (Mm)**	**profile**	**Pen Zone diameter (Mm)**	**profile**	**Tet Zone diameter (Mm)**	**profile**	**Cip Zone diameter (Mm)**	**profile**	**Ery Zone diameter (Mm)**	**profile**	**Met Zone diameter (Mm)**	**profile**
S. a 101	25	I	14	I	11	I	11	R	26	S	25	S	26	S	20	S
S. a 102	24	I	15	I	12	I	13	R	25	S	27	S	25	S	21	S
S. a 103	29	I	14	I	15	I	17	S	30	S	29	S	28	S	19	S
S. a 104	25	I	14	I	13	I	13	R	26	S	27	S	25	S	18	S
S. a 105	27	I	16	S	14	I	14	I	27	S	28	S	27	S	20	S
S. a 106	26	I	15	I	13	I	15	I	25	S	26	S	26	S	21	S
S. a 107	25	I	16	S	14	I	16	I	28	S	25	S	27	S	18	S
S. a 108	24	I	14	I	13	I	14	I	26	S	27	S	25	S	19	S
S. a 109	28	I	15	I	14	I	15	I	27	S	28	S	28	S	18	S
S. a 110	26	I	13	I	12	I	10	R	26	S	29	S	27	S	20	S
S. a 111	23	I	14	I	13	I	12	R	25	S	26	S	26	S	22	S
S. a 112	24	I	15	I	15	I	13	R	27	S	25	S	28	S	20	S
S. a 201	25	I	16	S	12	I	12	R	25	S	27	S	26	S	19	S
S. a 202	25	I	13	I	12	I	12	R	25	S	26	S	26	S	20	S
S. a 203	29	I	17	S	14	I	15	I	29	S	28	S	27	S	24	S
S. a 204	23	I	11	I	13	I	11	R	28	S	28	S	26	S	23	S
S. a 205	24	I	12	I	14	I	13	R	27	S	27	S	26	S	19	S
S. a 206	26	I	14	I	13	I	14	I	28	S	28	S	27	S	18	S
S. a 208	25	I	15	I	14	I	14	I	26	S	26	S	25	S	21	S
S. a 209	27	I	14	I	13	I	12	R	25	S	26	S	28	S	23	S
S. a 210	24	I	13	I	12	I	13	R	28	S	27	S	26	S	21	S
S. a 211	25	I	12	I	15	I	15	R	29	S	28	S	27	S	19	S
S. a 212	26	I	16	S	14	I	16	I	26	S	26	S	25	S	17	S

Chloramphenicol (Chl), Gentamycin (Gen), Vancomycin (Van), Penicillin G (Pen), Tetracycline (Tet), Ciprofloxacin (Cip), Erythromycin (Ery), Methicillin (Met)

4.6.2.2 Isolates from fermented milk

Zones of inhibition of *S. aureus* isolated from fermented milk when exposed to 8 different antibiotics showed that, there was significantly higher inhibition by Ciprofloxacin (mean inhibition zone 27.5mm), Tetracycline (mean 26.3mm), Erythromycin (mean 26.3 mm) and Chloramphenicol (mean 26.0 mm) than the other antibiotics (Table 4.7). Out of the 12 *S aureus* isolates, 5 isolates were sensitive to Gentamycin. All the 12 isolates were sensitive to Tetracycline, Ciprofloxacin, Erythromycin and Methicillin. There was no resistant isolate of *S. aureus* from fermented milk to any of the antimicrobial agents they were exposed to, f = 204.85, df = 7, p = 0.0001 (Table 4.8).

Table 4.7: Mean zones of inhibition of *S. aureus* in fermented milk by antimicrobial agents

***S. aureus* Isolate**	**Chl (Mm)**	**Gen (Mm)**	**Van (Mm)**	**Pen (Mm)**	**Tet (Mm)**	**Cip (Mm)**	**Ery (Mm)**	**Met (Mm)**
Mean	26.0d	15.3b	13.0a	13.8a	26.3d	27.5d	26.3d	19.7c
SE	0.59	0.38	0.33	0.44	0.38	0.42	0.41	0.50

Values indicated with similar letters are not significantly different ($p > 0.05$). Values indicated with different letters are significantly different ($p < 0.05$).

Chloramphenicol (Chl), Gentamycin (Gen), Vancomycin (Van), Penicillin G (Pen), Tetracycline (Tet), Ciprofloxacin (Cip), Erythromycin (Ery), Methicillin (Met)

Table 4.8: Sensitivity profiles of *S. aureus* obtained from fermented milk

***S. aureus* (S. a) Isolate**	**Chl Zone diameter (Mm) & profile**		**Gen Zone diameter (Mm) & profile**		**Van Zone diameter (Mm) & profile**		**Pen Zone diameter (Mm) & profile**		**Tet Zone diameter (Mm) & profile**		**Cip Zone diameter (Mm) & profile**		**Ery Zone diameter (Mm) & profile**		**Met Zone diameter (Mm) & profile**	
S. a 301	27	I	14	I	11	I	12	R	24	S	26	S	26	S	22	S
S. a 302	23	I	15	I	12	I	11	R	26	S	26	S	24	S	21	S
S. a 303	25	I	14	I	13	I	15	I	28	S	29	S	26	S	18	S
S. a 304	24	I	15	I	14	I	14	I	25	S	28	S	25	S	18	S
S. a 305	25	I	15	I	13	I	13	R	26	S	27	S	27	S	19	S
S. a 306	26	I	14	I	12	I	12	R	28	S	25	S	28	S	18	S
S. a 307	25	I	14	I	14	I	14	I	26	S	29	S	26	S	20	S
S. a 401	28	I	17	S	13	I	15	I	28	S	28	S	26	S	19	S
S. a 402	24	I	16	S	12	I	14	I	27	S	27	S	26	S	18	S
S. a 403	30	I	18	S	15	I	16	I	25	S	30	S	29	S	21	S
S. a 404	28	I	16	S	14	I	15	I	26	S	28	S	28	S	19	S
S. a 405	27	I	16	S	13	I	15	I	27	S	27	S	25	S	23	S

Chloramphenicol (Chl), Gentamycin (Gen), Vancomycin (Van), Penicillin G (Pen), Tetracycline (Tet), Ciprofloxacin (Cip), Erythromycin (Ery), Methicillin (Met)

4.6.2.3 Isolates from ice-cream

Out of two *S. aureus* isolates, one isolate was sensitive to Gentamycin, Both of the 2 isolates were sensitive to Tetracycline, Ciprofloxacin, Erythromycin and Methicillin. Only one isolate was resistant to Penicillin G (Table 4.9).

Table 4.9: Sensitivity of *S. aureus* obtained from ice-cream

***S. ureus* (S. a) Isolate**	**Chl diameter (Mm) profile**		**Gen Zone diameter (Mm) profile**		**Van Zone diameter (Mm) profile**		**Pen Zone diameter (Mm) profile**		**Tet Zone diameter (Mm) profile**		**Cip Zone diameter (Mm) profile**		**Ery Zone diameter (Mm) profile**		**Met Zone diameter (Mm) profile**	
501	25	I	16	S	13	I	15	I	26	S	27	S	27	S	20	S
502	26	I	14	I	12	I	12	R	25	S	26	S	25	S	21	S

Chloramphenicol (Chl), Gentamycin (Gen), Vancomycin (Van), Penicillin G (Pen), Tetracycline (Tet), Ciprofloxacin (Cip), Erythromycin (Ery), Methicillin (Met)

4.6.2.4 Isolates from raw milk

Zones of inhibition of the growth of *S. aureus* in raw milk when exposed to 8 different types of antimicrobial agents showed that, there was significantly higher inhibition by Tetracycline (mean inhibition zone 28.26 mm), Ciprofloxacin (mean inhibition 26.77 mm), Chloramphenicol (mean inhibition 25.97 mm), Gentamycin (mean inhibition 26.26 mm) and Erythromycin (mean inhibition 27.03 mm) than Methicillin (mean inhibition 22.66 mm), Penicillin (mean inhibition 15.60 mm) and Vancomycin (mean inhibition 14.09 mm), f = 442.50, df = 7, p = 0.0001 (Table 4.10).

Table 4.10: Mean zones of inhibition of *S. aureus* to antibiotics in raw milk

***S. aureus* Isolate**	**Chl (Mm)**	**Gen (Mm)**	**Van (Mm)**	**Pen (Mm)**	**Tet (Mm)**	**Cip (Mm)**	**Ery (Mm)**	**Met (Mm)**
Mean	25.97d	26.26d	14.09a	15.60b	28.26d	26.77d	27.03d	22.66c
SE	0.22	0.18	0.19	0.26	0.30	0.36	0.27	0.26

Values indicated with similar letters are not significantly different ($p > 0.05$). Values indicated with different letters are significantly different ($p < 0.05$)

Chloramphenicol (Chl), Gentamycin (Gen), Vancomycin (Van), Penicillin G (Pen), Tetracycline (Tet), Ciprofloxacin (Cip), Erythromycin (Ery), Methicillin (Met)

Thirty five *S. aureus* isolates were obtained from raw milk. Out of the 35 isolates of *S. aureus*, 3 isolates were resistant to Penicillin G, 10 isolates were sensitive to Penicillin G and 4 were sensitive to Vancomycin. All the 35 isolates were sensitive to Gentamycin, Tetracycline, Ciprofloxacin, Erythromycin and Methicillin. All were intermediate to Chloramphenicol. *S. aureus* isolates subjected to the eight antimicrobial agents were largely sensitive while the isolates were intermediate to other antibiotics used (Table 4.11).

Table 4.11: Sensitivity profiles of *S. aureus* obtained from raw milk

***S. aureus* (S. a) Isolate**	**Chl Zone diameter (Mm) & profile**		**Gen Zone diameter (Mm) & profile**		**Van Zone diameter (Mm) & profile**		**Pen Zone diameter (Mm) & profile**		**Tet Zone diameter (Mm) & profile**		**Cip Zone diameter (Mm) & profile**		**Ery Zone diameter (Mm) & profile**		**Met Zone diameter (Mm) & profile**	
S. a 601	26	I	27	S	15	I	18	S	28	S	22	S	25	S	25	S
S. a 602	27	I	28	S	14	I	18	S	32	S	32	S	30	S	24	S
S. a 603	24	I	25	S	13	I	14	I	31	S	29	S	26	S	23	S
S. a 604	25	I	27	S	14	I	15	I	27	S	27	S	26	S	22	S
S. a 605	27	I	26	S	15	I	16	S	29	S	30	S	27	S	20	S
S. a 606	26	I	26	S	13	I	13	R	25	S	26	S	25	S	21	S
S. a 607	25	I	25	S	16	S	16	I	27	S	27	S	26	S	24	S
S. a 608	24	I	27	S	15	I	15	I	28	S	28	S	25	S	23	S
S. a 609	27	I	28	S	13	I	18	S	27	S	29	S	28	S	22	S
S. a 610	28	I	28	S	14	I	14	I	30	S	30	S	27	S	20	S
S. a 611	26	I	27	S	13	I	15	I	28	S	27	S	26	S	22	S
S. a 612	28	I	25	S	15	I	16	I	29	S	28	S	28	S	24	S
S. a 613	25	I	26	S	14	I	18	S	27	S	25	S	26	S	23	S
S. a 614	26	I	27	S	13	I	14	I	26	S	26	S	27	S	20	S
S. a 615	24	I	26	S	13	I	16	I	29	S	24	S	30	S	24	S
S. a 616	27	I	27	S	15	I	17	S	31	S	28	S	29	S	23	S
S. a 617	26	I	25	S	14	I	15	I	28	S	27	S	28	S	22	S
S. a 618	26	I	26	S	16	S	16	I	27	S	28	S	25	S	22	S
S. a 619	25	I	27	S	13	I	14	I	28	S	23	S	27	S	25	S
S. a 620	24	I	25	S	14	I	17	S	30	S	29	S	26	S	23	S
S. a 621	25	I	26	S	15	I	13	R	29	S	24	S	28	S	25	S
S. a 622	25	I	26	S	14	I	14	I	26	S	26	S	27	S	22	S
S. a 623	26	I	27	S	15	I	15	I	31	S	28	S	26	S	24	S
S. a 624	27	I	26	S	12	I	16	I	29	S	26	S	25	S	20	S
S. a 625	25	I	25	S	13	I	16	I	28	S	25	S	27	S	23	S
S. a 626	27	I	24	S	13	I	18	S	26	S	26	S	30	S	20	S
S. a 627	25	I	26	S	15	I	15	I	28	S	27	S	28	S	24	S
S. a 628	28	I	27	S	16	S	16	I	29	S	28	S	29	S	23	S
S. a 629	28	I	26	S	13	I	17	S	30	S	26	S	25	S	25	S
S. a 630	27	I	28	S	13	I	18	S	29	S	27	S	28	S	22	S
S. a 631	28	I	27	S	15	I	13	R	27	S	27	S	28	S	23	S
S. a 632	26	I	25	S	12	I	15	I	28	S	23	S	29	S	23	S
S. a 633	27	I	26	S	15	I	16	I	31	S	25	S	28	S	22	S
S. a 634	24	I	27	S	16	S	15	I	26	S	26	S	27	S	21	S
S. a 635	25	I	25	S	14	I	14	I	25	S	28	S	24	S	24	S

Chloramphenicol (Chl), Gentamycin (Gen), Vancomycin (Van), Penicillin G (Pen), Tetracycline (Tet), Ciprofloxacin (Cip), Erythromycin (Ery), Methicillin (Met)

All isolates from the four different sources were 100 % susceptible to Tetracycline, Ciprofloxacin, Erythromycin and Methicillin. All isolates were 100 % intermediate to Chloramphenicol while all isolates from fresh whole milk, ice-cream and raw milk were 100 % intermediate to Vancomycin (Table 4.12 and Table 4.13).

Table 4.12: Numbers (%) of susceptible isolates

Milk type	Chl	Gen	Van	Pen	Tet	Cip	Ery	Met
Fresh whole milk	0.00	22.0	0.00	4.00	100.0	100.0	100.0	100.0
Yoghurt	0.00	58.0	0.00	0.00	100.0	100.0	100.0	100.0
Ice-cream	0.00	50.0	0.00	0.00	100.0	100.0	100.0	100.0
Raw milk	0.00	100.0	11.43	29.0	100.0	100.0	100.0	100.0

Chloramphenicol (Chl), Gentamycin (Gen), Vancomycin (Van), Penicillin G (Pen), Tetracycline (Tet), Ciprofloxacin (Cip), Erythromycin (Ery), Methicillin (Met)

Table 4.13: Numbers (%) of intermediate isolates from milk and milk products

	Antimicrobial agents							
Source of Isolates	**C30**	**CN30**	**VA5**	**P10**	**TE30**	**Cip5**	**E15**	**ME10**
Fresh Whole milk	100.00	78.26	100.00	39.00	0.00	0.00	0.00	0.00
Yoghurt	100.00	0.00	89.00	63.00	0.00	0.00	0.00	0.00
Ice-cream	100.00	50.00	100.00	50.00	0.00	0.00	0.00	0.00
Raw milk	100.00	42.00	100.00	67.00	0.00	0.00	0.00	0.00

Chloramphenicol (Chl), Gentamycin (Gen), Vancomycin (Van), Penicillin G (Pen), Tetracycline (Tet), Ciprofloxacin (Cip), Erythromycin (Ery), Methicillin (Met)

4.7 Risk assessment on specific milk food safety hazard

Six settlements selected in this study within Nairobi area had milk sold in four different types of milk. In Roysambu, Dagoreti, Kasarani, Langata, Embakasi and Westlands area, the major type of milk sold was pasteurized milk while no shop outlet was found selling ice-cream other than in the supermarkets (Table 4.14). There was no significant

difference on the type of milk sold across the six different settlements, chi-square = 7.69, df = 10, p = 0.659.

Table 4.14: Number of outlets selling milk and milk products within the study area

		Number of outlets selling milk and milk products			
Region Evaluated	**N**	**Raw milk**	**Pasteurized Milk**	**Fermented milk-Yogurt**	**Ice-cream**
Roysambu	20	7 (35%)	17 (85%)	9 (45%)	0 (0.0%)
Dagoreti	20	10 (50%)	18 (90%)	8 (40%)	0 (0.0%)
Kasarani,	20	8 (40%)	16 (80%)	7 (35%)	0 (0.0%)
Langata	20	14(70%)	15 (75%)	6 (30%)	0 (0.0%)
Embakasi	20	4 (20%)	19 (95%)	8 (40%)	0 (0.0%)
Westlands	20	7 (35%)	18 (90%)	9 (45%)	0 (0.0%)
		$X^2 = 7.69$	df = 10	p = 0.659	

Figures in brackets are percentages of the specific total sample (N=20)

Results showed that the major containers used to deliver raw milk to the milk outlets was plastic containers. Aluminum containers were used in low numbers compared to plastic containers (Table 4.15). However, there was no significant difference on the type of container used across the different regions, chi-square = 4.45, df = 5, p = 0.486.

Table 4.15: Types of container used in the Milk shops

Region Evaluated	N	Types of containers used to procure raw milk in the milk shops (outlets)	
		Plastic Container	Aluminum Container
Roysambu	20	16 (80%)	4 (20%)
Dagoreti	20	18 (90%)	2 (10%)
Kasarani	20	19 (95%)	1 (5%)
Langata	20	18 (90%)	2 (10%)
Embakasi	20	15 (75%)	5 (25%)
Westlands	20	17 (85%)	3 (15%)
	$X^2 = 4.45$	df = 5	p = 0.486

Figures in brackets are percentages of the specific total sample (N=20)

In these areas, milk is mainly transported by use of motor bikes. In Langata, 100 % of the milk is transported by use of motorbikes. In Roysambu, Dagoreti, Kasarani, Embakasi and Westlands, besides the motorbike, milk is also transported by use of pick-ups and other vehicles (Table 4.16).

Table 4.16: Means of transport used in carrying milk to the outlets

Region Evaluated	N	Means of transport used	
		Motor bike	Pick-up/ vehicle
Roysambu	20	17 (85%)	3 (15%)
Dagoreti	20	17 (85%)	3 (15%)
Kasarani	20	18 (90%)	2 (10%)
Langata	20	20 (100%)	0 (0.0%)
Embakasi	20	14 (70%)	6 (30%)
Westlands	20	15 (75%)	5 (25%)

Figures in brackets are percentages of the specific total sample (N=20)

In all the six areas, the major source of milk was from the farmers. A relatively higher proportion of milk (35 %) delivered to the shops in Langata was from the vendors who

obtained the milk from farmers (Table 4.17). However, there was no significant difference on the source of milk to the shops across the six settlements, chi-square = 5.96, df = 5, p = 0.310.

Table 4.17: Source of raw milk to the milk shops from farmers and vendors

		Source of milk to the milk shop			
Region Evaluated	**N**	**Farmers**		**Vendors**	
Roysambu	20	18	(90%)	2	(10%)
Dagoreti	20	12	(60%)	8	(40%)
Kasarani	20	15	(75%)	5	(25%)
Langata	20	13	(65%)	7	(35%)
Embakasi	20	16	(80%)	4	(20%)
Westlands	20	15	(75%)	5	(25%)
		$X^2 = 5.96$	df = 5	p = 0.310	

Figures in brackets are percentages of the specific total sample (N=20)

In all the regions evaluated, it was established that all the outlets preferred using casual method in checking for the quality of milk (Table 4.18).

Table 4.18: Methods and factors used in checking for the quality of raw milk

		Methods and factors used in checking for quality of raw milk			
Region Evaluated	**N**	**Lactometer/ Quality checking equipment**		**Color, smell & viscosity**	
Roysambu	20	0	(0.0%)	20	(100%)
Dagoreti	20	0	(0.0%)	20	(100%)
Kasarani	20	0	(0.0%)	20	(100%)
Langata	20	0	(0.0%)	20	(100%)
Embakasi	20	0	(0.0%)	20	(100%)
Westlands	20	0	(0.0%)	20	(100%)

Figures in brackets are percentages of the specific total sample (N=20)

In Roysambu, Embakasi and Westlands, raw milk is procured from the same source where as in Dagoreti, Kasarani and Langata not all the procuring of raw milk is done from the same source (Table 4.19).

Table 4.19: Bulking of raw milk from same and different sources

		Bulking of raw milk from source	
Region Evaluated	**N**	**Same source**	**Different sources**
Roysambu	20	20 (100%)	0 (0.0%)
Dagoreti	20	18 (90%)	2 (10%)
Kasarani	20	17 (85%)	3 (15%)
Langata	20	18 (90%)	2 (10%)
Embakasi	20	20 (100%)	0 (0.0%)
Westlands	20	20 (100%)	0 (0.0%)

Figures in brackets are percentages of the specific total sample (N=20)

Hygiene observation on the milk handlers indicated that most of the workers, 60.0 % had clean clothes. Toilets were available in all outlets. Hygienic hand driers and basins with running hot water were not available. Outlets had no sterilized equipment to procure milk (Table 4.20).

Table 4.20: Hygiene, milk handling practices and training for workers in milk shops

Hygiene practice	f (n = 120)	%
Workers clothes		
Clean	72	60.0
Dirty	48	40.0
Toilet available		
Available	120	100.0
Not available	0	0.0
Hand basin with running hot water		
Available	0	0.0
Not available	120	100.0
Hygiene hand drier		
Available	0	0.0
Not available	120	100.0
Soap for washing hands		
Available	78	65
Not available	42	35
Equipment sterilized		
Sterilized	0	0.0
Not sterilized	120	100
Cold storage (Freezer)		
Available	18	15
Not available	102	85

All the workers were not trained on hygienic practices. However, 1.67 % of the workers had other trainings from different fields such as computer studies (Table 4.21).

Table 4.21: Training of the workers serving in milk shops on food hygiene

Training	f	%
Food hygiene training		
Trained	0	0.0
Not trained	120	100
Training for personnel		
Trained	2	1.67
Not trained	118	98.0

The residents' milk consumers in this area of study, perceived milk quality attributes from formal and informal outlets mainly based on color, taste and smell as indicators of good quality (89.2 %). However, 86.7 % of them also considered environmental hygiene as an indicator while 61.75 % considered milk viscosity as an indicator of good quality milk (Table 4.22). There was no significant difference on consumer`s perception of milk quality attributes from formal and informal outlets, chi-square = 1.96, df = 10, p = 0.997.

Table 4.22: Consumers' perception of milk quality attributes from formal and informal outlets

Region Evaluated	N	Milk Viscosity as an indicator of good quality milk	Environmental Hygiene as an indicator of good quality milk	Color, taste, and smell as indicators of good quality milk
Roysambu	20	12(60.0%)	17(85.0%)	19(95.0%)
Dagoreti	20	13(65.0%)	16(80.0%)	16(85.0%)
Kasarani	20	11(55.0%)	18(90.0%)	18(90.0%)
Langata	20	9(45.0%)	18(90.0%)	18(90.0%)
Embakasi	20	15(75.0%)	17(85.0%)	17(85.0%)
Westlands	20	14(70.0%)	18(90.0%)	18(90.0%)
Mean		**61.7%**	**86.7%**	**89.2%**
		$X^2 = 1.96$	df = 10	p = 0.997

More, 70.0 % of the consumers in the area were aware of health risks associated with milk. Only 21.0 % were aware of disease associated with milk consumption while only 5.0 % had history of food borne disease associated with milk consumption in one year (Table 4.23). There was no significant difference on the level of awareness by the consumers across the six different settlements, Chi-square = 2.52, df = 10, p = 0.991.

Table 4.23: Consumer perceptions of health risks, awareness of diseases and history of food borne diseases associated with milk consumption within one year

Region Evaluated	N	Aware of health risks associated with milk (%)	Aware of diseases associated with milk Consumption (%)	History of food borne disease associated with milk consumption in one year (%)
Roysambu	20	65.0	22.0	12.0
Dagoreti	20	68.0	20.0	14.0
Kasarani	20	70.0	24.0	8.0
Langata	20	63.0	25.0	16.0
Embakasi	20	77.0	21.0	5.0
Westlands	20	79.0	26.0	6.0
Mean		**70.0**	**23.0**	**9.0**
		X^2= 2.52	df = 10	p = 0.991

Figure 4.3 shows that incidences of food poisoning associated with milk and milk products in Langata were much higher, 16.0 % than in other regions evaluated.

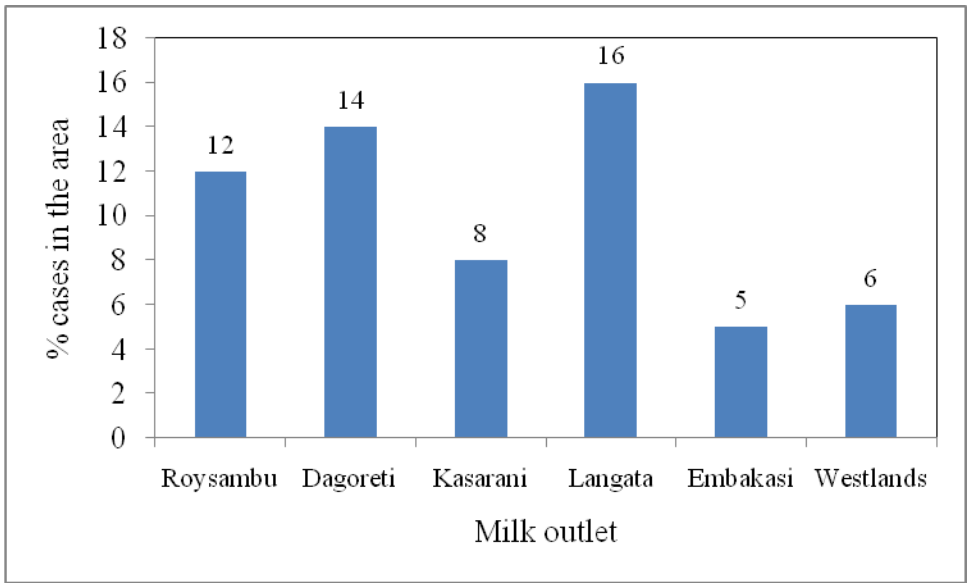


Figure 4.3: History of food borne disease associated with milk consumption in the outlets

CHAPTER FIVE

DISCUSSION

5.1 Prevalence of *S. aureus* in marketed milk and milk products within Nairobi County

Besides the targeted *S. aureus,* other bacteria and a fungus were isolated from the milk and milk products in this study. These were *Staphylococcus epidermidis*, *Staphylococcus saprophyticus, Bacillus* sp, and *E. coli* bacteria while the fungus was *Candida* (Table 4.2). Presence of *S. saprophyticus* in milk and milk products may have originated from the cows` urine in the farm during the milking process where the udder was not properly cleaned or dripping of urine down to the udder during the milking process. This is factually true as has been documented by Javaid *et al.* (2009) who reported that bacterial contamination of raw milk can originate from sources such as air, milking equipment, feed, soil, faeces and grass. He further pointed out that differences in feeding and housing strategies of cows may also influence the microbial quality of milk.

Staphylococcus epidermidis are a common resident of the skin and could therefore possibly originate from the milk-man or the cows` skin. *Escherichia coli* presence in raw milk alone denotes that the microorganisms could have possibly originated from the cows` faeces. Poor farm practices during milking and handling of milk are also possible causes of contamination (Javaid *et al.,* 2009). Some *Bacillus* sp are used in the fermentation process while others are environmental contaminants. This could therefore explain the evidence of their presence in fermented milk more than raw milk and the other milk products. These organisms have also been cited by Kaiza and Kurwijila (2011)

from Tanzania who reported isolation of *Bacillus* sp, *Proteus* sp, *E. coli, Staphylococcus* sp, *Enterobacteria* sp, *Corrynebacterium* and *Micrococcus* sp.

This study revealed a higher prevalence rate of *S. aureus* in raw milk 35 (64.81 %) compared to the other milk products investigated. Microbial contamination in milk marketed within Nairobi County could be associated with unhygienic milking and poor handling practices that could be promoting poor milk. Lack of proper basic training for the milk handlers could also be another factor promoting contamination. Mattias *et al.* (2013) reported that from the study he conducted, only 36 % of the employees had received basic training on food hygiene. The findings are comparably similar to those obtained by other investigators such as the results reported by Jorgensen *et al*. (2005) who found 68 %, Bendahon *et al*. (2008) found 40 % and Lilian *et al*. (2011) reported 68 %. However, the result was higher than the result reported by Shah *et al.* (2015) who found contamination in 25.53 % samples from a total of 47 raw milk samples collected.

In Pasteurized milk obtained from the supermarkets, 20.54 % of *S. aureus* isolates were detected and 10.71 % and 3.57 % in yoghurt and ice-cream respectively. Most of the bacterial contaminants must have been significantly eliminated during the process of pasteurization. However, presence of *S. aureus* in pasteurized milk indicates the process was not satisfactorily done. Its presence could also be due to exogenous contamination after pasteurization (Lilian *et al*., 2011). It has been cited elsewhere by Chapaval *et al.* (2010) that pasteurized milk may also be predisposed to the likely production of toxin because in various situations, the shop owners switch off the chillers at night to save

electricity, leaving the product exposed to variations in temperature. *Staphylococcus aureus* could also find access to pasteurized milk and milk products during cooling and packaging of the products into their various packets for branding and distribution to the outlets Chapaval *et al.* (2010). It has been documented that pasteurization does not completely free milk from bacteria (Karel *et al*., 2004).

Fermented milk is characterized by acid production, flavor additives and cultured bacteria. This environment could have therefore been competitively harsh for *S. aureus* to survive and thrive well and hence the possible cause of few isolates isolated compared to raw milk and pasteurized milk. Tortora *et al*. (2005) documented that low temperatures below 5˚C inhibit growth and multiplication of *S. aureus* and this could be the reason why its presence in ice-cream was notably very low compared to all other findings of this study. Results obtained from fermented milk are in accordance with the findings of other investigators such as the results according to Lilian *et al*. (2011) who reported 30 %, Ekici *et al*. (2004) reported 9.50 % , Kumar *et al.* (2012) reported 6.6 %, Shah *et al* (2015) reported 8.3 %, Singh *et al.* (2012) reported 10.34 % and Thaker *et al.* (2013) reported 10%.

According to Tortora *et al*. (2005) suitable refrigeration at temperatures below 5ºC is one of the ways of preventing *S. aureus* contamination and consequently the formation of staphylococcal toxin. This critical mistake was noted as one of the major reasons why high levels of contamination were detected in all the products except in ice-creams that require refrigeration to maintain their condition, taste and flavor. Presence of *S. aureus* in

raw milk, pasteurized milk, yoghurt and ice-cream was a clear indication that proper hygiene was not observed either in the herd and the surrounding, the milking process, the processing of milk into the various products or through the channels of distribution.

Contamination of the various milk and milk products as was found in this study revealed that the levels of contamination were significantly different as well as the number of samples contaminated. The microbial counts with regard to *S. aureus* were significantly lower in farmers` raw milk and highest in fermented milk. This was an implication that farmers milk was of a better quality but the quality deteriorated along the supply chain due to proliferation of the microorganisms initially present in milk or/and due to cross contamination. From the enumeration (Table 4.3), it is clearly demonstrated that the results on the level of contamination increased after send-off from the farm level by farmers. Increasing bacterial count in raw milk and the milk products, decreased their quality significantly. These results are in agreement with the findings by Omore *et al.* (2005) who reported that bacterial counts increase and subsequently milk quality decreases as milk passes through increasing numbers of intermediaries. Mattias *et al.* (2013) reported that milk quality in terms of microbial counts seemed to significantly decrease after send-off by farmers. He further points out that the decrease indicated that there was deterioration in quality of milk along the supply chain. In other shops and supermarkets, the milk products were openly found stocked in crates and open shelves where the ambient conditions could certainly favor growth of *S. aureus* and other microorganisms whose growth is inhibited by very low temperatures. Milk and milk

products are pre-disposed to contamination at any time if safety control measures are not put in place and risk assessment not carried out.

5.2 Antimicrobial susceptibility patterns of *S. aureus* isolates

From the findings of this study, *S. aureus* investigated were largely sensitive to the antimicrobial agents used. There was, however, occasional resistance to penicillin G. All *S. aureus* isolates investigated from the different sources (Pasteurized milk, yoghurt, Ice cream and raw milk) were found to be 100 % sensitive to Tetracycline, Ciprofloxacin, Erythromycin and Methicillin. None of the isolates was found sensitive to Chloramphenicol while in Vancomycin 11.00 % of the isolates were found sensitive in raw milk. This difference in activity by Vancomycin was thought to be brought about by the ability of *S. aureus* to acquire more resistance due to selective pressure. Lack of information on susceptibility patterns of *S. aureus* strains implicated in infections leads to poor management, resulting in treatment failures.

Resistance to Penicillin G was variant across the four different sources; Pasteurized milk, yoghurt, ice-cream and raw milk as 57 %, 33 %, 50 % and 8 % respectively. Resistance to Penicillin G antibiotics as presented in the current study could have been caused by the bacterial enzymes which destroy the antibiotic before it can act on the pathogen (Miller *et al.,* 2007). This mechanism is mostly used by microorganisms for defense against antimicrobial agents. In addition, frequent use of Penicillin G in treatment of both herds of cattle and in humans is also among the possible cause of emergence of more resistant strains of *S. aureus* and hence posing a serious challenge to public health. Thaker *et al.* (2013) investigated and detected resistance by *S. aureus* to Penicillin G (100.00 %) and

Gentamicin (10.00 %). According to Abdrezzak *et al.* (2008), all *S. aureus* isolates were susceptible to Ciprofloxacin, Gentamicin and Vancomycin. The isolates were resistant to Penicillin (56 %) and Tetracycline (22 %). *Staphylococcus aureus* resistance to penicillin is mediated by penicillinase (a form of β- lactamase) production. This enzyme cleaves the β-lactam ring of the penicillin molecule rendering the antibiotic ineffective (Miller *et al.*, 2007). Penicillinase-resistant β-lactam antibiotics such as methicillin, oxacillin, dicloxacillin and flucloxacllin are able to resist degradation by staphylococcal penicillinanse (Miller *et al.*, 2007). This is a possible explanation of high sensitivity of isolates to methicillin antibiotics used in this study.

Resistance to gentamicin (aminoglycosides) is due to the evolving mechanism of *S. aureus* strains to inhibit the aminoglycoside action which occurs via protonated amine and/or hydroxyl interactions with the ribosomal RNA of the bacterial 30s ribosomal subunit. These mechanisms of aminoglycoside resistance and genetic disorder exhibited by strains are either: aminoglycoside modifying enzymes, ribosomal mutations or active efflux of the drug out of the bacteria (Carter *et al.*, 2000).

Occasional resistance by *S. aureus* to antibiotics could be associated with earlier exposure of these drugs to isolates which may have enhanced development of resistance. It has been reported that there is a high level of antibiotic abuse arising from self-medication which is often associated with inadequate dosage and failure to comply with treatment Odugbemi *et al.* (1981) and availability of antibiotics to consumers over the counter without prescription (Paul *et al.*, 1982).

Failure to follow physicians` instructions resulting to frequent use of antibiotics can result in the emergence of multi-drug resistant strains. In addition, irresponsible use of antibiotics in animal husbandry could bring about antibiotic resistance by *S. aureus*. This wrong practice can result in mutation of the organisms` genes and therefore becoming more resistant to drugs commonly used in treatment. This should be an area of concern to the public health officials in order to curb the looming menace of *S. aureus* infections. MIC levels of ≤ 16µg/ml for CN30, ≤ 16µg/ml for VA5, ≤ 8µg/ml for E15, ≤ 16µg/ml for TE, ≤ 4µg/ml for Cip5, ≤ 32µg/ml for C30, ≤ 16µg/ml for ME and 0.25 ≤ µg/ ml for P10 were interpreted as resistant.

The findings of the current study in relation to sensitivity profiles were found to be in correlation with the results obtained by other researchers. Deka *et al*. (2012) established that all strains (100 %) were resistant to Penicillin G (PG) (10 µg), Ampicillin (AP) (10 µg), Amoxicillin-Clavulanic acid (AC) (30 µg), Ciprofloxacin (CIP) (5 µg), Erythromycin (E) (15 µg), Ceftriaxone (CRO) (30 µg), Trimethoprime-Sulfamethoxazole (TMP-SMZ) (25 µg) Oxacillin (Ox) (1 µg) and Vancomycin (V) (30 µg), 67.9 %, 70.9 %, 30.9 %, 0 %, 32.1 %, 23.1 %, 7.7 %, 60.3 % and 38.5 % respectively. The proportion of isolates resistant to CIP, TMP-SMZ, CRO, AC, E and V were low compared to AP, PG and Ox (Deka *et al*., 2012). The results on resistance to Penicillin G according to Deka *et al.* (2012) are high compared to the result in this study.

Broad-spectrum antibiotics were widely found being sold over the counter without a physician`s note in most of the regions within the study area. These broad-spectrum antibiotics decrease the load of the normal flora bacteria in the body of the individual host making him immuno-compromised (Schneider *et al*., 2009). In some countries, antibiotics are sold over the counter without a prescription which compounds the problem. Non-therapeutic use of antimicrobials corresponds to resistance rates (Schneider *et al*., 2009).

5.3 Risk factors associated with milk food safety hazard

Most of the milk shops sell raw milk, packaged pasteurized milk and fermented milk. Few consumers preferred packaged milk as they believed it was free from microbial contamination and therefore not at risk of contracting an infection. However, most consumers especially in Langata and Dagoreti settlements preferred raw milk to pasteurized milk due to its low cost compared to the other settlements (Table 4.14). This finding was comparably in agreement with the findings of SDP. (2004) who documented that unprocessed milk is also sold in desired quantities which give the low income earners access since they can buy as little as they can afford.

Milk shop owners obtained their milk directly from farmers while few bought from vendors. It was found that all the milk received by milk shops from farmers/vendors was not boiled. Major transport means of milk in the study area was motor cycle (an average of 85 %) and pick-up /vehicles (an average of 15 %) respectively. Those using motor cycles cited that it was easier to penetrate all the intended areas characterized by poor

road network and congestion due to space limitation. They in addition claimed that fuel consumption by motor cycle was very low compared to vehicles.

It was noted that all milk agents: farmers, vendors and owners of milk shops used plastic buckets (an average of 86 %) and aluminium gallons (an average of 14 %) for milk handling during procurement (Table 4.15, Table 4.16 and Table 4.17). Use of plastic containers which are not recommended for handling milk are known to be vulnerable and considered to be contributors of high contamination in raw milk (Soomro *et al.,* 2003). Plastic containers are also hard to clean adequately since they cannot be subjected to high temperatures for sterilization. In addition, if equipment is inadequately cleaned and milk residues are left on wet surfaces, it will result in microbial growth which could contaminate milk (Soomro *et al.,* 2003). Plastic containers are noted to scratch easily and provide hiding places for bacteria during cleaning. They are also poor conductors of heat and hence hinder effective sterilization (Soomro *et al.,* 2003). *Staphylococcus aureus* produces heat resistant toxins which need prolonged boiling / heating to be inactivated. Milk handling problems coupled with lack of quality assurance of milk delivered to most of the retailers and household consumers poses potential sources of public health risks to consumers. Omore *et al.* (2005) reported that the use of plastic containers was associated with high coliform counts in raw milk. This is likely due to the fact that plastic containers are difficult to clean and sterilize.

Use of plastic containers in procurement of raw milk in the findings of this study are higher compared to those reported by Omore *et al.* (2003), who found that in

Tanzania 41 %, 12 % and 8 % of the respondents claimed to use plastic buckets, plastic gallon and plastic jerry cans respectively. From the assessment made, none of the outlets sampled was found to have any equipment recommended in checking the quality of milk and 100 % confirmed using; color, smell and viscosity as the only method they used to determine whether the milk was fit for sale (Table 4.18). Muriuki *et al.* (2011) stated that milk handling equipment is one of the most significant sources of microbial contamination in milk. If equipment is inadequately cleaned and milk residues are left on wet surfaces it will result in microbial growth which could contaminate the milk.

Majority of the shop owners (94 %), reported that bulking milk from different farmers can result in low quality milk/and consequently increase health risks due to increased chances of contamination. Shop owners who preferred procuring milk from different sources said that they do so especially when the demand is high (Table 4.19). Bulking of milk from many sources increases the risk of infection with milk-borne zoonosis. This is especially so among people who drink milk without boiling it. Kleeberg *et al.* (1984), reported that the milk from an affected cow could contaminate milk from all the remaining healthy animals in the herd, or even milk from several other farms provided that the whole is mixed together. Risks from bacterial contamination has been reported to originate at farm level (Mathias *et al.,* 1998) and increases with bulking and number of agents handling milk before it reaches the consumer (Omore *et al.,* 2003).

Results on hygienic practices (Table 4.20 and Table 4.21) indicate that majority of workers (60 %) clothes were clean and (100 %) of kiosks reported that they have toilet facilities. It was noted with concern that none of the shop owners had hand basin with running hot water. None of the shops had a fitted hand drier while (65 %) of the shops had soap for washing hands. Owners of the shops claimed to only wash the milk reservoir equipment with running tap water or hot water as a way of sterilization. Only (15 %) of milk shops had cold facilities for storage of milk and milk products while (100 %) store milk separately from other foods. Only 2 (2 %) of the personnel in the milk shops had undergone a formal training on food hygiene.

Food handlers can be a source of the spread of food-borne disease caused by poor personal hygiene or cross-contamination. The study established that 118 (98 %) of food handlers did not receive any formal training regarding food hygiene and therefore did not have a high level of general food hygiene. The lack of training in food hygiene including milk may be a contributing factor to unhygienic milk handling by the informal sector traders and the subsequent cause of rise in contamination of milk and milk products. These findings were fairly in agreement with the findings by Mattias *et al.* (2013) who found that out of the shops he analyzed, (36 %) employees stated that they had attended at least one seminar held on hygiene. One (9 %) employee reported that she had been educated by her father who is a veterinarian whereas the rest (55 %) stated that they had no education within the food safety area (Mattias *et al.,* 2013). Consumers are at risk of any malpractice that occurs during milk handling practices. Majority of the consumers interviewed preferred taking milk to any other drink since they perceived it as being more

nutritive in value. Most consumers (Table 4.22) claimed that environmental hygiene (87 % on average), color, taste, and smell (89 % on average) respectively as their indicators of good quality milk.

Out of 120 respondents interviewed, an average of 84 (70 %) claimed to be aware of the health risks that could be associated with milk. The result on awareness of health risks associated with milk in this study compares well with that reported by Kaiza and Kurwijila (2011) from Tanzania who found 43 (71.67 %) of the respondents interviewed were aware. However, the result in this study is high compared to that reported by Karimuribo *et al*. (2005) who found 19 (20.7 %) of the respondents being aware. In this study 28 (23 %) claimed to be aware of diseases associated with consumption of contaminated milk. Most of them said they had encountered stomach disorders and diarrhea while others claimed to have experienced body rashes, severe headache and vomiting. On average, 8 (9 %) of the respondents claimed to have contracted a disease as a result of drinking contaminated milk within the last one year (Table 23). The result in this study on awareness of disease associated with consumption of contaminated milk is low compared with results reported by Kaiza and Kurwijila (2011) who found 30 (50 %). However, the result in this study is high compared with the results reported by Karimuribo *et al.* (2005) who found (21 %) being aware. Public awareness regarding health and safety of milk and milk products within Nairobi County should be considered as an initiative by the relevant authority mandated, since lack of awareness as reported is an impediment in accessing good quality milk.

Majority of the consumers claimed that they boiled the milk (raw milk) immediately after purchasing it in order to kill pathogenic microorganisms. This was the belief they all had stating that boiling milk destroys all microorganisms present in milk. It has been reported by Kaiza and Kurwijila (2011) that consumers prefer boiled milk served hot as they believe boiling kills pathogenic bacteria. Customers in this study also perceived that the general outlook of the milk shops and the attendants in terms of cleanliness determined their preference in purchasing milk and other food products from a particular seller. This attribute of consumer perception compares well with that reported by Kaiza and Kurwijila (2011) who reported that majority of respondents said they normally drink milk from a kiosk which has a good environment and its workers are generally clean. Those aware of health risk implications as a result of food poisoning (70 %) suggested that all milk outlets be thoroughly inspected by public health officials to ensure that proper food hygiene is maintained to guarantee food safety. The aspect of boiling raw milk from a home setting does no completely decontaminate raw milk and therefore a possible contributor of milk contamination. Microorganisms proliferate and exceed the maximum acceptable level set by Kenya Bureau of Standards at 2.0×10^6 CFU/ml (Omore *et al.*, 2005).

Contamination of raw milk by *S. aureus* was different across the six settlements where sampling was done. Higher contamination was detected in Langata (26 %) where it was also notably identified that more of raw milk was marketed compared to all other settlements investigated (Figure 4.1). More reported incidences (Figure 4.3) of foodborne diseases associated with milk consumption within one year in the same region (16 %) compared to other settlements investigated in this study. Almost similar results were

obtained in Dagoreti in the same study. Being settlements characterized by very high populations (densely populated) and poor drainage system compared to the other settlements in this study, the evidence of the results obtained indicate that these attributes are impediments to good quality milk supplied for consumption within these regions.

Poor environmental hygiene as observed in these settlements was in addition the possible cause of high contamination of raw milk. Drainage systems which are polluted with streams of dark, smelly and toxic sewage as were found in these settlements pose a health risk problem. They also pose a looming danger of contaminated drinking water and subsequently milk food. Poverty seems to be the main contributor for the lack of adequate resources and therefore appropriate innovations which are cost effective and affordable will be necessary to maintain proper milk handling (Mattias *et al.,* 2013).

The work of health inspection within the County is crucial to raise consumer awareness on the consumption of good-quality milk through educational programs and the inspection of establishments that insist on selling raw milk and milk products. Dairy inspection and adoption of HACCP principles and good fabrication practices can also contribute to a significant reduction in contamination of milk and milk products.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Presence of *S. aureus* in milk and milk products was found occurring in 72 (21.56 %) of the samples. The research hypothesis was rejected and it was concluded that milk and milk products marketed within Nairobi County are contaminated with *S. aureus.*

2. Isolates from the different sources were found to be occasionally resistant to Penicillin G. The isolates were sensitive to Tetracycline, Ciprofloxacin, Methicillin and Erythromycin. Occasional sensitivity was found in Gentamicin and Vancomycin while all isolates were found intermediate to Chloramphenicol.

3. Poor storage, use of plastic containers, non-confirmation of the quality of milk and procurement of raw milk from different sources were found to be risk factors for contamination of milk and milk products.

6.2 Recommendations

1. All processing procedures should be followed to the latter especially the pasteurization process so that the recommended temperatures are actually reached without compromise by employees in the plants. This will help alleviate the problem of milk and milk products contamination.

2. All retail shops and supermarkets (food outlets) selling milk and milk products to the final consumers be equipped with appropriate and functioning preservation facilities such as fridges, to ensure that milk and milk products remain under low temperatures ($\leq 4^{o}C$) that cannot pose threats of poisoning to the final consumers. Putting milk and milk products in open shelves should also be discouraged and public health officials be enforced to conduct regular check-ups to ensure that these measures are adhered to.

3. It is recommended that Tetracycline, Ciprofloxacin, Methicillin and Erythromycin antibiotics should continue to be used for treatment of *S. aureus.*

4. The competent authorities should adopt severe inspection measures in order to prohibit the informal sale of milk. It was therefore recommended that the government`s responsibility of ensuring up-to-date food legislation associated with milk and milk products relevant to the prevailing national problem be thoroughly enforced, since milk and milk product contamination can result to a serious outbreak in a region where the contaminated product has been supplied for consumption.

5. Education and public awareness regarding milk and milk products hygiene should be strengthened.

6. Finally, further research and study on hygiene practices in Kenya regarding milk and milk products and innovation of cost-effective ways of storage and preservation affordable to low-income earners should be done.

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APPENDICES

APPENDIX 1
QUESTIONNAIRE SURVEY FOR SELLERS OF MILK IN MILK SHOPS IN THE STUDY AREA

NAME OF ENUMERATOR: FILLED IN BY_____

Date: dd/ mm/yy {_____/____/_____}

Time started:_____ Time ended:_____

County: _____ Constituency :_____

Questionnaire No: _____

SECTION A: BACKGROUND INFORMATION

1. Respondent's name _____ Business Name (where applicable) _____

Sex of respondent	Age of respondent (yy)	Position in the shop/kiosk	Education level
(_____)	(_____) Yrs	(_____)	(_____)

Codes

Sex of respondent	Age of respondent (yy)	Position in the shop/kiosk	Education level
1. Male	1. =≤ 20	1.Owner	1.Primary School
2. Female	2. =21-30	2. Employee	2.Secondary School
	3. =31-50		3. Certificate
	4. =>50		4. Diploma
			5. University Degree

SECTION B: MARKETING INFORMATION AND HYGIENE

2. MARKETING INFORMATION

When did you start milk business? (_____) year(s)

Where do you get Milk for your shop?	Are you selling Ice-cream?	Are you selling raw milk?	Are you selling fermented milk?	Are you selling pasteurized milk
(____)	(____)	(____)	(____)	(____)

How do you assess the quality of milk before receiving it?

_____

_____

Codes

Where do you get milk for shop?	Are you selling i-cream?	Are you selling raw milk?
1. = Farmer	1. = YES	1. = YES
2. = Vendor	2. = NO	2. = NO

Are you selling fermented milk?	Are you selling pasteurized?
1. = YES	1. = YES
2. = NO	2. = NO

Are the walls, floor and ceiling in good condition and enable you to clean and disinfect them where necessary?	Do you have a cleaning schedule?	How often do you use the disinfectant?	If never, why?	Do you have a toilet on the premises?	Do you have a wash hand basin with a supply of running hot water, soap, and hygienic hand drying facilities?
(____)	(____)	(____)	(____)	(____)	(____)

If you have a cleaning schedule, how often does the premises receive a

(a) Deep cleaning _____

(b)General Cleaning_____

Codes

Walls, floor and ceiling able to be cleaned easily? cleaning schedule Use of disinfectant

1. = YES 1. = YES 1. = Everyday

2. = NO 2. = NO 2. = When available

3.= where necessary

4. = never

_____ _____

If never, why? Toilet availability Water basin, running water and soap

1. = Expensive 1. = YES 1. = YES

2. = Not available 2. = NO 2. = NO

3. = Not aware

SECTION C. INFORMATIUON ON TRANSPORT AND STORAGE

3. STORAGE

How is milk transported to your shop by your supplier?	What type of container do you use to procure milk?	Do you use any of the following in your premises?
(____)	(____)	(____)

Codes

How is milk transported to? your premise?	Are raw and ready to eat foods stored separately?	Do you use any of the following in your premise
1. =Moror bike	1. = Plastic container	1. = Fridges
2. = Pick up/ vehicle	2. = Aluminium container	2. = Freezers
		3. = Chilled display cabinets

4. Do you procure milk from the same source or different sources?

Codes: 1 (same source) 2 (different source)

SECTION D: TRAINING ON HYGIENE

5. How many employees do you have that handle food? _____

6. How many have acquired training on:

(a) Basic or foundation in hygiene training (6 hour course) _____

(b) Intermediate food hygiene training (2-3 day training course)_____

(c) Other similar food hygiene training (specify) _____

7. If no Food Handlers have been formally trained please state how you ensure they handle food hygienically? _____

Thank you for your assistance and co-operation.

APPENDIX 11

QUESTIONNAIRE SURVEY FOR CUSTOMERS /CONSUMERS OF MILK IN THE STUDY AREA

NAME OF ENUMERATOR: FILLED IN BY_____

Date: dd/mm/yy {_____/____/_____}

Time started:_____ Time ended:_____

County: _____ Constituency :_____

Questionnaire No: _____

SECTION A: BACKGROUND INFORMATION

1. Respondent's name _____

2. Background

Sex of respondent	Age of respondent (yy)	Position in the shop/kiosk	Education level
(_____)	(_____) Yrs	(_____)	(_____)

Codes

Sex of respondent	Age of respondent (yy)	Position in the shop/kiosk	Education level
1. Male	1. =≤ 20	1.Owner	1.Primary School
2. Female	2. =21-30	2. Employee	2.Secondary School
	3. =31-50		3.Certificate
	4. =>50		4.Diploma
			5.University Degree

SECTION B: CONSUMER PERCEPTION ON MILK QUALITY

3. Why do you prefer milk instead of other drinks such as soda?

_____

How many times do you take milk in a week?	Why do you prefer this place among many other milk shops around this place?
(____)	(____)

Codes

How many times you take milk in a week? many	Why do you prefer this place among other milk shops around this place?
1. = All the days 2. = 2 – 3 days 3. = When I have money	1. = low price 2. = good customer care 3. = quality of milk 4. = Clean environment

Do you think milk viscosity is an indicator of good quality milk?	Is environmental hygiene an indicator of milk quality?	Do you use color, taste and smell to qualify milk as good for consumption?
1(Yes) 2 (No)	**1(Yes) 2 (No)**	**1(Yes) 2 (No)**

Are you aware of health risks associated milk?	Are you aware of diseases associated with milk consumption?	Have you ever contracted an infection after consuming milk in the last one year?
1(Yes) 2 (No)	**1(Yes) 2 (No)**	**1(Yes) 2 (No)**

Thank you for your assistance and co-operation

APPENDIX III

PREPARATION OF MEDIA USED

1) Mannitol salt agar

Direction

Suspend 111.0g of powder in 1 litre of distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. Dispense the content into Petri-dishes as desired.

2) Blood agar

Direction

Suspend 40.0g of powder in 1 litre of distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. Allow to cool to about 40°C and then add 80 ml of de-fibrinated Sheep blood under aseptic measures. Dispense the media into sterile Petri-dishes as desired.

3) Mueller Hinton ager

Direction

Suspend 35.0g of powder in 1 litre of distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. Dispense aseptically into Petri-dishes as desired.

4) Tryptone soya broth

Direction

Suspend 30.0g of powder in 1 litre of distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. Dispense into universal bottles as desired aseptically.

5) Preparation of Mc Farland 0.5 Standard

This is half the density of a Mc Farland number one Standard and is prepared by adding:

0.5 ml. of 0.048 M $BaCl_2$ (1.1755w/v $BaCl_2$. $2H_2O$) to 99.5 ml of 0.36 N H_2SO_4 (1% V/V)

PRACTICE INVOLVED

Plate 1: The process of obtaining Sheep`s blood

APPENDIX 1V

One-way ANOVA: Zone versus Antibiotics

Analysis of Variance for Zones in pasteurized milk

Source	DF	SS	MS	F	P
antimicr	7	6410.52	915.79	421.45	0.000
Error	176	382.43	2.17		
Total	183	6792.96			

Individual 95% CIs For Mean
Based on Pooled StDev

Level	N	Mean	StDev	-----+---------+---------+---------+-
c	23	25.435	1.674	(*)
CIP	23	26.913	1.203	(*)
cn	23	14.261	1.484	(-*)
E	23	26.391	1.033	(*)
ME	23	20.000	1.809	(*)
P	23	13.478	1.780	(*)
TE	23	26.696	1.490	(*-)
va	23	13.261	1.096	(-*)

Pooled StDev = 1.474 -----+---------+---------+---------+- 15.0 20.0 25.0 30.0

Analysis of Variance for Zones in fermented milk

Source	DF	SS	MS	F	P
Antibiotic	7	3280.67	468.67	204.85	0.000
Error	88	201.33	2.29		
Total	95	3482.00			

Individual 95% CIs For Mean
Based on Pooled StDev

Level	N	Mean	StDev	------+---------+---------+---------+
c	12	26.000	2.045	(-*-)
Cip	12	27.500	1.446	(-*-)
cn	12	15.333	1.303	(-*)
E	12	26.333	1.435	(-*)
ME	12	19.667	1.723	(*-)
p	12	13.833	1.528	(-*)
te	12	26.333	1.303	(-*)
VA	12	13.000	1.128	(-*-)

Pooled StDev = 1.513 ------+---------+---------+---------+ 15.0 20.0 25.0 30.0

Analysis of Variance for Zones in raw milk

Source	DF	SS	MS	F	P
Antibiotic	7	7385.26	1055.04	442.50	0.000
Error	272	648.51	2.38		
Total	279	8033.77			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
C	35	25.971	1.294	(*)
Cip	35	26.771	2.129	(*)
CN	35	26.257	1.039	(-*)
E	35	27.029	1.599	(*)
ME	35	22.657	1.533	(*)
P	35	15.600	1.538	(*)
TE	35	28.257	1.788	(-*)
VA	35	14.086	1.147	(*)

Pooled StDev = 1.544

15.0 20.0 25.0 30.0

APPENDIX V

Distribution of *S. aureus* isolated within 9 weeks of investigation and biochemical analysis

Table 1: Distribution of *S. aureus* isolated within 9 weeks of investigation

	Time (Weeks)					
Source	**Week 1**	**Week 3**	**Week 5**	**Week 7**	**Week 9**	**Total**
Raw milk	6 (8%)	8 (11%)	7 (10%)	6 (8%)	8 (11%)	35 (64.81%)
Pasteurized milk	4 (6%)	5 (7%)	5 (7%)	4 (6%)	5 (7%)	23 (20.5%)
Fermented milk	3 (4%)	2 (3%)	3 (4%)	2 (3%)	2 (3%)	12 (10.7%)
Ice-cream	0 (0%)	0 (0%)	1(2%)	0 (0%)	1(2%)	2 (3.6%)

Figures in brackets are percentages of the total sample

Table 2: Biochemical test results

	Organisms		
Test	***Staphylococcus aureus***	***Staphylococcus epidermidis***	***Staphylococcus saprophyticus***
Coagulase	Positive	Negative	Negative
Catalase	Positive	Positive	Positive

APPENDIX VI

Risk factor assessment: Statistical outputs

Table 3: Number of outlets selling milk and milk products

Expected: contingency table

	A	B	C
1	8.25	17.0	7.75
2	9.00	18.5	8.46
3	7.75	16.0	7.29
4	8.75	18.0	8.22
5	7.75	16.0	7.29
6	8.50	17.5	7.99

Chi-square = 7.69
degrees of freedom = 10
probability = 0.659

Table 4: Types of container used to procure raw milk

Expected: contingency table

	A	B
1	17.2	2.83
2	17.2	2.83
3	17.2	2.83
4	17.2	2.83
5	17.2	2.83
6	17.2	2.83

Chi-square = 4.45
degrees of freedom = 5
probability = 0.486

Table 5: Source of milk to the milk shops

Expected: contingency table

	A	B
1	14.8	5.17
2	14.8	5.17
3	14.8	5.17
4	14.8	5.17
5	14.8	5.17
6	14.8	5.17

Chi-square = 5.96
degrees of freedom = 5
probability = 0.310

Table 6: Consumer perception of milk quality attributes from formal and informal outlets

Expected: contingency table

	A	B	C
1	12.5	17.6	17.9
2	11.7	16.5	16.8
3	12.2	17.2	17.5
4	11.7	16.5	16.8
5	12.8	17.9	18.3
6	13.0	18.3	18.7

Chi-square = 1.96
degrees of freedom = 10
probability = 0.997

Table 7: Consumer perception of health risks, awareness and history of food borne diseases associated with milk consumption within one year

Expected: contingency table

	A	B	C
1	13.0	4.14	1.84
2	14.4	4.57	2.03
3	14.4	4.57	2.03
4	14.4	4.57	2.03
5	13.7	4.35	1.94
6	15.1	4.79	2.13

Chi-square = 2.52
degrees of freedom = 10
probability = 0.991

EQUIPMENT USED

Plasma obtained from Rabbit`s blood was used in carrying out Coagulase test.

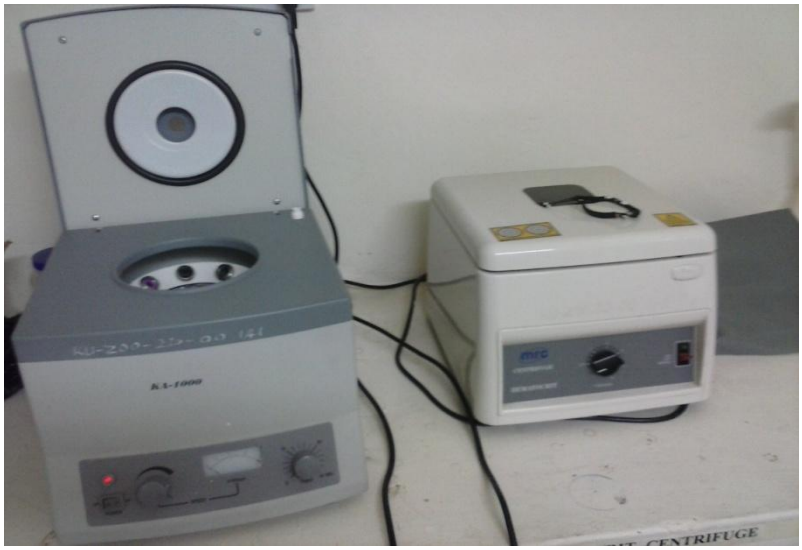

Plate b: A centrifuge used in obtaining Rabbit plasma.

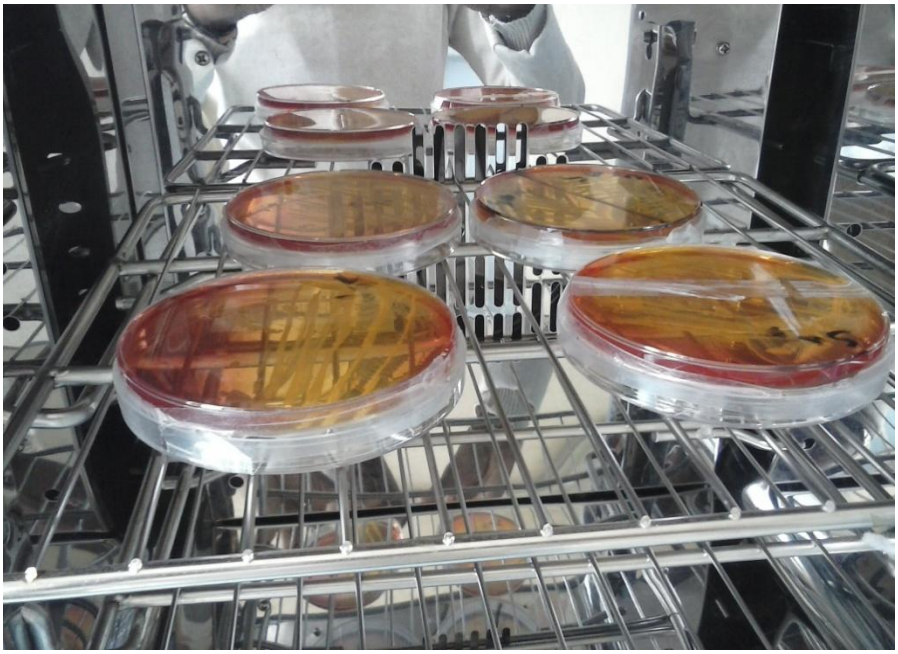

Plate c: Growing Microorganisms in an incubator at 37°C