FACTORS ASSOCIATED WITH PREVALENCE OF ANTIMICROBIAL RESIDUES AND COMPOSITIONAL QUALITY OF INFORMALLY MARKETED RAW COW MILK IN LAMU WEST SUB-COUNTY, KENYA

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2019

Factors Associated with Prevalence of Antimicrobial Residues and Compositional Quality of Informally Marketed Raw Cow Milk in Lamu West Sub-County, Kenya

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A thesis submitted in partial fulfilment for the Degree of Master of Science in Applied Epidemiology in the Jomo Kenyatta University of Agriculture and Technology

2019

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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This thesis has been submitted for examination with our approval as university supervisors

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DEDICATION

I dedicate this thesis with much love and appreciation to my family. I thank the Almighty God for every blessing.

ACKNOWLEDGEMENT

I would like to acknowledge and give thanks to the following persons and institutions for their much appreciated support and encouragement during the study: my academic supervisors Professors J. K. Gikunju and J. N. Ombui, and The Kenya Field Epidemiology and Laboratory Training Programme (K-FELTP) faculty for their invaluable inputs towards completion of this work.

I would also like to thank K-FELTP for the scholarship opportunity and financial sponsorship of the study; Lamu County Hospital Medical Officer of Health Dr. Victor Tole and laboratory staff: Mr Jamleck Safari, Mr David Gitau and Mr Ignatius Kamau for hosting the study at his facility and technical support; Voi Milk Analysis Station for equipment support. Many thanks to my office supervisor, Dr Charles Gichohi, for providing ample time to enable me complete this study.

A big thank you goes to my family, friends and colleagues who encouraged me through the course of the work. To the farmers and milk vendors I extend my sincere gratitude for your invaluable input without which this work would not have been completed. Thank you so much and keep up the good work.

To all those whom I have not mentioned by name and who, in one way or another, contributed to the success of this work, receive my sincere thanks.

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LIST OF ACRONYMS

ADI	Acceptable Daily Intake
AOR	Adjusted Odds Ratio
CAC	Codex Alimentarius Commission
CAC-MRLs	Codex Alimentarius Commission Maximum Residue Limits
CAIS	Central Artificial Insemination Station
CI	Confidence Interval
EU-MRLs	European Union Maximum Residue Limits
FAO	Food and Agriculture Organization
GART	Golden Valley Agricultural Research Trust
GDP	Gross Domestic Product
ILRI	International Livestock Research Institute
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KCC	Kenya Cooperative Creameries
KeBS	Kenya Bureau of Standards
KNH	Kenyatta National Hospital
KPHC	Kenya Population and Housing Census
LME	Liquid Milk Equivalent

MRL	Maximum Residue Limit
SNF	Solid Non Fat
UON	University of Nairobi
VRB	Violet Red Bile agar
WHO	World Health Organization
ZDU	Zoonotic Disease Unit

DEFINITION OF TERMS

Antimicrobial resisues	Remnants of either the parent antibiotic compounds or their metabolites that may accumulate, deposit or be stored in cells, tissues, organs or in edible products (such as milk and eggs) of an animal following their use prophylactically
Informal raw milk vendor	Any person (male/female) who obtained milk from own farm or bought milk from one or more farms / milk outlets and sold it by hawking along the pathways or at raw milk selling shops within the milk supply catchment of the selected urban centres and willing to participate in the study.
Informally marketed raw cow milk	Milk that is sold directly as obtained from the cow; without any form of processing or quality assurance.
Livestock farmer	Any person (male / female) with lactating cattle and offers milk for sale within his /her farm, and willing to participate in the study.

ABSTRACT

Unadulterated milk, free of antimicrobial residues is important for industrial processing and consumers' health. Antimicrobial residues in foods of animal origin can cause adverse public health effects like drug resistance and hypersensitivity. Milk produced in Lamu West sub-county is sold raw directly to consumers. A cross sectional study was carried out to estimate the compositional quality and prevalence of antimicrobial residues in informally marketed raw cow milk in Lamu West Sub-County, Kenya. One hundred and fifty two (152) vendors and 207 farmers were recruited from four randomly selected urban centres and interviewed using a pretested standardized questionnaire. A 100-ml raw milk sample was aseptically collected from each vendor and farmer and tested for antimicrobial residues using Charm Blue Yellow II kit following the European Union Maximum Residue Limits (EU-MRLs) while an Ekomilk® Analyzer was used to measure compositional quality. Samples with solid not fat (SNF) <8.5 or those with added water ≥0.01% or both were considered adulterated. Data was analyzed using univariate analysis and unconditional logistic regression to calculate odds ratios (OR) and 95% Confidence intervals (CI). Thirty-two (15.5%) of the 207 samples from farmers and 28 (18.4%) of the 152 samples from vendors had antimicrobial residues above the MRLs. Thirty-six (17.4 %) samples from farmers and 38 (25.0%) from vendors were found to be adulterated with water. Farmer awareness of the danger of consuming milk with antimicrobial residues and training on good milking practices were protective against selling milk with antimicrobial residues (adjusted OR and 95% CI 0.20, 0.07–0.55 and 0.33, 0.11–0.99, respectively). Farmers having secondary level of education and above (AOR 3.03, 95% CI: 1.44-6.39) and being a pastoralist farmer (AOR 3.20, 95% CI: 1.05-9.71) were retained as independent risk factors against marketing of milk of poor compositional quality. Being a male vendor was retained as the only independent risk factor associated with marketing of milk of poor compositional quality amongst vendors (AOR 2.73. 1.22-6.08) The antimicrobial residues above EU-MRLs and adulteration of raw marketed cow milk observed in this study provide evidence for routine testing of marketed milk and educating farmers to observe antimicrobial withdrawal period.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Agriculture remains the backbone of the Kenyan economy as it is the single most important sector contributing approximately 25% of the GDP, and employing 75% of the national labour force. Over 80% of the Kenyan population live in the rural areas and derive their livelihoods, directly or indirectly from agriculture (Alila & Atieno, 2006). The dairy sub-sector accounts for 3.5% of the national GDP and contributes to the livelihoods of about four million Kenyans through food, income and employment (Kimenchu *et al.,* 2014). This shows that the dairy industry plays a very key role in the national economy.

The dairy industry in Kenya has evolved over time. Originally, it was dominated by large scale herds that were kept by white settlers in early 1900's in the high agricultural potential zones of the then Rift Valley and Central provinces. To assist in controlling livestock diseases and promote animal husbandry research services, in 1903 the government started the Veterinary Research Laboratory in Kabete and the Animal Husbandry Research Station in Naivasha. Other livestock support institutions followed up later; namely: the Kenya Cooperative Creameries (KCC) in 1925, to take the sole responsibility of processing and marketing milk; Central Artificial Insemination (CAIS) in Kabete in 1946 to control reproductive diseases and regulate breeding; and the Kenya Dairy Board in 1958 to regulate milk marketing (Omore *et al*., 1999). It is worth noting that, in 1954 indigenous Kenyans were allowed to venture into commercial agriculture courtesy of the Swynnerton Plan of 1954 (Omore *et al.,* 1999). This increased the general ownership of dairy cattle by idigenious Kenyans. A further major boost in the industry was observed in 1992 when marketing of milk was liberalized; ending the marketing monopoly of Kenya Cooperative Creameries (KCC) thus causing an increase in small scale trading in fresh milk (Owango *et al.,* 1998).

With the changes that came in at independence (1963), some of the white settler farmers opted out and sold their farms. This resulted in reduction of the dairy cattle population in the large scale farms and a rapid growth of the exotic herd and cross breeds with the local East African zebu. To boost smallholder

development, the government gave various incentives such as free / subsidized clinical services, artificial insemination and open access to KCC by the smallholder farmers(Thorpe *et al.* 2000).

The 1992 (Republic of Kenya 2013), policy change which ended the milk marketing monopoly of KCC especially in urban areas resulted in the rise in the private formal and informal milk marketing systems thus increasing demand for milk and a further growth in the smallholder dairy farming (Omore *et al.*, 1999). It is estimated that Kenya has the largest dairy industries in the Sub Saharan Africa with a well developed production and processing capacity based on over five million heads of improved dairy cattle. As it was during the white settlers' time, the former Rift Valley and Central Provinces still dominate in the dairy cattle population. Rift Valley province has 53% whereas Central province has 25% of the national dairy herd (Staal *et al*., 2008; Muriuki *et al.,* 2001).

A study by Omore *et al.* (2005) on marketing of milk produced in Kenya revealed that, 86% of the milk is informally marketed (that is, without going through the pasteurization process); 42% of which is sold raw directly to consumers, 23% is sold through mobile traders, 15% is sold through milk bars and kiosks, and 6% sold through cooperatives. The formal milk market (the pasteurized milk) accounts for only 14% of all sold milk and are made up of 30 processors (Omore *et al.* 2005). Public health concerns have been raised in Kenya on the poor compositional quality and presence of harmful substances such as antimicrobial residues in marketed milk (Omore *et al*., 1989; Kang'ethe *et al*., 2005).

1.2 Statement of the Problem

Compared to countries within the Africa zone, Kenya has compareably very high per capita milk consumption, 90Kg Liquid Milk Equivalent (LME) per annum whereas Uganda has 22Kg LME, Ghana has 5Kg LME, and Tanzania has 28Kg LME. (Kang'ethe *et al.,* 2005) . With such high per capita milk consumption, there is a high likelihood of exposure to public health hazards including antimicrobial residues due to consumption of milk of unknown compositional quality since the bulk of the milk is sold through informal markets without any form of quality testing or processing (Omore *et al.,* 2005).

To protect the public against possible health risks caused by consumption of milk of unacceptable quality regulations have been developed both locally and internationally to ensure observance of withdrawal

periods after antimicrobial therapy and proper handling and marketing of milk. International regulations include European Union Maximum Residue Limits (EU MRLs) and the Codex Alimentarious Commission Maximum Residue Limits (CAC-MRLs) (Codex Alimentarius Commission 2009;The European Commission 2010) **(Appendix IX)**. In Kenya, quality and safety of milk is regulated by the Dairy Industry Act (Republic of Kenya 2007), Public Health Act (Republic of Kenya, 2012), and the Standards Act (Republic of Kenya 2008). However, such regulations might not be adhered to or enforced, as is the case in many developing countries (Aning *et al.*, 2007).

A study by Kang'ethe *et al.* (2005), in Nairobi, Nakuru, Kiambu and Narok, found 16% of marketed milk with unacceptable concentrations of antimicrobial residues. The occurrence of antimicrobial residues in milk causes harmful public health effects such as drug resistance and hypersensitivity and huge economic losses in milk processing industries like in the manufacture of cultured products such as yoghurt and cheese through inhibition of starter cultures (Kang'ethe *et al.*, 2005). A study by Omore *et al*. (1989) on the quality of marketed milk in rural and urban locations of Nakuru, Kiambu and Nairobi, found that 60-80% of milk samples could not meet the standards set by the Kenya Bureau of Standards.

1.3 Study Justification

The dairy industry in Lamu County is in early stages of development. As of 2015, all the milk produced in Lamu West sub-county was sold raw directly to consumers without undergoing any quality assessment and safety assurance against presence of antimicrobial residues. Unlike in Lamu county, in areas where the dairy industry is well developed such as in Kiambu county, there are many milk processors who conduct quality assurance on most of the milk produced in the region and give evidence-based feedback to farmers through regular extension messages resulting in marketing of good quality milk (Ombui, 1994). In Lamu, the only intermediary between the farmer and the consumer is a small scale milk trader/ vendor / hawker. No investigations have been carried out to assess the extent and nature of the risks consumers of marketed raw cow milk in Lamu County may be exposed to. This study assessed the compositional quality of the marketed raw cow milk and estimated the prevalence of antimicrobial residues in the milk in Lamu West Sub-County. Findings of this study will be used to create awareness amongst farmers, vendors and consumers on the quality of milk sold in Lamu County and enable implementation of appropriate policies to enhance marketing of quality milk.

1.4 Research Questions

1. What is the compositional quality of the informally marketed raw cow milk in Lamu West Sub-County?

2. What is the prevalence of antimicrobial residues present in the informally marketed raw cow milk?

3. What factors are associated with poor compositional quality of informally marketed raw cow milk in Lamu West Sub-County?

4. What factors are associated with the prevalence of antimicrobial residues in marketed raw cow milk in Lamu West Sub-County?

1.5 Objectives

1.5.1 General Objective

To determine the compositional quality and the prevalence of antimicrobial residues in informally marketed raw cow milk in Lamu West Sub-County, Kenya.

1.5.2 Specific Objectives

1. To determine the compositional quality of informally marketed raw cow milk in Lamu West Sub-County.

2. To determine the prevalence of antimicrobial residues in the informally marketed raw cow milk in Lamu West Sub-County.

3. To identify factors associated with the poor compositional quality of informally marketed raw cow milk in Lamu West Sub-County.

4. Identify factors associated with the prevalence of antimicrobial residues in marketed raw cow milk in Lamu West Sub-County.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Milk is as the product resulting from complete and continuous milking of healthy cows, well fed and given sufficient rest, under good conditions of hygiene (Hogeveen *et al.,* 2001). It is meant to be the first and main food for mammalian offspring and contains a balanced form of all the necessary and digestible elements for building and maintaining human and animal body plus immunoglobulins which protect the newborn against diseases (Pandey & Voskuil, 2011). Milk is a very important source of nutrients required by infants, children, pregnant and lactating mothers, the elderly and the immunocompromised. In the industrial or manufacturing sector, milk contains various properties which make it easy to convert into different products (such as yoghurt, butter, ghee) or use as an ingredient in the manufacture of other foods (such as cakes). It is also worth noting that various communities have their own traditional ways of using milk and preparing different products (Pandey & Voskuil, 2011).

2.2 Milk and its Constituents

A wide variation is usually observed in the composition of milk depending on the individual cow, breed, stage of lactation, age and health status. Herd management practices (such as feeding) and environmental conditions (such as climate) also influence milk composition. Milk is composed of water and total solids (fat, proteins, lactose and minerals). Total solids without milk fat are referred to as Solids Non Fat (SNF) (Connor, 1995a). Table 2.1 shows the average chemical composition of cow milk while table 2.2 shows breed specific average chemical composition of milk.

Table 2.1: Average chemical composition of cow milk

Main Component	Range (%)	Mean (%)
Water	85.5 – 89.5	87.0
Total Solids	10.5 – 14.5	13.0
Fat	2.5 – 6.0	4.0
Proteins	2.9 – 5.0	3.4
Lactose	3.6 – 5.5	4.8
Minerals (Ash)	0.6 -0.9	0.8

Source: ILRI Training Manual 1: Rural Dairy Technology, 1995.

Table 2.2: Breed specific average chemical composition (%) of cow milk

Breed	Fat	Protein	Lactose	Ash
Zebu	5.6	3.1	4.6	0.71
Aryshire	3.8	3.4	4.8	0.70
Fresian	3.4	3.2	4.6	0.74
Guernsey	4.9	3.8	4.8	0.75
Jersey	5.1	3.8	4.9	0.75
Shorthorn	3.6	3.4	4.8	0.70

Source: ILRI Training Manual 1: Rural Dairy Technology, 1995.

2.3 Physico-chemical properties of milk

2.3.1 Acidity / Alkalinity of milk (pH)

The pH or hydrogen ion concentration of milk refers to the acidity or alkalinity of milk. Normal milk has a pH range of 6.6 – 6.8. However, a lower pH can be observed as a result of breakdown of lactose to form lactic acid which is indicative of milk spoilage. A lower pH less than 6.6 can also be observed as a result of phosphates, citrates and carbon dioxide present in the milk. The pH value of milk can be higher than 6.8 due to mastitis (Ogola *et al.,* 2007). The acidity or alkalinity of milk can be measured directly (using indicator dyes, titratable acidity or use of pH meters) or indirectly (through clot on boiling and alcohol tests).

2.3.2 Density / specific gravity of milk/ Lactometer test

Specific gravity of milk is the ratio of the density of milk to that of water. Normally, using a lactometer, the specific gravity of milk should range between 1.026 – 1.036 g/ml. Specific gravity of milk is measured when milk is at 20 ^{0}C. Approximately 250 ml of milk is put in a measuring cylinder and a lactometer is inserted. Readings are taken from just above the milk surface. Milk with specific gravity not within the range is considered to be adulterated and is usually rejected. Lower lactometer readings can be observed following addition of water and cream into the milk, whereas higher readings are observed in cases where skim milk has been added into the milk or fat has been removed (Connor, 1995a). Farmers, milk traders, transporters and shopkeepers often add water and other substances to milk with the aim of increasing their profits. This is easily detected by a lactometer as its specific gravity shall fall outside the normal range; hence such milk is considered adulterated (Das *et al.,* 2016).

2.3.3 Freezing point of milk

Milk freezes at a lower temperature than water with its freezing point lying between -0.525^{0}C and -0.565^{0}C. Variation of milk freezing point temperature is influenced by levels of soluble constituents. Presence of soluble constituents lowers the freezing point. A small adulteration of milk with water will cause detectable elevation of the freezing point of the milk. Freezing temperatures can be determined using a cryostat, cryoscope or through the use of a lactoscan. It is worth noting that freezing point of milk is the most constant physical property of milk (Henno *et al.,* 2008) (Ruegg & Reinemann, 2002).

2.4 Milk Hygiene

Milk is highly nutritious and is usually safe, but its quality is easily tarnished and rendered unsafe for consumption by foreign agents which come in as a result of contamination and poor storage conditions. The hygienic quality of milk is determined by the bacterial population present in milk; where a high bacterial count is an indication of poor hygiene and a long lag-time between milking and sale of milk hence reduced shelf life of the milk and enhanced risk of milk-borne bacterial infections(Donkor *et al.*, 2007; Omore *et al*., 1989). Poor milk hygiene often arises from poor handling and bacterial contamination from faeces, milk handlers, containers and adulteration by addition of contaminated water thus reducing the compositional, nutritional, and processing quality of the milk (Donkor *et al*., 2007).

2.5 Poor Compositional Quality of Milk

Unadulterated high quality milk that is free of antimicrobial residues is of interest to farmers, consumers and milk processing companies. Such milk enables farmers to get a fair price for their produce while processors are assured of a raw material suitable for manufacture of various dairy products and consumers are guaranteed of getting a healthy product at a good value (Tessema & Tibbo, 2009)]. Compositional quality of milk is determined by measuring its constituents and physico-chemical properties including: added water, butter fat, solid non-fat (SNF), protein, specific gravity and freezing point (Connor, 1995a)]. Adulteration of milk refers to the alteration of the natural composition of milk by extraction of one or more of its components (such as butter fat) or addition of some substances (such as water). Milk adulteration by addition of substances such as water interferes with the hygienic, compositional, nutritional and processing qualities of the milk, while extraction of components from milk lowers the value for money paid by consumers or processors (Donkor *et al.,* 2007).

Most of the times, milk adulteration is done intentionally for selfish economic gains. For example, when low value ingredients such as water or liquid whey are added to milk, they increase the volume of the milk but at the same time reduces its nutritional value. If the water used is contaminated, there is a risk of causing health problems (Das *et al.,* 2016). Several substances have been reported to be used as milk adulterants to achieve various intended results. Some of the substances include: chlorine, antibiotics, non-

milk proteins, low value milk, milk powder, colour, preservatives, urea, liquid whey and water (Das *et al.*, 2016; Jha *et al.*, 2016; Singh & Gandhi, 2015) .

Milk adulteration has been reported world over. In north eastern Brazil, 41.2% of goat milk presented to the market was found to contain bovine milk (Rodrigues *et al.*, 2012). In Ghana, 18% of marketed milk was found to be adulterated by addition of water (Donkor *et al.* 2007). In Kenya, a study by Omore *et al.* (2002) in Nakuru, Narok, Nairobi and Kiambu districts found 4.7% of milk samples from households (farms) and 10.4% from marketing agents to be adulterated by addition of water (Omore *et al.*, 2002). There is no published data on the compositional quality of marketed cow milk in Coastal Kenya Counties. A byproduct from the cottage cheese industry called liquid whey has been reported to be used as a milk adulterant to increase the volume of milk after extracting proteins and fat (Motta *et al.*, 2014) .

2.6 Quality assurance tests of milk

Milk quality control or assurance tests are used to ensure that milk and its products are safe, healthy and meet the set standards for chemical composition, purity, levels of bacteria and other microorganisms. Milk quality assurance tests entails checking the physico-chemical properties of milk (density/ specific gravity, acidity/ alkalinity and freezing point) as described above and performing various specific tests as detailed below.

2.6.1 Organoleptic testing of milk

Organoleptic testing of milk is done by simply looking and smelling milk. Fresh milk has a pleasant soft and sweet taste and carries hardly any smell. Milk flavour is a very important determinant of consumer acceptance of milk. Milk that has a bad smell, abnormal colour or contains particles is usually rejected or discarded. Off flavors and odours in milk may be produced by: Feed and weed flavours, strong smelling plants (onion, garlic), cow barn flavours, rancidity flavours due to excessive agitation during transportation, flavours from chemicals such as chlorine, acaricides, medications; high acidity flavours, strong flavoured feed stuffs such as poor quality silage, and oxidized flavours due to contact with copper or exposure to sunlight (Tessema & Tibbo, 2009; Pandey & Voskuil, 2011).

Apart from smelling of milk, visual observations can be done (sediment test) to check for insoluble dirt in milk. This may indicate the degree of sanitary care taken during production. The test consists of straining a quantity of milk through a cotton pad and observing the amount and type of residue remaining. Decisions are made on whether to accept or reject the milk in reference to standards set by the receiving institution. However, the presence or absence of a residue does not necessarily indicate the bacteriological quality of the milk; and the test is valueless if the milk has been carefully strained before delivery to the processing centre. In addition to that, erroneous results may be obtained if the milk is not well mixed before a representative sample is taken (Connor, 1994).

2.6.2 Clot on boiling

Clot on boiling is used to identify milk with high acidity (pH<5.8), colostral or mastitic milk. Colostral milk is usually rejected because it has a very high percentage of whey proteins which are made up of a number of distinct proteins most important being β-lactoglobulin and lactoglobulin (Connor, 1995a). When milk is heated to a temperature of over 75^0C, the β-lactoglobulins form a complex with k-casein which affects the functional properties of the milk. Milk that clots on boiling is usually rejected as it cannot withsatand the heating done during milk processing. The test is done by heating 2 ml of milk in a test tube and making observations for any clots or coagulation (Tessema & Tibbo, 2009).

2.6.3 Alcohol test

This test is based on instability of the proteins when the levels of acid and/or rennet are increased and acted upon by alcohol. It checks for the acidity of milk, but it is more sensitive than clot on boiling test. It is able to detect acidity from a pH<6.4. It is able to capture both mastitic and colostral milk. It is done by putting equal volumes of milk and 68% alcohol in a test tube (2 ml milk + 2 ml of 68% alcohol) and inverting the test tube severally to mix; with a thump pressed tightly on the open end of test tube. Clots will be observed if the milk has a pH less than 6.4 (Kurwijila, 1997).

2.6.4 Resazurin test

This test is based on colour changes that occur on a dye mixed with a milk sample within a time frame. The duration of reduction is considered as a measure of the number of organisms in the milk. The colour

change observed is due to oxidation reductions resulting from metabolism of the organisms present in the milk. The greater the number of organisms in the milk the quicker the oxygen is consumed and hence the faster the colour disappears (Connor, 1994). The test takes a very short time (ten minutes) and hence can be carried out as a screening test at the platform during milk collection. To carry out the test one needs to have: Resazurin tablets, test tubes, 1 ml pipettes, thermostatically controlled water bath and a Lovibond comparator with a Resazurin disc 4/9 (Connor, 1994; Kurwijila, 1997).

2.6.5 The Gerber Butterfat test

This test determines the fat content of milk. It is very important in many countries where the rate of payment for milk supplied by farmers to processors is a factor of the fat content of the milk supplied. Fat content of milk is also used to calculate the correct amount of feed ration for high yielding cows. To carry out the test one needs: Gerber butyrometers, rubber stoppers for butyrometers, 11 ml pipettes for milk, 10 ml pipettes for Gerber acid, 1 ml pipettes for Amyl alcohol and stands for the butyrometers. Butter fat content of milk can also be determined by running a milk sample through a milk analyzer (Muzira *et al*., 2006; Kurwijila , 1997).

2.6.6 Standard Plate Count (Total Plate Count)

Standard plate count is the official regulatory test used for estimating bacterial population of raw milk and milk products. Standards have been set on the maximum acceptable bacterial population in milk. A high standard plate count is indicative of poor hygiene in handling, milking equipments or errors in milk cold chain. This test is performed by pipetting diluted milk into Petri dishes, adding Standard Plate Count Agar, then incubating the plates for 48 hours at 32 ^{0}C. Bacterial colonies are then counted using a variety of methods depending on the colony types present. The Standard Plate Count is calculated based on the dilution and the number of colonies present(Shunda *et al*., 2013; Addo *et al.,* 2011).

2.6.7 Total Coliform Count

Presence of coliforms in milk is indicative of poor hygienic preparedness before milking and unhygienic environment around the cow. This test is performed by culturing dilutions of raw milk on Violet Red Bile

agar and incubated at 37 ^{0}C for 24 hours. Coliform counts are calculated based on the dilution and number of colonies present (Mwangi *et al.,* 2000; Njehu *et al*., 2014).

2.6.8 Test for Presence of Antimicrobial Residues in Milk

A number of tests kits are available to test for the presence of antimicrobial residues in milk. The most common ones include: Charm Blue-Yellow II test kit and Charm SL ROSA (Rapid One Step Assay) test kit (Charm Sciences Incorporated, MA, USA) (Omore *et al.,* 2009); Trisensor Rapid test (Unisensor S.A.); Delvotest® kit (DSM Food Specialties B.V.) (Adesiyun *et al,.* 2007) and HPLC (High Performance Liquid Chromatography), (Agilent Technologies Inc.)

2.7 Antimicrobial Residues in milk

Antimicrobial residues are remnants of either the parent antibiotic compounds or their metabolites that may accumulate, deposit or be stored in cells, tissues, organs or in edible products (such as milk and eggs) of an animal following their use prophylactically, therapeutically or to enhance production. Residues in animal tissues or products of animal origin can also result from unintentional administration of drugs, feed additives; or accidental exposure to chemicals in the environment (Seri, 2013).

Dairy cattle that have been treated with antibiotics produce milk containing residues for a period after treatment. Hence, such cows are required to be excluded from the milk supply chain for a specific period to ensure that the antibiotic residues no longer appear in their milk. Milk from treated cows is supposed to be discarded during this period. This period of exclusion is called the milk withdrawal period or milk discard time (Seri, 2013; Nisha, 2008) This is the time from cessation of treatment to the time it takes for the residues of the drug in milk to deplete below the safe concentration (Maximum Residue Limit, MRL). A maximum residual limit is the maximum concentration of residue resulting from the use of a veterinary medicinal product which may be legally permitted or recognized as acceptable in food. It is based on the type and amount of residue considered to be without any toxicological hazard for human health as expressed by the acceptable daily intake (ADI) (World Health Organization and Food and Agriculture Organization of the United Nations, 2011; Seri, 2013; Nisha, 2008).

Although antimicrobials are useful for treatment of infections, their occurrence in foods of animal origin as residues can cause adverse public health effects such as drug resistance (McDermott *et al.,* 2002; Ungemach *et al.,* 2006) and hypersensitivity caused by penicillins and sulphonamides (Dewdney *et al.,* 1991; Seri, 2013). Their occurrence in milk also causes huge economic losses in processing industries by interfering with the manufacture of cultured products such as yoghurt and cheese through inhibition of starters and rejection of milk from farmers that test positive for antimicrobials (Kang'ethe *et al.* 2,005). Antibiotics used in veterinary practice are identical or closely related to those used in human medicine. Hence, any improper use or exposure in either can easily result in cross-resistance (Ungemach *et al.,* 2006)
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A healthy milking herd forms the basis for good quality milk, among which the sick lactating cows are attended to by an animal health professional and are only incorporated into the milk supply chain after observing the recommended withdrawal period of the antibiotics used to treat the animals. There are several different groups of antibiotics which are available for treatment of dairy cattle. The most common groups of antibiotics used for cows include the beta-lactams (penicillin), sulphonamides (sulfamethazine), aminoglycosides (streptomycin) and tetracyclines (Barlow, 2011). These antimicrobials may be used singly, or in combinations.

Antimicrobial residue occurrence in milk and other foods of animal origin have been reported in various countries. However, in countries with effective quality assurance systems, reports of residues in foods destined for the market are minimal or non-existent (Kang'ethe *et al.,* 2005). For example, in Brazil, a study to assess hazards in unpasteurized marketed milk at farm level found a prevalence of antimicrobial residues of 11.5% (Nero *et al,.* 2004). A study in the peri-urban areas of Accra and Kumasi in Ghana (Aning *et al.,* 2007) found that 35.5% of samples of raw marketed milk were contaminated with antimicrobial residues. In Tanzania, a prevalence of 36% was observed in a study to investigate the risk of exposure to antimicrobial residues present in marketed raw milk in Mwanza and Dar es Salaam (Kurwijila *et al.,* 2006). In Kenya,(Kang'ethe *et al.,* 2005) a study assessing marketed milk in contrasting urban and rural locations of Nakuru, Nairobi, Narok and Kiambu districts found a prevalence of 11.1% in urban and 16% in rural locations.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Lamu West Sub-County of Lamu County, the North Coastal part of Kenya, bordering Lamu East to the North, Indian Ocean to the East, Tana River County to the South West and Garissa County to the North West. Lamu lies 2^0 16' 42'' South of the Equator and 40^0 54'12'' East of the Greenwich. Lamu County is made up of two sub-counties: Lamu West and Lamu East. Lamu West Sub-County is made up of four administrative divisions: Amu, Hindi, Mpeketoni and Witu. Amu Division is an island in the Indian Ocean, whereas the other three divisions are on the mainland. The Sub-County has six urban centers, namely: Lamu, Mokowe, Hindi, Mpeketoni, Kibaoni and Witu. The Sub-County covers an area of 4,503.7 square kilometers with low lying altitudes ranging between 0m and 50m above sea level (Figure 3.1).

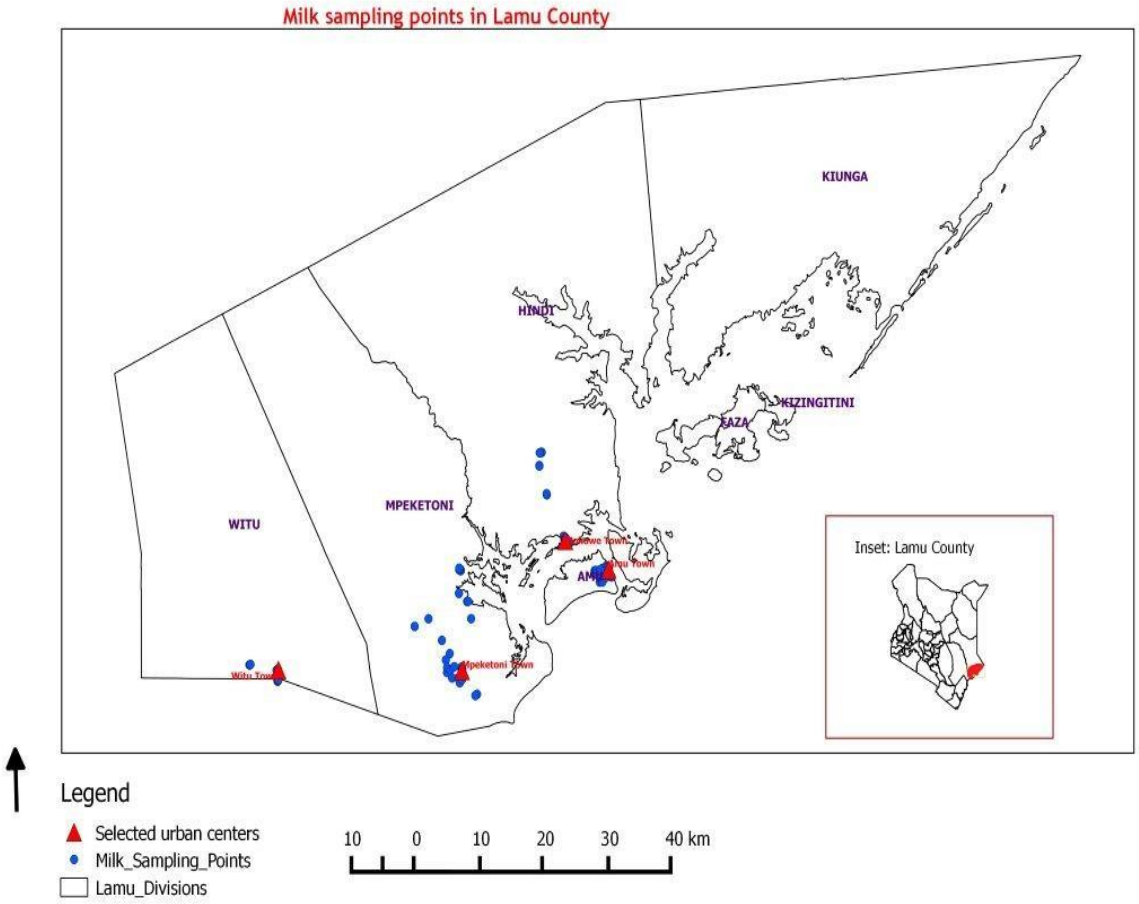

Figure 3.1: Map of Lamu County showing sampling sites

GPS references for the selected urban centres for this study: Amu (-2.271099379; 40.90262004); Mokowe (-2.3719039; 40.847748916); Mpeketoni (-2.385382522; 40.69676394) and Witu (-2.388310237; 40.43949659).

Lamu County receives rain in two seasons, these are, the long rains season between March and April and the short rain season between November and December. Annual rainfall ranges between 850mm -1110mm with temperatures ranging between 23^0C and 32^0C. Lamu West Sub-County has a population of 80,000 persons. The main economic activities in this region are fishing, tourism, livestock and crop farming. The livestock species kept here are mainly cattle, sheep, goats, donkeys and poultry. Lamu West has an estimated cattle population of 126,250. The cattle rearing systems practiced here are: pastoralism, semi-

zero grazing and zero grazing. Pastoralism here entails grazing large herds of local breeds of cattle (Boran and Zebu) in public or communally owned land and occasionally migrating to neighboring counties in search of greener pastures and drinking water. Pastoralism is practiced in Hindi, Mpeketoni and Witu divisions. Semi-zero grazing is practiced in Hindi, Mpeketoni and Amu divisions and entails grazing cattle in the fields and providing supplementary feeding with fodder or commercial feeds. The breeds of cattle kept in this type of farming are cross-breeds and exotic breeds (Jersey, Guernsey, and Friesian). Zero grazing is practiced mainly in Amu division where cattle are kept in enclosures and rely on fodder and commercial feeds. The types of cattle kept here are cross-breeds and exotic breeds. In Lamu County, the milk from these cattle is sold raw to consumers who then boil it before consumption. The only link between the producer (farmer) and the consumer are small scale traders or milk vendors.

3.2 Study Design

Cross-Sectional Study

3.3 Study Population

1. Informal raw cow milk vendors operating within the milk catchment area of the selected urban centres in Lamu West Sub-County.
2. Livestock farmers with lactating cattle and offering milk for sale within their farms within the milk catchment area of the selected urban centres in Lamu West Sub-County.

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion criteria:

A livestock farmer: Any person (male / female) with lactating cattle and offerered milk for sale within his /her farm, and was willing to participate in the study.

A milk vender: any person (male/female) who obtained milk from own farm or bought milk from one or more farms / milk outlets and sold it by hawking along the pathways or at raw milk selling shops within the milk supply catchment of the selected urban centres and willing to participate in the study.

3.4.2 Exclusion criteria:

A farmer or a vendor absent during the visit; or unwilling to participate in the study, or unwilling to give required information, or did not have milk for sampling at the time of the visit; or had no time for the questionnaire interview.

3.5 Sample Size Determination

Sample sizes for vendors and farmers were determined separately using the Cochran formula of 1977, using estimated prevalence (p) of antimicrobial residues of 16% for milk from farms and 11.1% for milk from vendors (Kang'ethe *et al.* 2005).

Sample size for farmers was calculated as: $$n = \frac{z^2 p(1-p)}{d^2}$$

Where Z value for 95% confidence level is 1.96,

P is the known prevalence at 16 % and

d is the precision (margin of error) at 5%.

$$\frac{1.96^2 \text{ X } 0.16 \text{ X } 0.84}{0.05^2}$$

n = 206.5

A total of 207 livestock farmers were estimated as the sample sample size needed to achieve a power of 80%.

Using the same formula and a prevalence of 11.1%, the required sample size from vendors was determined:

$$\frac{1.96^2 \text{ X } 0.111 \text{ X } 0.889}{}$$

$$0.05^2$$

$$n = 151.6$$

A total of 152 milk vendors were estimated as the sample size needed to achieve a power of 80%.

3.6 Sampling Design

Four of the six urban centres in the sub county were selected for the study. These urban centres were: Amu, Mokowe, Mpeketoni and Witu. A preliminary visit was made to the selected market centres and with the help of key informants (the Public Health Officers, Livestock Production Officers, Veterinary Officers, Local Authority Trade Officers, milk marketing groups or associations and milk selling points), a sampling frame of informal raw milk vendors was created for each selected urban centre. A milk vendor was defined as any person who obtained milk from own farm or bought milk from one or more farms or milk outlets and sold it by hawking along the pathways or at raw milk selling shops within the milk supply catchment of the selected urban centres. Using the same key informants, another sampling frame was created comprising of livestock farmers, where a legible livestock farmer was defined as any person with lactating cattle within the milk supply catchment area of each selected urban centre and offered milk for sale at their farms. The established sampling frames were made of two groups: 784 livestock farmers with lactating cattle and 251 vendors. The number of farmers and vendors sampled from each centre were determined proportionate to number of farmers and vendors in each selected centre (Appendix V). Sampling units were individual vendors and farmers. Those who participated in the study were selected using simple random sampling, (Table 3.1). Those who refused to consent or participate in the study were replaced from the sampling frame using simple random sampling.

Table 3.1: Farmers and vendors selected for the study from the four urban centres; 2015

	Gender	Amu	Mokowe	Mpeketoni	Witu	Total
Farmers	Male	25	30	39	69	**163**
	Female	2	10	13	19	**44**
	Total	**27(13.0)**	**40(19.3)**	**52(25.1)**	**88(42.6)**	**207**
Vendors	Male	20	10	19	2	**51**
	Female	26	29	25	21	**101**
	Total	**46(30.3)**	**39(25.7)**	**44(28.9)**	**23(15.1)**	**152**

3.7 Milk Sample Collection

After obtaining consent and appropriately compensating the farmers and vendors, 100 ml of milk was aseptically collected into sterile bottles from each respondent, stored them in ice-packed cool boxes and transported them to Lamu County hospital laboratory where they were analyzed on the same day they arrived. The hospital laboratory was selected as it was the most established laboratory facility in the County and provided ample environment for hosting the equiments needed for our study.

3.8 Administration of questionnaires

A pretested structured questionnaire translated into Kiswahili was administered to each vendor and farmer, through face to face interview, to collect data on demographics and practices that might affect the compositional quality of the marketed milk and occurrence of antimicrobial residues such as livestock treatment practices, observance of milk withdrawal period following antibiotic therapy, training in good milking and milk handling practices, practices used to prolong freshness of milk; and methods used by vendors in selling milk.

3.9 Compositional quality analysis

The 152 samples from vendors and 207 from farmers were analyzed for compositional quality using Ekomilk® Ultrasonic Milk Analyzer (EON Trading LLC USA), as per manufacturer's instructions and as similarly done by (Kunda *et al*., 2015). The milk analyzer was obtained from Voi Milk Analysis Station for purposes of this study. The milk sample vials were brought out of the cool box and allowed to thaw to room temperature. Each milk specimen vial was shaken gently to thoroughly mix the contents after which 20 ml of the milk specimen was transferred into the analyzer cup. The cup was placed below the aspiration tube of the Ekomilk® Ultrasonic Milk Analyzer and connected to power to start the analysis. The parameters estimated by the analyzer were: added water, butter fat, solid non-fat (SNF), protein percentage, specific gravity and freezing point. Adulterated milk was identified using standard values (Connor 1995b; Kunda *et al*., 2015) by having SNF <8.5%, added water ≥0.01%, specific gravity outside the normal range (1.026–1.036 Kg/l, butterfat <3.3% or >7.0% ; and freezing point outside the normal range of between -0.525^{0}C and -0.565^{0}C.

3.10 Testing for presence of Antimicrobial Residues

Charm Blue Yellow II kit (imported from the manufacturer, Charm Sciences Inc.) was used to test for presence of antimicrobial residues using a standard method as described by the manufacturer (Sciences Charm, 2010). A 100-ml milk specimen obtained from Witu Veterinary Farm from a cow that had not been exposed to antibiotics either therapeutically, prophylactically or as feed additives for the past 12 months was used as a negative control. The specimen was confirmed negative by the Charm Blue Yellow II test kit. A positive control was prepared by reconstituting the provided 4 parts per billion (ppb) Penicillin G Standard with 10.0 ml of a negative control, shaken and allowed to stand refrigerated for 15 minutes. The controls were put in 0.5 ml aliquots in clean vials and frozen at -15^{0}C for later use. Whenever the controls were needed, they were slowly thawed overnight in a refrigerator and shaken well before use.

Each milk specimen (or control) was shaken and 50 µl was pipetted into the purple agar portion of the well. A clear sealing tape was applied and pressed firmly to seal the rim of each well to prevent them from drying. The prepared wells were put into an air incubator at 64±1^{0}C for 2 hours 55 minutes. After incubation, the wells were removed from the air incubator, allowed to settle for 5 minutes on the desk, for

colour development. Colour observation was done in comparison with the reference colours provided by the manufacturer(Sciences Charm, 2010). Yellow or yellow/green wells were interpreted as negative, whereas blue/purple wells were interpreted as positive. Grey coloured wells, (referred to as 'Caution' by the manufacturer) were interpreted as positive. From the initial positive results, 600 µl of milk was heated in a test tube to boiling point for 3 minutes. Then they were allowed to cool to room temperature and shaken. The heated specimens were run in duplicate along with a negative and positive control and unheated milk specimen in the same procedure as above. Specimens which tested positive after heat treatment were interpreted as 'Blue Yellow II Test positive' hence contained antibiotics **(Appendix IX).** Specimens that tested negative after heat treatment were considered to contain a non-antibiotic heat sensitive inhibitor.

3.11 Data Analysis

The data was entered, cleaned and analyzed in Ms Excel™ 2007 and EPI Info 7™. In univariate analyses, proportions were calculated for categorical variables and means and medians for continuous variables. Bivariate analysis (Pearson chi square and Fischer's exact tests) was carried out to examine the association between the presence of antimicrobial residues or compositional quality of raw marketed milk and other factors with p-value ≤ 0.05 considered statistically significant. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Factors in bivariate analyses with p-value ≤ 0.1 were included in a forward selection unconditional logistic regression model to control for confounders and identify independent factors associated with the occurrence of antimicrobial residues in milk and milk adulteration as identified by adjusted odds ratios (AORs) and 95% CIs. Factors with p-value <0.05 in the final model were considered significant. Comparison of proportions was made using a 2-sample z-test with two tailed comparisons at 0.05 level of significance.

3.12 Ethical Approvals and Considerations

The aim and procedures of the study were explained to the study participants who were required to give written informed consent prior to their voluntary participation in the study. Milk specimens were collected from only those who consented and the specimens were only used to assess quality characteristics and antimicrobial residues. Confidentiality of laboratory information and data was observed and maintained

through password protected computers and observing good professional conduct. Ethical clearance and approval for this study was obtained from Jaramogi Oginga Odinga Teaching and Referral Hospital Ethical Review Committee, Ref. ERC.1B/VOL.1/158. Approval was also given from the Board of post graduate studies of Jomo Kenyatta University of Agriculture and Technology (JKUAT), the Lamu County Veterinary Officer and the Lamu County Director of Health, to use the institution's laboratory facility.

3.13 Study Limitations

In interpreting the findings of this study, it should be noted that sampling of farmers was independent from sampling of vendors. Milk could not be followed along the market value chain, from individual farms to individual vendors, to determine the source or point of adulteration and the antimicrobial residues in the milk.

CHAPTER FOUR

RESULTS

4.1 Socio-demographic characteristics of respondents

Participants enrolled included 152 vendors and 207 farmers from Amu, Mokowe, Mpeketoni and Witu urban centres of Lamu West Sub-County. The socio-demographic characteristics of the study participants varied by age, sex, level of education, type of livestock production system, and mode of milk vending business (Table 4.1).

Table 4.1: Socio-demographic characteristics of study participants in Lamu West Sub County, 2015

Characteristics	**Total Farmers (N=207) n (%)**	**Total Vendors (N=152) n (%)**
Sex		
Female	44 (21.3)	101 (67.1)
Male	163 (78.7)	51 (33.5)
Age groups (years)		
<45 (19-44)	93 (44.9)	102 (67.1)
≥ 45 (45-80)	114 (55.1)	50 (32.9)
Livestock production System		
Pastoralism	157 (75.9)	-
Non pastoralism	50 (24.1)	-
Level of education		
< Secondary Education	130 (62.8)	114 (75.0)
≥ Secondary Education	77(37.2)	38 (25.0)
Type of vendor		
Kiosk-Shop	-	15 (9.9)
Hawker (mobile trader)	-	137 (90.1)

4.1.1 Vendor characteristics

Majority of the milk vendors (78%) obtained milk from more than one farm or outlet and dispensed by either scooping or pouring. They accessed the milk market by various means, including on foot, bicycles, motorcycles, donkeys, vehicles and boats; depending on the geographicall location of the urban centre. On

handling of surplus milk at the end of sales day, most vendors (80%) opted to consume it with the family. Majority of the vendors (93%) were using plastic jerricans in transportation of milk to the market, which were said to be cheaper and easily portable. Although the vendors were in the milk selling business, none had been licenced to carry out the trade.

4.1.2 Farmers' characteristics

Farmers in the study site dispensed milk by pouring or scooping to buyers (consumers/ vendors) who who came for the milk from the farm. Surplus milkwas consumed by the family. Milk for sale at farm level was stored in plastic jerricans which the farmers said were cheaper and readily available, as compared to alluminium cans. In regard to treatment of animals, around half (55.1%) of the farmers said their animals were being treated by a veterinary professional.

4.2 Compositional quality of milk

The median butterfat content of the marketed raw milk from farms was 5.21 (range 2.02–9.47) whereas that from vendors was 5.25 (range 2.26–9.34). An acceptable range of butterfat (3.3–7.0%) was observed in the raw milk from 92.7% (192/207) of farmers and 92.1% (140/152) of vendors. Unacceptable (out of range) values of SNF, specific gravity, added water and freezing points were also observed in samples from both farmers and vendors. Overall, 82.6% (95% CI: 77.0-87.3) of marketed raw cow milk from farms and 75.0% (95% CI 66.7-81.4) from vendors were of acceptable compositional quality (Table 4.2).

Table 4.2: Compositional quality of informally marketed raw cow milk, Lamu West, 2015

	Farmers, N=207		Vendors, N=152		
Milk Component	Median (Range)	No.(%) out of normal range	Median (Range)	No.(%) out of normal range	Normal Range
Butter Fat %	5.21 (2.02-9.47)	15/207 (7.2)	5.25 (2.26-9.34)	12/152 (7.9)	3.3-7.0
SNF	9.32 (5.86-12.1)	36/207 (17.4)	9.29 (5.45-12.4)	38/152 (25.0)	>8.5-12.0
Specific Gravity kg/l	1.030 (1.018-1.037)	36/207 (17.4)	1.029 (1.018-1.037)	38/152 (25.0)	1.026-1.036
Added water %	14.28 (0.37-27.90)	36/207 (17.4)	14.04 (0.48-27.6)	38/152 (25.0)	0.00
Freezing point (0C)	-0.597 (-0.401- -0.733)	36/207 (17.4)	-0.595 (-0.402 – -0.649)	38/152 (25.0)	-0.525 - -0.565
Protein %	3.53 (2.26-5.27)	5/207 (2.4)	3.52 (2.30-4.68)	3/152 (2.0)	2.9-5.0

The compositional quality of informally marketed raw cow milk varied across the selected four study sites. In Amu town, 7.4% of the milk from farms and 47.8% milk from vendors were found to be of poor compositional quality as a result of addition of water. (Table 4.3).

Table 4.3: Compositional quality of informally marketed raw cow milk, Amu town, Lamu West, 2015

	Farmers, N=27		Vendors, N=46		
Milk Component	Median (Range)	No.(%) out of normal range	Median (Range)	No.(%) out of normal range	Normal Range
Butter Fat %	3.73 (2.02-5.73)	1/27 (3.7)	3.97 (2.26-6.60)	5/46 (10.9)	3.3-7.0
SNF	9.00 (7.84-9.69)	2/27 (7.4)	8.58 (5.45-9.43)	22/46 (47.8)	>8.5-12.0
Specific Gravity kg/l	1.030 (1.024-1.035)	2/27 (7.4)	1.029 (1.019-1.034)	22/46 (47.8)	1.026-1.036
Added water %	0.00 (0.00-7.15)	2/27 (7.4)	0.00 (0.00-24.6)	22/46 (47.8)	0.00
Freezing point (0C)	-0.587 (-0.516- -0.643)	2/27 (7.4)	-0.564 (-0.416 – -0.649)	22/46 (47.8)	-0.525 - -0.565
Protein %	3.40 (2.96-3.65)	0	3.27 (2.92-3.80)	0	2.9-5.0

In Mokowe, 27.5% of the milk from farms and 7.69% from vendors were found to be of poor compositional quality due to adulteration by addition of water (Table 4.4).

Table 4.4: Compositional quality of informally marketed raw cow milk, Mokowe, Lamu West, 2015

	Farmers, N=40		**Vendors, N=39**		
Milk Component	Median (Range)	No.(%) out of normal range	Median (Range)	No.(%) out of normal range	Normal Range
Butter Fat %	6.35 (2.13-9.47)	4/40 (10.0)	5.92 (2.30-9.00)	5/39 (12.8)	3.3-7.0
SNF	9.59 (6.71-10.4)	11/40 (27.5)	9.30 (6.69-10.8)	3/39 (7.69)	>8.5-12.0
Specific Gravity kg/l	1.030 (1.018-1.033)	11/40 (27.5)	1.029 (1.019-1.036)	3/39 (7.69)	1.026-1.036
Added water %	0.00 (0.00-16.8)	11/40 (27.5)	0.00 (0.00-18.4)	3/39 (7.69)	0.00
Freezing point (0C)	-0.603 (-0.426- -0.639)	11/40 (27.5)	-0.595 (-0.454 – -0.645)	3/39 (7.69)	-0.525 - -0.565
Protein %	3.73 (2.60-3.97)	2/40 (5.0)	3.52 (2.57-4.07	2/39 (5.12)	2.9-5.0

In Mpeketoni, 19.2% of milk from farms and 18.2% from vendors were found to be of poor compositional quality due to adulteration by addition of water (Table 4.5).

Table 4.5: Compositional quality of informally marketed raw cow milk, Mpeketoni, Lamu West, 2015

	Farmers, N=52		**Vendors, N=44**		
Milk Component	Median (Range)	No.(%) out of normal range	Median (Range)	No.(%) out of normal range	Normal Range
Butter Fat %	5.33 (2.79-9.30)	5/52 (9.6)	5.43 (3.43-9.52)	0	3.3-7.0
SNF	9.52 (7.32-11.5)	10/52 (19.2)	9.40 (6.00-12.4)	8/44 (18.2)	>8.5-12.0
Specific Gravity kg/l	1.031 (1.022-1.037)	1052 (19.2)	1.030 (1.018-1.037)	8/44 (18.2)	1.026-1.036
Added water %	0.00 (0.00-13.1)	10/52(19.2)	0.00 (0.00-27.6)	8/44 (18.2)	0.00
Freezing point (0C)	-0.612 (-0.483- -0.733)	10/52 (19.2)	-0.602 (-0.402 – -0.644)	8/44 (18.2)	-0.525 - -0.565
Protein %	3.62 (2.77-4.38)	1/52 (1.9)	3.57 (2.92-4.68	0	2.9-5.0

In Witu, 14.8% of milk from farms and 21.7% from vendors were found to be of poor compositional quality due to adulteration by addition of water (Table 4.6).

Table 4.6: Compositional quality of informally marketed raw cow milk, Witu, Lamu West, 2015

	Farmers, N=88		**Vendors, N=23**		
Milk Component	Median (Range)	No.(%) out of normal range	Median (Range)	No.(%) out of normal range	Normal Range
Butter Fat %	5.16 (2.66-7.98)	5/88 (5.7)	5.10 (2.36-8.00)	2/23 (8.7)	3.3-7.0
SNF	9.07 (5.86-12.1)	13/88 (14.8)	9.31 (6.83-10.4)	5/23 (21.7)	>8.5-12.0
Specific Gravity kg/l	1.029 (1.017-1.036)	13/88 (14.8)	1.030 (1.021-1.035)	5/23 (21.7)	1.026-1.036
Added water %	0.00 (0.00-27.9)	13/88(14.8)	0.00 (0.00-19.7)	5/23 (21.7)	0.00
Freezing point (0C)	-0.583 (-0.401- -0.691)	13/88 (14.8)	-0.597 (-0.446 – -0.646)	5/23 (21.7)	-0.525 - -0.565
Protein %	3.45 (2.26-5.27)	2/88 (2.3)	3.53 (2.59-3.90)	1/23 (4.3)	2.9-5.0

Overall, 17.4% (95%CI: 12.7-23.0) of the milk samples from farmers and 25.0% (95%CI: 20.6-36.6) from vendors were found to be of poor compositional quality due to adulterattion by addition of water (p=0.786). A difference was observed between compositional quality of milk sold by farmers and vendors in Amu (7.4% vs 47.8%; p<0.001) and Mokowe (27.5% vs 7.7%; p=0.021) respectively (Table 4.7).

Table 4.7: Comparison between prevalence of poor compositional quality of informally marketed raw cow milk per urban centre from farmers and vendors, Lamu West Sub-County, 2015

Urban centre	**No. of milk samples**		**Poor compositional quality**		
	From farmers	From vendors	Poor compositional quality milk samples from **farmers** n (%)	Poor compositional quality milk samples from **vendors** n (%)	p-value
Amu	27	46	**2/27 (7.4)**	**22/46 (47.8)**	**<0.001**
Mokowe	40	39	**11/40 (27.5)**	**3/39 (7.7)**	**0.021**
Mpeketoni	52	44	10/52 (19.2)	8/44 (18.2)	0.900
Witu	88	23	13/88 (14.8)	5/23 (21.7)	0.424
Total	**207**	**152**	**36/207 (17.4)**	**38/152 (25.0)**	0.786

4.3 Prevalence of antimicrobial residues

Overall, 15.5% (95% CI: 11.0-20.9) of the samples from farmers and 18.4% (95% CI: 12.9-25.2) of the samples from vendors were found to have antimicrobial residues above the EU MRLs (p-value=0.467). A significant difference between the prevalence of antimicrobial residues in milk sold by farmers compared to that sold by vendors was only observed in Witu (12.5% vs 30.4%; p=0.038) (Table 4.8).

Table 4.8: Comparison between prevalence of antimicrobial residues in informally marketed raw cow milk per urban centre from farmers and vendors, Lamu West Sub-County, 2015

Urban centre	**No. of milk samples**		**Antimicrobial residues**		
	From farmers	From vendors	Positive milk samples from **farmers** n (%)	Positive milk samples from **vendors** n (%)	p-value
Amu	27	46	6(22.2)	8(17.4)	0.615
Mokowe	40	39	7(17.5)	5(12.8)	0.560
Mpeketoni	52	44	8(15.3)	8(18.2)	0.704
Witu	88	23	**11(12.5)**	**7(30.4)**	**0.038**
Total	**207**	**152**	**32(15.5)**	**28(18.4)**	0.467

From the interview findings, 28.5% (59/207) of the farmers and 8.6% (13/152) of the vendors acknowledged to be using a herbal substance with a local name "*mpingo"* which they applied by smoking the inner side of wooden milk handling containers, to serve as a milk preservative. On laboratory analysis using Charm Blue Yellow test, 20% (41/207) of the milk samples from farmers and 5.9% (9/152) of samples from vendors indicated the presence of a non-antibiotic heat-sensitive inhibitor. Of the 41 positive milk samples from farmers, 63.4% (26/41) were from Witu and 21.9% (9/41) from Mpeketoni.

4.4 Comparison between compositional quality and prevalence of antimicrobial residues in milk marketed by farmers and vendors

Overall, 70.5% (95% CI: 64.1-76.4) samples from farmers and 63.2% (95% CI: 55.3-70.6) from vendors were both of good compositional quality and free of antimicrobial residues. However 3.4% (95%CI: 1.5-6.6) of milk samples from farmers and 6.6% (95%CI 3.4-11.4) from vendors contained antimicrobial residues and were of poor compositional quality (p=0.159).

4.5 Factors associated with presence of antimicrobial residues in marketed raw cow milk among farmers

Farmers who had less than secondary level of education were three times more likely to sell milk with antimicrobial residues (OR 2.98, 95% CI: 1.16-7.56) compared to farmers who had secondary level of education and above. Farmers who were aware of dangers of consuming milk with antimicrobial residues were less likely to sell milk with antimicrobial residues compared to those farmers who were not aware (OR 0.20, 95% CI: 0.07-0.55). Those farmers who had some training on good milking practices were less likely to sell milk with antimicrobial residues compared to those farmers who did not have any training on good milking practices (OR 0.32; 95% CI: 0.11-0.96). Farmers' awareness of dangers of consuming milk with antimicrobial residues and farmers' training on good milking practices were retained as independent factors protective against selling milk with antimicrobial residues (Table 4.9).

Table 4.9: Factors associated with presence of antimicrobial residues in informally marketed raw cow milk by farmers in Lamu West Sub County, 2015

Characteristics	Residues Positive n (%)	Residues Negative n (%)	Crude OR (95%CI)	p-value	Adjusted OR (95%CI)	p-value
Age Group						
≥45 years (45-80)	20 (17.5)	94 (82.5)	1.43 (0.66-3.12)	0.440	-	-
<45 years (19-44)	12 (12.9)	181(87.1)	1.00			**<0.001**
Level of education						
<Secondary Education	26 (20.0)	104(80.0)	**2.98 (1.16-7.56)**	**0.027**	1.49(0.52-4.31)	0.461
≥ Secondary Education	6 (7.8)	71 (92.2)	1.00			
Who treats your sick livestock						
Veterinarian	14 (12.3)	100 (87.7)	0.58 (0.27-1.25)	0.180	-	-
Other	18 (19.4)	75 (80.6)	1.00			
Sex						
Male	22(13.5)	141(86.5)	0.53(0.23-1.22)	0.158	-	-
Female	10(22.7)	34(77.3)	1.00			
Aware of danger of the residues						
Aware of danger	5 (5.6)	84(94.4)	**0.20(0.07-0.55)**	**<0.001**	**0.20(0.07-0.55)**	**0.002**
Not aware of danger	27 (22.9)	91(77.1)	1.00			
Livestock Production System						
Pastoralist	23 (14.6)	134 (85.4)	0.78 (0.33-1.82)	0.653	-	-
Non-Pastoralist	9 (18)	41 (82)	1.00			
Training on good milking practices						
Trained	4 (6.9)	54 (93.1)	**0.32(0.11-0.96)**	**0.033**	**0.33(0.11-0.99)**	**0.048**
Not trained	9 (18.8)	121 (81.2)	1.00			

4.6 Factors associated with poor compositional quality of marketed raw cow milk among farmers and vendors

Farmers who had at least secondary level of education were three times more likely to market milk of poor compositional quality (OR 2.88, 95% CI: 1.38-5.99) compared to those with primary level of education or no formal education. Pastoralist farmers were three times more likely to sell milk of poor compositional quality (OR 2.94, 95% CI: 0.99-8.78) as compared to non-pastoralist farmers. Of the 32 pastoralist farmers found selling milk of poor compositional quality 19 (59.4%) had attained at least secondary level of education. Adjusting for factors simultaneously, farmers having secondary level of education and above (AOR 3.03, 95% CI: 1.44-6.39) and being a pastoralist farmer (AOR 3.20, 95% CI: 1.05-9.71) were retained as independent risk factors against marketing of milk of poor compositional quality (Table 4.10).

Table 4.10: Factors associated with poor compositional quality of informally marketed raw cow milk by farmers in Lamu West Sub County, 2015

Characteristics	Poor Quality n (%)	Good Quality n (%)	Crude OR (95%CI)	p-value	Adjusted OR (95%)	p-value
Age Group						
≥45 years (45-80)	18 (19.4)	75 (80.6)	1.28 (0.62-2.63)	0.581	-	-
<45 years (19-44)	18 (15.8)	96 (84.2)	1.00			**<0.001**
Level of education						
≥Secondary Education	21 (27.3)	56 (56)	**2.88(1.38-5.99)**	**0.007**	**3.03(1.44-6.39)**	**0.004**
< Secondary Education	15 (11.5)	115 (88.5)	1.0	-	-	-
Sex						
Male	27 (16.6)	136 (83.4)	0.77(0.33-1.79)	0.511	-	-
Female	9 (20.5)	35(79.5)	1.00			
Training on good milking practices						
Trained	10 (17.2)	48 (82.8)	0.84(0.40-1.99)	0.842	-	-
Not trained	26 (19.0)	111 (81.0)	1.00			
Livestock Production System						
Pastoralist	32 (20.4)	125(79.6)	2.94(0.99-8.78)	**0.053**	**3.20(1.05-9.71)**	**0.040**
Non-Pastoralist	4(8)	46 (92)				

Male vendors were three times more likely to market milk of poor compositional quality (OR 3.46, 95% CI: 1.61-7.47) compared to female vendors. At bivariate analysis, vendors who had been trained on good milk handling practices were more likely to market milk of poor compositional quality (OR 17.12 CI: 1.93-151.7). However, at multi-variate analysis, this finding was not statistically significant (AOR 9.05 CI: 0.97-84.3). Of the 152 vendors, only 6 (3.9%) male vendors had been trained on good milk handling practices of which 5 (83.3%) were found to be selling milk of poor compositional quality.Being a male vendor was retained as the only independent risk factor associated with marketing of milk of poor compositional quality amongst vendors (AOR 2.73. 1.22-6.08) after adjusting for vendor-training on good milk handling practices (Table 4.11).

Table 4.11: Factors associated with poor compositional quality of informally marketed raw cow milk by vendors in Lamu West Sub County, 2015

Characteristics	Poor Quality n (%)	Good Quality n (%)	Crude OR (95%CI)	p-value	Adjusted OR (95%CI)	p-value
Age Group						
≥45 years	23 (22.6)	79 (77.4)	0.68(0.32-1.46)	0.326	-	-
<45 years	15 (30.0)	35 (70.0)	1.00			
Level of education						
<Secondary Education	27 (23.7)	87 (76.3)	0.76(0.33-1.74)	0.522	-	-
≥ Secondary Education	11 (30.0)	27 (70.0)	1.00			
Sex						
Male	21 (41.2)	30 (58.8)	**3.46(1.61-7.47)**	**0.002**	**2.73(1.22-6.08)**	**0.014**
Female	17 (16.8)	84 (83.2)	1.00			
Trained on good milk handling practices						
Trained	5 (83.3)	1 (16.7)	**17.12(1.93-151.7)**	0.004	9.05(0.97-84.3)	0.053
Not trained	33 (22.6)	113 (77.4)	1.00			
Type of trade						
Kiosk/shop	0 (0)	8(100)	0.00	-	-	-
Hawker	38(26.4)	106 (73.6)				

<0 001

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This is the first study in the northern coastal Kenya to assess the compositional quality and milk safety with regard to presence of antimicrobial residues. Results of this study demonstrated that consumers of marketed raw cow milk in this region are at risk of being exposed to public health problems associated with presence of antimicrobial residues in food of animal origin and consumption of adulterated milk. The study identified factors associated with the occurrence of the residues and the milk adulteration. Both farmers and vendors were found to use a herbal substance in preservation of milk.

Water was identified as the only adulterant. Other studies have also identified water as the most common adulterant in the milk industry (Das *et al*. 2016). Addition of water lowers the nutritional value of the milk, interferes with processing qualities and poses a risk of contaminating the milk. Adulteration of milk by addition of water can easily be detected in the field using a lactometer (Mwangi *et al*. 2000). Other substances have been reported as milk adulterants, such as: chlorine, antibiotics, non-milk proteins, low value milk, milk powder, colour, preservatives, urea and liquid whey (Das *et al*. 2016; Jha *et al*. 2016; Singh & Gandhi 2015) . In north eastern Brazil, 41.2% of goat milk presented to the market was found to contain bovine milk (Rodrigues *et al*. 2012). A by-product from the cottage cheese industry called liquid whey has been reported to be used as a milk adulterant to increase the volume of milk after extracting proteins and fat (Motta *et al.* 2014). Because of the wide variety of adulterants reportedly used in the dairy industry with diverse effects, there is need for routine monitoring of the milk market value chain right from farm level to assure safety of milk to the consumers.

This study found a higher rate of milk adulteration compared to that reported from a study done in Nakuru, Narok, Nairobi and Kiambu districts of Kenya by Omore *et al* (2002) where 4.7% of milk specimens from household farms and 10.4% from marketing agents were found to be adulterated by addition of water. The higher prevalence observed in our study can be associated with the young dairy industry in Lamu County where most of the milk produced is marketed raw directly to consumers as compared to Nakuru, Narok and

Kiambu where bulk of the milk produced is sold to milk processing companies who are very strict on the quality of milk purchased. Milk of poor compositional quality is usually rejected by processors resulting in huge economic losses to farmers (Connor 1995a; Muzira *et al.* 2006). Milk processors also carry out extension services to farmers promoting good milking practices, a service lacking in Lamu County as there are no local milk processors.

A butter fat percentage above normal range was observed in a few samples. This could be attributed to poor mixing of milk before sampling or poor shaking of milk before putting it in the Ekomilk Analyser. Below normal butter fat could be suggestive of adulteration by removal of cream. However, as cows grow older, fat content of milk decreases by 0.02 percentage units per lactation while SNF falls by 0.04 percentage units. Underfeeding as well as mastitis has been found to reduce both the butter fat content and the SNF of milk (Connor 1995a).

This study observed a SNF below 8.5%. This was indicative of adulteration which was confirmed with the presence of added water in such milk samples. However, one sample had SNF of 12.1%, slightly above normal. This could be treated as a marginal error of the machine. However, above normal SNF is usually observed when adulterants such as urea are used to prepare synthetic milk; or high urea content in milk due to unbalanced feed for cows (Das *et al.*, 2016). A below normal specific gravity was observed in some samples. This was attributed to adulteration by addition of water, since all samples that were positive for added water had a specific gravity below 1.026kg/l.

A protein content of as low as 2.26% was observed in this study. This can be taken as a normal observation as milk protein content varies tremendously from one herd to another. In other studies, (Das *et al.* 2016), a herd average milk protein ranging from 1.57% to 4.66% with an average of 3.05% has been recorded. Freezing point of cow's milk is relatively constant and has been used widely to detect the use of water in adulteration of milk. In this study, samples that had a freezing point above -0.525^0C also were detected to be containing added water. Freezing point of milk is not entirely constant. It is influenced by variation in environment, management, breed of the cow, stage of lactation and Rumen Protein Balance (RPB) (Henno *et al.*, 2008).

A statistically significant difference was observed between the compositional quality of milk marketed by farmers and vendors in Amu and Mokowe urban centres. Farmers in Amu were more likely to sell unadulterated milk as compared to vendors where close to 50% was found to be adulterated. The high level of milk adulteration by vendors in Amu can be attributed to unscrupulous traders out to enrich themselves without giving priority to customer safety and satisfaction. However, the case was different in Mokowe centre where a high rate of adulteration was observed amongst farmers than vendors. With such kind of mixed scenarios, it is important to design public health messages targeting both the farmers and vendors as both are likely to be involved in the act of compromising the quality of marketed milk.

This study demonstrated the occurrence of antimicrobial residues in marketed raw cow milk indicating that consumers are likely to be exposed to antimicrobial residues above EU MRLs each time they consumed the milk. The presence of antimicrobial residues in food is of concern as it can contribute to development of drug resistance of human pathogens, allergic reactions and interference with growth of starter cultures in the milk processing industry (Addo *et al.,* 2011; Tollefson & Flynn, 2002; Dewdney *et al.,* 1991). The observed prevalence of residues in our study indicates a need to begin to address the problem both at the farm and market levels. This can be done through raising awareness amongst policy makers and implementers, farmers, vendors and consumers through specific extension messages (Kang'ethe *et al.*, 2005). A study in 1994 (Ombui, 1994) conducted in Kiambu detected no residues in milk being supplied to milk cooperative societies in the district and was attributed to the high level of awareness and strict adherence to the withdrawal periods by farmers. This is consistent with findings in our study, where farmers who were aware of the danger of consuming milk with antimicrobial residues and those who had been trained on good milking practices were less likely to sell milk with antimicrobial residues.

This study found a higher prevalence of antimicrobial residues in milk marketed by farmers and vendors; compared to a study by (Kang'ethe *et al.,* 2005) conducted in Nairobi, Nakuru and Narok, Kenya, where a prevalence of 11.1% amongst milk vendors and 16% amongst farmers was observed. The higher prevalence of antimicrobial residues observed in our study was attributed to lower levels of awareness of withdrawal periods amongst farmers.

In this study, presence of antimicrobial residues was observed across all the four selected study sites. However, a statistically significant difference was observed in the prevalence of antimicrobial residues

amongst farmers and vendors in Witu town where a higher prevalence was observed amongst vendors as compared to farmers. The higher prevalence observed amongst vendors was attributed to the pooling together of milk by vendors from various farms hence resulting in 'contaminating' milk that previously did not have antibiotics; and the possibility of vendors using antibiotics as well to prevent early spoilage of milk.

Antimicrobial residue occurrence in milk has been reported globally. However, in countries with effective quality assurance systems, reports of residues in foods destined for the market are minimal or non-existent (Kang'ethe *et al.,* 2005). For example, in Brazil, a study to assess hazards in unpasteurized marketed milk at farm level found a prevalence of antimicrobial residues of 11.5% (Nero *et al.,* 2004). A study in the peri-urban areas of Accra and Kumasi cities in Ghana (Aning *et al.,* 2007) found that 35.5% of samples of raw marketed milk, collected from different marketing agents including farmers, processors, wholesalers and retailers, were contaminated with antimicrobial residues. In Tanzania, a prevalence of 36% was observed in a study to investigate the risk of exposure to antimicrobial residues present in marketed raw milk in Mwanza and Dar es Salaam (Kurwijila *et al.,* 2006). With such high prevalence reports in various countries, there is need to intensify safety assurance efforts both at farm and market levels, promote prudent use of antibiotics and observance of drug withdrawal period.

This study noted the existence of a non-antibiotic heat-sensitive inhibitor (Sciences Charm, 2010) in milk from Witu and Mpeketoni towns. From the interview findings, locals acknowledged to be using a herbal substance they called *mpingo* to preserve milk. This practice of using natural antimicrobials in milk preservation has been reported elsewhere (Tiwari *et al.,* 2009; Khusniati *et al.,* 2008; Lucera *et al.,* 2012). Such substances are likely to affect growth of starter cultures in the milk industry, if the milk is not properly heated before start of processing. In addition, little is known about the *mpingo* herb, which could have side effects to consumers.

In interpreting the findings of this study, it should be noted that sampling of farmers was independent from sampling of vendors. We could not follow milk along the market value chain, that is, from individual farms to individual vendors, to determine the source or point of adulteration or entry of the antimicrobial residues in the milk.

5.2 Conclusion

1. About 17.4 % and 25.0% of informally marketed raw cow milk by farmers and vendors respectively in Lamu West Sub-county were found to be adulterated by addition of water.
2. Approximately 15.5% and 18.4% of informally marketed raw cow milk from farmers and vendors respectively were found to contain antimicrobial residues above the acceptable MRLs.
3. Farmers having secondary level of education and above, and being a pastoralist farmer were identified as independent risk factors against marketing of milk of poor compositional quality. Being a male vendor was identified as a risk factor of marketing milk of poor compositional quality amongst vendors.
4. Farmer awareness of the danger of consuming milk with antimicrobial residues and training on good milking practices were protective against selling milk with antimicrobial residues.

5.3 Recommendations

The following are the recommendations of the study:

1. There is need for routine testing of marketed milk for various forms of adulteration and compositional quality (through collaborative efforts of The Kenya Dairy Board, Public Health, Veterinary & Livestock Production departments and law enforcemet agencies); and imposition of stiff penalties on those adulterating milk;
2. Training of farmers and milk vendors in the County on good milking and milk handling practices;
3. Education of farmers on the source and impact of antimicrobial residues in milk and the need for strict adherence to withdrawal periods after animal treatment;
4. Further research to be done on the health effects of *Mpingo* herb that is used by farmers and vendors in the County to preserve milk inorder to gather adequate information to inform future policies on use of the herb.

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APPENDICES

Appendix I: Questionnaire (Vendors)

The questionnaire was administered through observation and face-to-face interview of the respondents in Kiswahili as it is the main language of communication used in the Sub-County.

Questionnaire No. ……

Factors associated with Prevalence of Antimicrobial Residues and Compositional Quality of Informally Marketed raw cow Milk in Lamu West Sub-County, Kenya

Name of the vendor: .....

Name of interviewer: .....

Date of interview: .....

Time of interview: .....

Market Centre.....

GPS readings ……..…….

Age of vendor ……..

1. What is your highest level of formal education?
    - None
    - Lower Primary
    - Completed Primary
    - Early Secondary
    - Completed Secondary
    - Tertiary

2. Type of trade:

    - Milk bar
    - Kiosk/ shop
    - Mobile trader/ individual (hawker)
    - Dairy cooperative
    - Other (specify)

3. What volume of milk did you bring to the market today? ……..
4. In a good day, what is the highest volume of milk you have sold at this outlet in 1 day? ……
5. How do you dispense your milk? Pouring Scooping
6. Is the milk you are selling solely from one farm? ○Yes ○No
7. If not, from how many farms or agents do you collect your milk?

    - ≤2
    - 3
    - 4
    - ≥5

8. For how long have you been in this milk trading business?

    - ≤1year
    - 2years
    - 3years
    - 4 years
    - ≥5years

9. How often do you bring milk to the market?

    - Daily, morning only
    - Daily, evening only
    - Daily, morning and evening
    - Once a week
    - Occasionally

10. What means of transport do you use to get milk to your selling point (market)?

    - On foot
    - Bicycle
    - Motorbike
    - Donkey
    - Boat
    - Vehicle

11. What do you do when you have surplus milk at the end of the day?

    - Boil and store for sale the next day
    - Consumed by family
    - Give it out on credit
    - Mix it with fresh supply and sell
    - Add some preservative (probe, which one)
    - Sell it at a lower price
    - Store in a refrigerator for sale next day
    - Other, (specify)

12. What other thing do you do to the milk to make it stay fresh for longer? (Probe) ……

13. What do your co-traders do to the milk to prevent early spoilage?

……

14. Have you ever been trained on milk handling practices? ○Yes ○ No
15. If yes, who was the training agent? …….
16. How long ago was the training done? …….
17. Are you licensed to handle and trade in milk? ○Yes ○No
18. If yes, who licensed you? ……..
19. What type of containers do you use to transport your bulk milk to the market?

- Plastic containers
- Aluminum cans / metal cans
- Other, specify ……

20. If you use plastic containers, why do you prefer using such containers?

- Cheap
- Easily portable
- Minimal spillage
- Other, (specify) ……

21. Do you have access to refrigeration facilities? Yes No
22. Are there times when the owners of the farms decline to sell to you milk?

0 Yes 0 No

23. If yes, what are some of the reasons for this refusal? (do not prompt)

- Cows under treatment
- Non-payment for previous supplies
- Cows have dried up, hence not much is available for sale
- Cows are sick

- Other, (specify)

24. Can you consume milk from a cow under treatment? Yes No

25. Are you aware of any danger in consuming milk from cows under treatment? Yes No

26. If yes, which ones ……..

27. Has your supplier ever refused to sell milk to you because cows are under treatment? Yes No

Appendix II: Questionnaire (Farmers)

The questionnaire was administered through observation and face-to-face interview of the respondents in Kiswahili as it is the main language of communication used in the Sub-County.

Questionnaire No. ……..

Factors associated with Prevalence of Antimicrobial Residues and Compositional Quality of Informally Marketed raw cow Milk in Lamu West Sub-County, Kenya

Name of the farm: .....

Name of interviewer: .....

Age of farmer ……

Date of interview: .....

Time of interview: .....

Ward.....

GPS readings …….

1. What is your highest level of formal education?
    - None
    - Lower Primary
    - Completed Primary
    - Early Secondary
    - Completed Secondary
    - Tertiary

2. Type of livestock farming:

    - Pastoralism
    - Zero grazing
    - Semi-zero grazing

3. Breed of cattle:

    - Exotic
    - Local
    - Crossbreed

4. What volume of milk do you get from your cows in one milking? ……
5. What volume of milk do you take to the market for sale? ……..
6. Who does the milking? Self, Son/Daughter, Herdsman, Spouse, Neighbour, Other (specify)
7. How do you dispense your milk? ○ Pouring ○Scooping
8. How do you sell your milk?

    - Consumers come for it
    - Vendors come for it
    - You take it for sale at the market
    - Take it to the consumers' doorstep
    - Other (specify)

9. What do you do when you have surplus milk at the end of the day?

    - Boil and store for sale the next day
    - Consumed by family
    - Give it out on credit
    - Mix it with fresh supply and sell
    - Add some preservative (probe, which one)
    - Sell it at a lower price

- Store in a refrigerator for sale next day
- Other, (specify)

10. Do you usually boil the milk before bringing it for sale? ○ Yes ○ No
11. What other thing do you do to the milk to make it stay fresh for longer? (Probe) ……
12. What do other farmers do to the milk to prevent early spoilage?

    …….

13. Have you ever been trained on good milking practices? ○Yes ○No
14. If yes, who was the training agent? …….
15. How long ago was the training done? …….
16. What type of containers do you use to transport your bulk milk to the market?

    - Plastic containers
    - Aluminum cans / metal cans
    - Other, specify ……

17. If you use plastic containers, why do you prefer using such containers?

    - Cheap
    - Easily portable
    - Minimal spillage
    - Other, (specify) ……

18. Who treats your cattle when they are sick?

- Self
- A veterinarian
- Neighbour
- Other (specify)

19. Are you aware of any changes to be observed in utilization of milk from cows under treatment?

Yes ○ No ○

20. If yes, what are the changes? ……

21. What do you do to milk from a cow under treatment?

- Discard it
- Give to dogs/ cat
- Give to calves
- Sale it
- Consume it at home
- Other (specify)

22. In your opinion, do you consider it necessary to discard the milk? ○ Yes ○ No

23. Are you aware of any danger in consuming milk from cows under treatment? Yes No

24. If yes, which ones:

- Allergic reactions
- Drug resistance
- Interference with industrial use of milk (manufacturing)
- Other (specify)

25. Do you know of farmers in this area who do not observe milk withdrawal period? Yes No

26. Have you ever discarded milk because it was from a cow under treatment? Yes No

27. Have you ever added water to milk to increase its volume before selling? Yes No

28. Do you know people in this area who add water into milk for sale? Yes No

Appendix III: Consent form

Title of study:

Factors associated with Prevalence of Antimicrobial Residues and Compositional Quality of Informally Marketed raw cow Milk in Lamu West Sub-County, Kenya

Introduction:

My name is George Kiage Ondieki. I am trying to learn more about the compositional quality and prevalence of antimicrobial residues in raw cow milk sold in urban and rural centers in Lamu West Sub-County. Milk is a good source of essential nutrients for people of all ages. However, milk obtained from cows under treatment may contain antimicrobial residues as original molecules or as metabolites. These cause harmful effects to the consumers and economic losses to milk processers. Milk whose composition has been altered, gives low value for money to those purchasing it and may also result in exposure to public harzards when unsafe water is added into the milk, as a form of adulteration.

Purpose of study:

Due to the great public health importance of the risks posed by the compositional quality and the antimicrobial residues present, I am requesting for your participation in this study whose main objective is to determine the compositional quality and prevalence of antimicrobial residues in informally marketed raw cow milk in Lamu West Sub-County, and identify factors associated. The study shall form a basis for the relevant authorities to find ways of dealing with the public health risks posed by the marketed raw milk in this area. You are being asked to join this study because you were picked by chance among other milk vendors / farmers in this area.

Expectations of the study:

If you agree to participate in the study, I wish to test some of the milk you are selling; to determine its compositional quality and test for presence of antimicrobial residues. If you agree to take part in the study, I will sample 100ml of the milk in your bulk container. The milk sample will be transported to Lamu County Hospital laboratory where I will test for compositional quality and antimicrobial residues. I shall then ask you some questions which are written on a paper on handling, transportation and storage of milk. The test results shall be availed as soon as possible to the Sub-County Veterinary officer and Public Health officer, Lamu West who shall forward them to you and advice on any necessary control measures if need be.

Risks:

There are no envisaged risks from participating in this study. We shall compensate you appropriately for the 100 ml milk sample. However, you shall experience a minor economic loss of 100ml milk from your milk for sale and 30 minutes of your time.

Benefits:

The results of this study will be communicated and disseminated to the people concerned to take action on the recommendations that will come out from the study.

Confidentiality:

Any information obtained from you will be kept confidentially and used solely for purposes of this research only. The results of this research may be published in scientific journals or presented at medical or veterinary meetings, but your identity will not be disclosed.

Compensation:

If you accept to take part in this study, there will be no payment for participation.

Alternatives:

You have a choice to agree or not to agree to participate in this study. If you agree to participate in study, you are allowed to withdraw at any time if you so wish without any consequences whatsoever.

Note: If you have any questions or concerns about the study, please contact:

Ondieki George Kiage

P. O. Box 78700 code 00507 Nairobi

Email: drondiekigk@gmail.com

Cellphone: 0722-391294.

For any questions pertaining to the rights as a research participant, please contact:

The Secretary

The Jaramogi Oginga Odinga Teaching and Referral Hospital-Ethics & Research Committee (JOOTRH – ERC)

P O BOX 849, Kisumu, Kenya

Email: ercjootrh@gmail.com

Tel: 0723-016372

Consent:

I have been fully informed about the study, the risks and benefits of it. I had the opportunity to ask questions which were satisfactorily answered. I therefore consent to voluntarily participate in the study.

Name of participant……

Signature/ thumb print of participant……..

Date……..

Name of witness ……..

Signature…….. Date …….

Appendix IV: Ethical Review Committee Approval

MINISTRY OF HEALTH

Telegrams: "MEDICAL", Kisumu
Telephone: 057-2020801/2020803/2020321
Fax: 057-2024337
E-mail: ercjootrh@gmail.com
When replying please quote

JARAMOGI OGINGA ODINGA TEACHING & REFERRAL HOSPITAL
P.O. BOX 849
KISUMU

Ref: ERC.1B/VOL.I/158

Date 26th February, 2015

George Kiage Ondieki,
TM-312/2386/2013,
JKUAT.

Dear George,

RE: FORMAL APPROVAL TO CONDUCT RESEARCH TITLED: *"ASSESSMENT OF QUALITY OF INFORMALLY MARKETED RAW COW MILK IN URBAN AND RURAL CENTRES IN LAMU WEST SUB-COUNTY, KENYA"*

The JOOTRH ERC (ACCREDITATION NO. 01713) has reviewed your protocol and found it ethically satisfactory. You are therefore, permitted to commence your study immediately. Note that this approval is granted for a period of one year (26th February, 2015 to 27th February, 2016). If it is necessary to proceed with this research beyond the approved period, you will be required to apply for further extension to the committee.

Also note that you will be required to notify the committee of any protocol amendment(s), serious or unexpected outcomes related to the conduct of the study or termination for any reason.

Finally, note that you will also be required to share the findings of the study in both hard and soft copies upon completion.

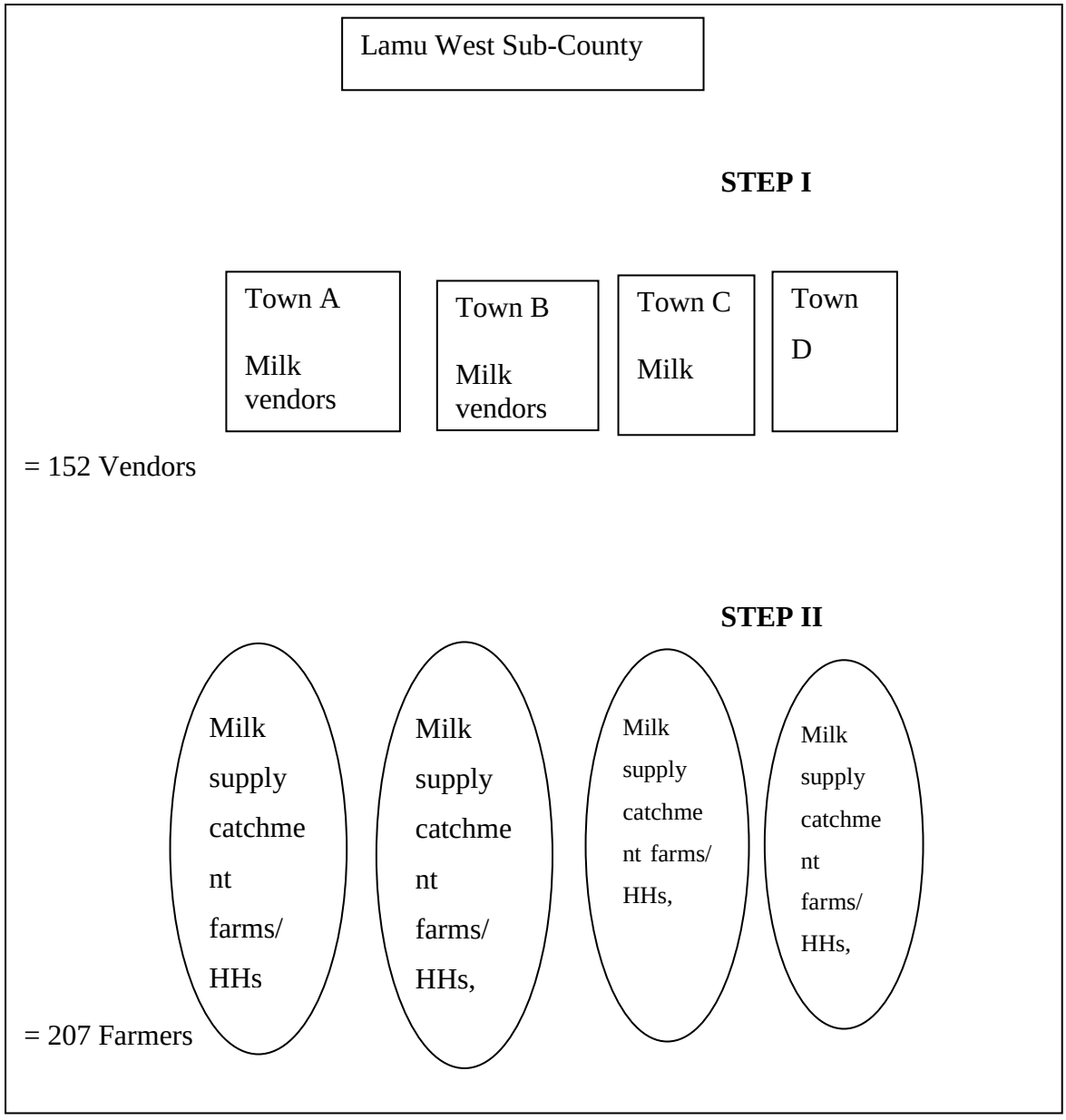
The JOOTRH ERC takes this opportunity to thank you for choosing the institution and wishes you the best in your endeavours.

Yours sincerely,

FRED OUMA AKWATTA,
SECRETARY - ERC,
JOOTRH – KISUMU.

JOOTRH ETHICS & REVIEW COMMITTEE
P. O. Box 849 - 40100
KISUMU

Appendix V Sampling Structure



Appendix VI: Translated Questionnaire (Vendors)

The questionnaire was administered through observation and face-to-face interview of the respondents in Kiswahili as it is the main language of communication used in the Sub-County.

Utafiti kuchunguza ubora wa maziwa mabiji ya ng'ombe yanayouzwa mijini na vijijini katika Kaunti ndogo ya Lamu Mgaharibi, nchini Kenya

Jina la muuzaji: .....

Jina la mtaalamu: .....

Tarehe ya mahojiano: .....

Umri wa muuzaji ……..

Saa ya mahojiano: .....

Jina la soko.....

GPS ……..

1. Umefikia kiwango gani cha masomo?

    - Sikuenda shuleni
    - Shule ya msingi, mwanzoni
    - Nilikamilisha Shule ya msingi
    - Shule ya upili, mwanzoni
    - Nilikamilisha shule ya upili
    - Chuo cha kadri ama chuo kikuu

2. Biashara yako ya maziwa ni ya aina gani?

    - Duka la kuuza maziwa na bidhaa zake
    - Kioski/ ama duka
    - Kuuza kwa kunadi ama kutembeza maziwa
    - Shirika la kununua na kuuza maziwa

3. Ni kiazi gani cha maziwa ulileta kuuza sokoni hii leo? ……..
4. Katita siku nzuri, ni kiazi gani cha juu zaidi cha maziwa ushauza hapa kwa siku? ……
5. Je unawatiliaje wateja wako maziwa? Ku○amiminia Kuwa○hotea
6. Je, maziwa unayouza ni kutoka kwa shamba / wakala moja? Ndio○ La○
7. Kama ni kutoka mashamba/ wakala wengi; ni kama wangapi?

    - ≤2
    - 3
    - 4
    - ≥5

8. Umekuwa katika hii biashara ya maziwa kwa mda upi (miaka)?

    - ≤1
    - 2
    - 3
    - 4
    - ≥5

9. Huwa unaleta maziwa sokoni mara ngapi?

    - Kila siku, asubuhi pekee
    - Kila siku, jioni pekee
    - Kila siku, asubuhi na jioni
    - Mara moja kwa wiki

- Mara moja moja

10. Unatumia mbinu gani ya usafiri kufikisha maziwa sokoni?

    - Natembea kwa miguu
    - Baiskeli
    - Pikipiki
    - Punda
    - Boti/ mashua/jahazi
    - Gari

11. Wewe huwa unafanya nini na maziwa yanayobaki ukishafunga kazi ya siku?

    - Chemsha na kuweka ili iuzwe siku inayofuatia
    - Tunaitumia nyumbani na familia
    - Naipeana kwa watecha kwa mkopo
    - Nachanganya na maziwa fresh na kuuza
    - Naongezea chemikali ya kuzuia isiaribike (chunguza)
    - Naiuza kwa bei ya chini
    - Naiweka kwa friji na kuuza siku inayofuata
    - Njia zingine, (gani)

12. Je, wewe hufanya nini ili kuzuia maziwa isiharibike haraka? (chunguza) ……..
13. Wafanyibiashara wenzako hufanya nini ili kuzuia maziwa isiharibike haraka? …….. …….
14. Ushahudhuria mafunzo kuhusiana na mbinu nzuri za kusafirisha maziwa? ○ Ndio ○ La
15. Kama ushafunzwa, ulifunzwa na nani? …….
16. Ni kitambo gani ulipokea hayo mafunzo? …….
17. Je, umesajiliwa kuhudumu na kuuza maziwa? Ndio ○ La ○
18. Kama umesajiliwa, umesajiliwa na nani? ……..

19. Unatumia vyombo ama mitungi ya aina gani kusafirisha maziwa kuja sokoni?

- Vyombo vya plastiki
- Mitungi ya Aluminum
- Zingine, (gani) ……

20. Kama unatumia mitungi ya plastiki, kwa nini?

- Iko rahisi
- Inabebeka kwa urahisi
- Haimwagi maziwa kwa urahisi
- zingine, (gani) ……

21. Je, uko na friji ambayo wewe hutumia kuhifadhi maziwa? ○Ndio ○ La

22. Je, kuna wakati wafugaji wanakataa kukuuzia maziwa?

○ Ndio ○ La

23. Kama ndivyo; kwa nini?

- Ng'ombe zinaendelea na matibabu
- Kutolipia maziwa
- Maziwa yamepungua
- Ng'ombe ni wagonjwa
- Zingine, (gani)

24. Je, unaweza tumia maziwa kutoka kwa ng'ombe inayoendelea na matibabu?

Ndio ○ La ○

25. Je, unadfahamu madhara yeyote yanayotokana na kutumia maziwa kutoka kwa ng'ombe inayoendelea na matibabu? Ndio ○ La ○

26. Kama unafahamu, ni madhara gani? …….

27. Je, mkulima anayekuuzia maziwa ashawahi kataa kukuuzia maziwa kwa sababu ng'ombe anaendelea na matibabu? Ndio o La o

Appendix VII Translated Questionnaire (Farms/ Households)

The questionnaire was administered through observation and face-to-face interview of the respondents in Kiswahili as it is the main language of communication used in the Sub-County.

Utafiti kuchunguza ubora wa maziwa mabiji ya ng'ombe yanayouzwa mijini na vijijini katika Kaunti ndogo ya Lamu Mgaharibi, nchini Kenya

Jina la mwenye shamba: .....

Jina la mtaalamu: .....

Umri wa mkulima ……..

Tarehe ya mahojiano: .....

Saa ya mahojiano: .....

Eneo.....

GPS …….

1. Umefikia kiwango gani cha masomo?

    - Sikuenda shuleni
    - Shule ya msingi, mwanzoni
    - Nilikamilisha Shule ya msingi
    - Shule ya upili, mwanzoni
    - Nilikamilisha shule ya upili
    - Chuo cha kadri ama chuo kikuu

2. Unatumia mfumo gani wa ufugaji:

    - Pastoralism
    - Zero grazing
    - Semi-zero grazing

3. Ng'ombe wako ni wa aina gani:

    - Wa gredi
    - Kienyeji
    - Crossbreed

4. Ni kiwango gani cha maziwa unapata kutoka kwa ng'ombe wako? ……
5. Ni kiwango gani wewe hupeleka sokoni? ……..
6. Nani hukamua ng'ombe, hapa shambani kwako? (Wewe, Wanao, Mchungaji, Bwana/bibi, Jirani, yeyote)
7. Je unawatiliaje wateja wako maziwa? ○ Kuwamiminia ○Kuwachotea
8. Je ni mbinu gani wewe hutumia kuuza maziwa kutoka shamba lako?

    - Wateja watumiaji huja kununua
    - Wateja wauzaji, madalali huja hapa shambani kununua
    - Hupeleka mimi mwenyewe sokoni
    - Huwapelekea wateja wanunuzi hadi nyumbani mwao
    - zingine (gani)

9. Wewe huwa unafanya nini na maziwa yanayobaki ukishafunga kazi ya siku?

    - Chemsha na kuweka ili iuzwe siku inayofuatia
    - Tunaitumia nyumbani na familia
    - Naipeana kwa watecha kwa mkopo
    - Nachanganya na maziwa fresh na kuuza
    - Naongezea chemikali ya kuzuia isiaribike (chunguza)

- Naiuza kwa bei ya chini
- Naiweka kwa friji na kuuza siku inayofuata
- Njia zingine, (gani)

10. Je, wewe huwa unachemusha maziwa kabla ya kupeleka sokoni kuuza? Ndio ○ La…….
11. Je wewe hufanya nini (kingine) kwa maziwa ili isiharibike haraka? (Chunguza) ……. …….. …….
12. Je, wafugaji wengine hutumia mbinu gani ili kuzuia maziwa isiharibike haraka?

    …….

13. Ushahudhuria mafunzo kuhusiana na mbinu nzuri za kukamua na kuhifadhi maziwa? Ndio ○ La ○
14. Kama ushafunzwa, ulifunzwa na nani? …….
15. Ni kitambo gani ulipokea hayo mafunzo? …….
16. Unatumia vyombo ama mitungi ya aina gani kusafirisha maziwa kwenda sokoni?

    - Vyombo vya plastiki
    - Mitungi ya Aluminium
    - Zingine, (gani) ……

17. Kama unatumia mitungi ya plastiki, kwa nini?

    - Iko rahisi
    - Inabebeka kwa urahisi
    - Haimwagi maziwa kwa urahisi
    - Zingine, (gani) ……

18. Je, nani hutibu ng'ombe zako zikigonjeka?

    - Mimi mwenyewe

- Daktari wa mifugo
- Jirani
- Mwingine (nani)

19. Je, wafahamu mabadiliko yeyote kuhusu matumizi ya maziwa wakati ng'ombe iaendelea kupewa matibabu? Ndio ○ La ○
20. Kama unafahamu, ni gani? ……
21. Je, wewe hufanya nini na maziwa wakati ng'ombe wako anaendelea kupewa matibabu?

- Humwaga chini maziwa
- Pea mbwa na paka
- Pea ndama
- Ninauza
- Tuaitumia nyumbani
- Zingine (gani)

22. Kwa maoni yako, unaona ni muhimu binadamu kutotumia maziwa haya? Ndio ○ La
23. Je wafahamu madadhara yanayoweza kutokana na matumizi ya maziwa kutoka kwa ng'ombe anayeendelea kupewa matibabu? Ndio La ○ ○
24. Kama wafahamu, ni madhara gani unajua:

- Husababisha kujikunakuna
- Husababisha ugumu wa matibabu ya magonjwa yanayosababishwa na viini.
- Hutatiza utengenezaji wa bidhaa za maziwa viwandani.
- Zingine (elezea).

25. Je unafahamu bahadi ya wakulima wafugaji katika eneo hili waotumia maziwa kutoka kwa ng'ombe anayeendelea kupewa matibabu? Ndio La ○ ○
26. Je wewe, usha wahi kukosa kutumia maziwa kwa sababu ilikuwa inatoka kwa ng'ombe ambayo ilikuwa inatibiwa ? Ndio La ○ ○
27. Je, wewe, ushawahi ongezea maji kwa maziwa kabla ya kuuza? Ndio ○ La ○

28. Je, unafahamu watu katika eneo hili ambao huongezea maziwa maji kabla ya kupeleka sokoni?

Ndio ○ La ○

Appendix VIII: Translated Consent form

Title of study:

Utafiti kuchunguza ubora wa maziwa mabiji ya ng'ombe yanayouzwa mijini na vijijini katika Kaunti ndogo ya Lamu Mgaharibi, nchini Kenya

Utangulizi:

Jina langu ni George Kiage Ondieki. Ningependa kufanya utafiti kuhusu hali ya usafi, ubora na uwepo wa madawa yanayotumika katika matibabu ya mifugo nadani ya maziwa ya ng'ombe mabiji yanayouzwa mijini na vijijini katika Kaunti ndogo ya Lamu Magharibi. Maziwa yana virutubisho muhimu kwa binadamu wa umri wowote. Walakini, maziwa kutoka ng'ombe wagonjwa Walakini, maziwa kutoka ka ng'ombe wagonjwa na maziwa yanayowekwa kwa hali duni za kiafya wakati wa kukamuliwa, kusafirishwa au kuhifadhiwa, yanaweza kuwa chanzo cha maradhi kwa watakayoyatumia. Maziwa kutoka kwa ng'ombe zilizochini ya matibabu inaweza kuwa na baadhi ya chemikali au madawa yanayotumika katika matibabu. Chemikali hizi husababisha madhara kwa watakayoyatumia na pia hasara kwa matumizi viwandani.

Madhumuni ya matibabu:

Kutokana na umuhimu mkuu wa madhara kwa uma yanayotokana na hali ya usafi, ubora na uwebo wa chemikali za tiba ndani ya maziwa, ninaomba kukuhuzisha katita utafiti huu ambao nia yake kuu ni kuchunguza hali ya usafi, ubora na uwepo wa chemikali za tiba ndani ya maziwa mabiji ya ng'ombe yanayouzwa mijini na vijijini katika Kaunti ndogo ya Lamu Magharibi. Utafiti huu pia utachunguza mienendo inazochangia hali halisi. Utafiti huu utakuwa msingi kwa wahusika wasimamizi kutafuta mbinu za kupambana na madhara kwa uma yanayotokana na maziwa mabiji ya ng'ombe yanayouzwa katika eneo hili. Ninakuomba ujiunge na utafiti huu kwa vile ulichaguliwa kutoka kwa wauzaji maziwa / wakulima wafugaji wengine katika eneo hili.

Matarajio ya utafiti huu:

Kamam utakubali kujiunga na utafiti huu, ningependa kuchunguza maziwa hayo unayouza ili kujua hali yake ya usafi, ubora na uwepo wa chemikali za tiba. Kama utakubali kujiunga na utafiti huu, nitachukua sambuli ya 100 ml ya maziwa kutoka kwa mtungi wako. Sambuli hii itasafirishwa hadi maabara ya Utafiti wa Afya ya Mifugo iliyoko Witu ambako uchunguzi utafanyika. Mbali na hayo, nitakuuliza maswali ambayo yamenakiliwa kwa karatasi kuhusiana na usafirishaji na uhifadhi wa maziwa. Matokeo ya utafiti huu yatatolewa punde tu yakiwa tayari kupitia Ofisi ya Idara ya Afya ya Mifugo pamoja na ofisi inayohusiana na Afya ya Uma katika Kaunti ndogo ya Lamu Magharibi, ambao watayaleta kwako na kukushauri vilivyo.

Madhara:

Hakuna madhara yeyoye yanayotarajiwa kutokana na wewe kukubali kuhusika katika utafiti huu. Licha ya hayo, utapata hasara ya maziwa mililita 100 kutoka kwa maziwa yako na mda wako wa dakika thelatini.

Umuhimu:

Matokeo ya utafiti huu yatawazilishwa kwa wahusika wasimamizi ili kutekeleza mapendekezo yatakayojitokeza kutokana na utafiti huu.

Kuhifadhi siri:

Habari zozote zitakazochukuliwa kutoka kwako zitahifadhiwa kwa siri na zitatumika kwa huu utafiti peke yake. Matokeo ya utafiti huu yatachapishwa kwa majaridha ya kisayansi ama kusungumuziwa katika mikutano ya madaktari bila kutaja jina lako ama kukutambua.

Fidhia:

Ukikubali kuingia katika utafiti huu, hakutakuweko na malipo yeyote kwa kuhusika.

Uhuru wa uamuzi:

Kumbuka wewe uko huru kukubali au kukataa kuhusika katika utafiti huu. Ukikubali kuhusika katika utafiti huu, kumbuka ya kwamba uko huru pia kutoka wakati wowote bila madhara ya aina yeyote kwako.

Kibali cha utafiti:

Kibali cha kutekeleza utafiti huu kimetolewa na:

This study will be approved by:

The Jaramogi Oginga Odinga Teaching and Referral Hospital-Ethics & Research Committee (JOOTRH – ERC)

P O BOX 849, Kisumu, Kenya

na

Board of Post graduate studies

Jomo Kenyatta University of Agriculture and Technology

P.O. Box 62000, Juja, Kenya

Kama una swali lolote ama jambo ungependa ufahamishwe sahidi, unaweza andikia wakurugenzi wa mashirika yaliyoko hapo juu.

Kukubali:

Nimefaamishwa kikamilifu kuhusu utafiti huu, madhara yake na umuhimu wake. Nimekuwa na nafasi ya kuuliza maswali ambayo nimejibiwa kwa ukamilifu. Sasa ninakubali kwa hiari yangu kuhusika katika utafiti huu.

Jina la muhusika……

Sahihi / alama ya kidole cha muhusika ……..

Tarehe……..

Jina la mtafiti/ mtafiti msaidizi……..

Sahihi…….. Tarehe …….

Appendix IX: Charm Blue Yellow II Sensitivity Levels, Concentration of Antimicrobial residues detected in milk, (µg/kg); Codex Alimentarius Commission Maximum Residual Limits (CAC-MRLs) and European Union Maximun Residual Limits (EU-MRLs).

Common veterinary antimicrobials	**Charm Blue Yellow II Sensitivity levels (µg/kg)**	**CAC-MRLs (µg/kg)**	**EU-MRLs (µg/kg)**
Ampicillin	2-3	4	4
Amoxicillin	2-3	4	4
Benzyl Penicillin/ Procaine Penicillin, Penicillin G	2-3	4	4
Cefalexin	60-100	100	100
Closacillin	10-20	30	30
Diclosacillin	10-30	30	30
Dihydrostreptomycin/ Streptomycin	150-200	200	200
Erythromycin	100-150	40	40
Gentamycine	75-100	200	100
Neomycin	75-150	1500	500
Sulphadiazine	80-100	100	100
Sulphamethoxine	50-75	100	100
Tetracycline, Oxytetracycline	100	100	100
Tylosin	20-30	50	50

Sources: Adapted from Charm Sciences Inc., USA and Codex Alimentarius Commission, July 2009; Maximum Residue Limits for Veterinary Drugs in Foods. Pp 1-36.; Current EU Antibiotic Maximum Residue Limits, March 2011.

Appendix X: Publication

Antimicrobial residues and compositional quality of informally marketed raw cow milk, Lamu West Sub-County, Kenya, 2015

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Abstract

Introduction: unadulterated milk, free of antimicrobial residues is important for industrial processing and consumers' health. Antimicrobial residues in foods of animal origin can cause adverse public health effects like drug resistance and hypersensitivity. Milk produced in Lamu West sub-county is sold raw directly to consumers. We estimated the compositional quality and prevalence of antimicrobial residues in informally marketed raw cow milk in Lamu West Sub-County, Kenya.

Methods: we randomly recruited 152 vendors and 207 farmers from four randomly selected urban centers in a cross-sectional study and interviewed them using a pretested standardized questionnaire. A100-ml raw milk sample was aseptically collected from each vendor and farm and tested for antimicrobial residues using Charm Blue Yellow II kit following the European Union Maximum Residue Limits (EU-MRLs) while an Ekomilk® Analyzer was used to measure compositional quality where samples with either solid not fat (SNF) < 8.5 or added water ≥ 0.01% or both were considered adulterated. We analyzed data using univariate analysis and unconditional logistic regression to calculate odds ratios (OR) and 95% confidence intervals (CI).

Results: thirty-two of the 207 (15.5%) samples from farmers and 28 (18.4%) of the 152 samples from vendors tested positive for antimicrobial residues. Thirty-six (17.4 %) samples from farmers and 38 (25.0%) from vendors were found to be adulterated with water. Farmers' awareness of the danger of consuming milk with antimicrobial

residues and farmers having training on good milking practices were protective against selling milk with antimicrobial residues (adjusted OR and 95% CI 0.20, 0.07-0.55 and 0.33, 0.11-0.99, respectively).

Conclusion: the antimicrobial residues above EU MRLs and adulteration of raw marketed cow milk observed in this study provide evidence for routine testing of marketed milk and educating farmers to observe antimicrobial withdrawal period.

Introduction

Unadulterated high quality milk that is free of antimicrobial residues is of interest to farmers, consumers and milk processing companies. Such milk enables farmers to get a fair price for their produce while processors are assured of a raw material suitable for manufacture of various dairy products. Consumers are also guaranteed of getting a healthy product at a good value [**1**].

Compositional quality of milk is determined by measuring its constituents and physico-chemical properties including: added water, butter fat, solid non-fat (SNF), protein, specific gravity and freezing point [**2**]. Adulteration of milk refers to the alteration of the natural composition of milk by extraction of one or more of its components (such as butter fat) or addition of some substances (such as water). Milk adulteration by addition of substances such as water interferes with the hygienic, compositional, nutritional and processing qualities of the milk, while extraction of components from milk lowers the value for money paid by consumers or processors [**3**].

Although antimicrobials are useful for treatment of infections, their occurrence in foods of animal origin as residues can cause adverse public health effects such as drug resistance [**4**,**5**] and hypersensitivity caused by penicillins and sulphonamides antibiotic groups [**6**,**7**]. Their occurrence in milk also causes huge economic losses in milk processing industries by interfering with the manufacture of cultured products such as yoghurt and cheese through inhibition of starters and rejection of milk from farms that test positive for antimicrobials [**8**]. Antibiotics used in veterinary practice are identical or closely related to those used in human medicine. Hence, any improper use or exposure in either can easily result in cross-resistance [**5**].

To protect the public against possible health risks caused by antimicrobial residues and consumption of milk of unacceptable compositional quality, regulations have been developed both locally and internationally to ensure observance of withdrawal periods after antimicrobial therapy and proper handling and marketing of milk. International regulations include European Union Maximum Residual Limits (EU MRLs) and the Codex Alimentarious Commission (CAC) [**9**,**10**]. In Kenya, quality and safety of milk is regulated by the Dairy Industry Act [**11**], Public

Health Act [12] and the Standards Act [13]. However, such regulations might not be adhered to or enforced, as is the case in many developing countries [14].

The dairy industry in Lamu County is in its early stages of development. As of 2015, all the milk produced in Lamu West sub-county was sold raw directly to consumers without undergoing any quality assessment and safety assurance against presence of antimicrobial residues. No investigations have been carried out to assess the extent and nature of the risks consumers of marketed raw cow milk in Lamu County may be exposed to. This study assessed the compositional quality of milk and estimated the prevalence of antimicrobial residues in informally marketed raw cow milk in Lamu West Sub-County with the goal of providing feedback to farmers, vendors, consumers, policy makers and enforcers.

Methods

Study area and design

A cross-sectional study was conducted in Lamu West Sub County of Lamu County, in the northern coastal region of Kenya during the months of July to November 2015. Lamu County is made up of two sub-counties: Lamu East and Lamu West. Lamu West Sub-County is made up of four administrative divisions, namely: Amu, Hindi, Mpeketoni and Witu; and six urban centres, namely: Amu, Mokowe, Hindi, Mpeketoni, Kibaoni and Witu, of which Amu is an island in the Indian Ocean (**Figure 1**).

Lamu West Sub-County has a population of 80,000 persons. The main economic activities in this region are fishing, tourism, livestock and crop farming. The livestock species kept here are mainly cattle, sheep goats, donkeys and poultry. Lamu West has an estimated cattle population of 126,250. The cattlerearing systems practiced here are: pastoralism, semi-zero grazing and zero grazing. Pastoralism, practiced in Hindi, Mpeketoni and Witu divisions, entails grazing large herds of local breeds of cattle (Boran and Zebu) in public or communally owned land and occasionally migrating to neighboring counties in search of greener pastures and drinking water. Semi-zero grazing, practiced in Hindi, Mpeketoni and Amu divisions, entails grazing cattle in the fields and providing supplementary feeding with fodder or commercial feeds. The breeds of cattle kept in this type of farming are cross-breeds and exotic breeds (Jersey, Guernsey, and Friesian). In zero grazing, practiced in Amu division, cattle are kept in enclosures and rely of fodder and commercial feeds. The types of cattle kept here are cross-breeds and exotic breeds. In Lamu County the milk from these cattle is sold raw to consumers who then boil it before consumption. The only link between the producer (farmer) and the consumer are small scale traders or milk vendors.

Sample size calculation and sampling procedure

Sample sizes for vendors and farmers were determined separately using the Cochran formula of 1977, using estimated prevalence of antimicrobial residues of 16% for milk from farms and 11.1% for milk from vendors [**8**]. We assumed Z-value for 95% confidence level as 1.96, and the precision (margin of error) at 5%. A total of 152 milk vendors and 207 livestock farmers were estimated as the sample size needed to achieve power of 80%.

Four of the six urban centres in the sub county were randomly selected for this study. These urban centres were: Amu, Mokowe, Mpeketoni and Witu. A preliminary visit was made to the selected market centres and with the help of key informants (the Public Health Officers, Livestock Production Officers, Veterinary Officers, Local Authority Trade Officers, milk marketing groups or associations and milk selling points), a sampling frame of informal raw milk vendors was created for each selected urban centre. A milk vendor was defined as any person who obtained milk from own farm or bought milk from one or more farms or milk outlets and sold it by hawking along the pathways or at raw milk selling shops within the milk supply catchment of the selected urban centers. Using the same key informants, another sampling frame was created comprising of livestock farmers, where a legible livestock farmer was defined as any person with lactating cattle within the milk supply catchment area of each selected urban centre and offered milk for sale at their farms. The established sampling frames were made of two groups: 784 livestock farmers with lactating cattle and 251 vendors. The number of farmers and vendors sampled from each centre were determined proportionate to number of farmers and vendors in each selected centre. Sampling units were individual vendors and farmers. Those who participated in the study were randomly selected using simple random sampling. Those who refused to consent or participate in the study were replaced from the sampling frame using simple random sampling.

Milk specimen and data collection

After obtaining consent and appropriately compensating the farmers and vendors for the milk, 100 ml milk specimens were aseptically collected in sterile bottles from each respondent, stored in ice-packed cool boxes and transported to Lamu County hospital laboratory where they were analyzed on the same day they arrived. A pretested structured questionnaire translated into Kiswahili was administered to each vendor and farmer to collect data on demographics and practices that might affect the compositional quality of the milk and occurrence of antimicrobial residues such as livestock treatment practices, observance of milk withdrawal period following antibiotic therapy, training in good milking and milk handling practices, practices used to prolong freshness of milk and methods used by vendors in selling milk.

Compositional quality

The milk specimens from vendors and from farmers were analyzed for compositional quality using Ekomilk® Ultrasonic Milk Analyzer (EON Trading LLC USA), as per manufacturer's instructions and as similarly done by Kunda *et al* (2015) [**15**]. The milk specimen vials were brought out of the cool box and allowed to thaw to room temperature. Each milk specimen vial was shaken gently to thoroughly mix the contents after which 20 ml of the milk specimen was transferred into the analyzer cup. The cup was placed below the aspiration tube of the Ekomilk® Ultrasonic Milk Analyzer and connected to power to start the analysis. The parameters estimated by the analyzer were: added water, butter fat, solid non-fat (SNF), protein percentage, specific gravity and freezing point. Adulterated milk was identified using standard values [**2**,**15**] by having SNF < 8.5%, added water ≥ 0.01%, specific gravity outside the normal range (1.026-1.036 Kg/l, butterfat < 3.3% or > 7.0%;and freezing point outside the normal range of between -0.525°C and -0.565°C.

Testing for antimicrobial residues

Charm Blue Yellow II kit was used to test for presence of antimicrobial residues using a standard method as described by the manufacturer [**16**]. A 100-ml milk specimen obtained from Witu Veterinary Farm from a cow that had not been exposed to antibiotics therapeutically, prophylactically or as feed additives for the past 12 months was used as a negative control and was confirmed negative by the Charm Blue Yellow II test kit. A positive control was prepared by reconstituting the provided 4 parts per billion (ppb) Penicillin G Standard with 10.0 ml of a negative control, shaken and allowed to stand refrigerated for 15 minutes. The controls were put in 0.5 ml aliquots in clean vials and frozen at -15°C for later use. Whenever the controls were needed, they were slowly thawed overnight in a refrigerator and shaken well before use.

Each milk specimen (or control) was shaken and 50 µl was pipetted into the purple agar portion of the well. A clear sealing tape was applied and pressed firmly to seal the rim of each well to prevent them from drying. The prepared wells were put into an air incubator at 64 ± 1°C for 2 hours 55 minutes. After incubation, the wells were removed from the air incubator, allowed to settle for 5 minutes on the desk, for colour development. Colour observation was done in comparison with the reference colours provided by the manufacturer [**16**]. Yellow or yellow/green wells were interpreted as negative, whereas blue/purple wells were interpreted as positive. Grey coloured wells, (referred to as 'Caution' by the manufacturer) were interpreted as positive. From the initial positive results, 600 µl of milk was heated in a test tube to boiling point for 3 minutes. Then they were allowed to cool to room temperature and shaken. The heated specimens were run in duplicate along with a negative and positive control and unheated milk specimen in the same procedure as above. Specimens which tested positive after heat treatment were interpreted as 'Blue Yellow II Test positive' hence contained antibiotics. Specimens that tested negative after heat treatment were considered to contain a non-antibiotic heat sensitive inhibitor.

Statistical analysis

The data was entered, cleaned and analyzed in Ms Excel™ 2007 and EPI Info 7™. In univariate analyses, proportions were calculated for categorical variables and means and medians for continuous variables. Bivariate analysis (Pearson chi square and Fischer's exact tests) was carried out to examine the association between the presence of antimicrobial residues or compositional quality of raw marketed milk and other factors with factors with p-value ≤ 0.05 considered statistically significant. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Factors in bivariate analyses with p-value ≤ 0.1 were included in a forward selection unconditional logistic regression model to control for confounders and identify independent factors associated with the occurrence of antimicrobial residues in milk and milk adulteration as identified by adjusted odds ratios (AORs) and 95% CIs. Factors with p-value < 0.05 in the final model were considered significant. Comparison of proportions was made using a 2-sample z-test with two tailed comparisons at 0.05 level of significance. Analysis for antimicrobial residue was not done for vendors since the assumption was that majority of antimicrobial residue occurred at the farm level due to lack of observance of antimicrobial withdrawal periods by farmers; and vendors had no role in occurrence of antimicrobial residues in milk as much as the study found antimicrobial residues in milk marketed by vendors.

Ethical clearance

The aim and procedures of the study were explained to the study participants who were required to give written informed consent prior to their voluntary participation in the study. Milk specimens were collected from only those who consented and the specimens were only used to assess quality characteristics and antimicrobial residues. Confidentiality of laboratory information and data was observed and maintained through password protected computers and observing good professional conduct. Ethical clearance and approval for this study was obtained from Jaramogi Oginga Odinga Teaching and Referral Hospital Ethical Review Committee, Ref. ERC.1B/VOL.1/158. Approval was also obtained from the Board of post graduate studies of Jomo Kenyatta University of Agriculture and Technology (JKUAT), the Lamu County Veterinary Officer and the Lamu County Director of Health, to use the institution's laboratory facility.

Results

Socio-demographic characteristics of respondents

Only three vendors and two farmers declined to participate in the study due to lack of time to respond to the questionnaire, and were replaced by randomly sampling again from the established sampling frames. One hundred and fifty two vendors and 207 farmers were enrolled into the study from Amu, Mokowe, Mpeketoni and Witu urban

centers of Lamu West Sub-County. The socio-demographic characteristics of the study participants varied by age, sex, level of education, type of livestock production system, and mode of milk vending business (**Table 1**).

Compositional quality of milk

The median butterfat content of the marketed raw milk from farms was 5.21 (range 2.02-9.47) whereas that from vendors was 5.25(range 2.26–9.34).An acceptable range of butterfat (3.3-7.0%) was observed in the raw milk from 92.7% (192/207) of farmers and 92.1% (140/152) of vendors. Unacceptable values of SNF, specific gravity, added water and freezing points were also observed in samples from both farmers and vendors (**Table 2**). Overall, 82.6% (95% CI: 77.0-87.3) of marketed raw cow milk from farms and 75.0% (95% CI 66.7-81.4) from vendors were of acceptable compositional quality.

Prevalence of antimicrobial residues and poor compositional quality

Overall, 15.5% (95% CI: 11.0-20.9) of the samples from farmers and 18.4% (95% CI: 12.9-25.2) of the samples from vendors were found to have antimicrobial residues above the EU MRLs (p-value = 0.467). A significant difference between the prevalence of antimicrobial residues in milk sold by farmers compared to that sold by vendors was only observed in Witu (12.5% vs 30.4%; p = 0.038) (**Table 3**).

From the interview findings, 28.5% (59/207) of the farmers and 8.6% (13/152) of the vendors acknowledged to be using a herbal substance with a local name *"mpingo"* which they applied by smoking the inner side of wooden milk handling containers, to serve as a milk preservative. On laboratory analysis using Charm Blue Yellow test, 20% (41/207) of the milk samples from farmers and 5.9% (9/152) of samples from vendors indicated the presence of a non-antibiotic heat-sensitive inhibitor. Of the 41 positive milk samples from farmers, 63.4% (26/41) were from Witu and 21.9% (9/41) from Mpeketoni. Of the milk samples from farmers, 17.4% (95%CI: 12.7-23.0) and 25.0% (95%CI: 20.6-36.6) from vendors were found to be of poor compositional quality, adulterated by addition of water (p = 0.786). A difference was observed between compositional quality of milk sold by farmers and vendors in Amu (7.4% vs 47.8%; p < 0.001) and Mokowe (27.5% vs 7.7%; p = 0.021) respectively (**Table 4**).

Comparison between compositional quality and prevalence of antimicrobial residues in milk marketed by farmers and vendors

Overall, 70.5% (95% CI: 64.1-76.4) samples from farmers and 63.2% (95%CI: 55.3-70.6) from vendors were both of good compositional quality and free of antimicrobial residues. However 3.4% (95%CI: 1.5-6.6) of milk samples from farmers and 6.6% (95%CI 3.4-11.4) from vendors contained antimicrobial residues and were of poor compositional quality (p = 0.159).

Factors associated with presence of antimicrobial residues in marketed raw cow milk among farmers

Farmers who had less than secondary level of education were three times more likely to sell milk with antimicrobial residues (OR 2.98, 95% CI: 1.16-7.56) compared to farmers who had secondary level of education and above. Farmers who were aware of dangers of consuming milk with antimicrobial residues were less likely to sell milk with antimicrobial residues compared to those farmers who were not aware (OR 0.20, 95% CI: 0.07-0.55). Those farmers who had some training on good milking practices were less likely to sell milk with antimicrobial residues compared to those farmers who did not have any training on good milking practices (OR 0.32; 95% CI: 0.11-0.96). Farmers' awareness of dangers of consuming milk with antimicrobial residues and farmers' training on good milking practices were retained as independent factors protective against selling milk with antimicrobial residues (**Table 5**).

Factors associated with poor compositional quality of marketed raw cow milk among farmers and vendors

Farmers who had at least secondary level of education were three times more likely to market milk of poor compositional quality (OR 2.88, 95% CI: 1.38-5.99) compared to those with primary level of education or no formal education. Pastoralist farmers were three times more likely to sell milk of poor compositional quality (OR 2.94, 95% CI: 0.99-8.78) as compared to non-pastoralist farmers. Of the 32 pastoralist farmers found selling milk of poor compositional quality 19 (59.4%) had attained at least secondary level of education. Adjusting for factors simultaneously, farmers having secondary level of education and above (AOR 3.03, 95% CI: 1.44-6.39) and being a pastoralist farmer (AOR 3.20, 95% CI: 1.05-9.71) were retained as independent risk factors against marketing of milk of poor compositional quality (**Table 6**).

Male vendors were three times more likely to market milk of poor compositional quality (OR 3.46, 95% CI: 1.61-7.47) compared to female vendors. Vendors who had been trained on good milk handling practices were more likely to market milk of poor compositional quality (OR 17.12 CI: 1.93-151.7). Being a male vendor was retained as the

only independent risk factor associated with marketing of milk of poor compositional quality amongst vendors (AOR 2.73. 1.22-6.08) after adjusting for vendor-training on good milk handling practices (**Table 7**). Of the 152 vendors, only 6 (3.9%) male vendors had been trained on good milk handling practices of which 5 (83.3%) were found to be selling milk of poor compositional quality.

Discussion

This is the first study in the northern coastal Kenya to assess the compositional quality and milk safety in regard to presence of antimicrobial residues. Our results demonstrated that consumers of marketed row cow milk in this region were at risk of being exposed to public health problems associated with presence of antimicrobial residues in food of animal origin and consumption of adulterated milk. This study identified factors associated with the occurrence of the residues and the milk adulteration, and observed use of a herbal substance by both farmers and vendors in preservation of milk.

This study identified water as the main adulterant, which has also been identified by other studies elsewhere as the most common adulterant in the milk industry [**17**]. Water lowers the nutritional value of the milk, interferes with processing qualities of milk and poses a risk of contaminating the milk. Adulteration of milk by addition of water can easily be detected in the field using a lactometer [**18**].Other substances have been reported as milk adulterants, such as: chlorine, antibiotics, non-milk proteins, low value milk, milk powder, colour, preservatives, urea, liquid whey and water [**17**,**19**,**20**]. In north eastern Brazil, 41.2% of goat milk presented to the market was found to contain bovine milk [**21**]. A by-product from the cottage cheese industry called liquid whey has been reported to be used as a milk adulterant to increase the volume of milk after extracting proteins and fat [**22**]. Because of the wide variety of adulterants reportedly used in the dairy industry with diverse effects, there is need for routine monitoring of the milk market value chain right from farm level to assure food safety to consumers.

Findings of this study were higher than those of a study done in Nakuru, Narok, Nairobi and Kiambu counties of Kenya by Omore et al (2002) where 4.7% of milk specimens from household farms and 10.4% from marketing agents were found to be adulterated by addition of water [**23**]. The higher prevalence observed in our study can be

associated with the young dairy industry in Lamu County where most of the produced milk is marketed raw directly to consumers as compared to Nakuru, Narok and Kiambu where bulk of the produced milk is sold to milk processing companies who are very strict on the quality of milk purchased. Milk of poor compositional quality is usually rejected by processors resulting in huge economic losses to farmers [**2**,**24**,**25**]. Milk processors also carry out extension services to farmers promoting good milking practices, a service lacking in Lamu County as there are no local milk processors.

This study demonstrated the occurrence of antimicrobial residues in marketed raw cow milk indicating that consumers are likely to be exposed to antimicrobial residues above EU MRLs each time they consumed the milk. The presence of antimicrobial residues in food is of concern as it contributes to development of drug resistance of human pathogens, allergic reactions and interference with growth of starter cultures in the milk processing industry [**6**,**26**,**27**]. The observed prevalence of residues in our study indicates a need to begin to address the problem both at the farm and market levels. This can be done through raising awareness amongst policy makers and implementers, farmers, vendors and consumers through specific extension messages [**8**]. A study in 1994 [**28**] conducted in Kiambu detected no residues in milk being supplied to milk cooperative societies in the county and was attributed to the high level of awareness and strict adherence to the withdrawal periods by farmers. This is consistent with findings in our study, where farmers who were aware of the danger of consuming milk with antimicrobial residues and those who had been trained on good milking practices were less likely to sell milk with antimicrobial residues.

This study observed higher prevalence of antimicrobial residues in milk marketed by farmers and vendors; compared to a study in 2005 [**8**] conducted in Nairobi, Nakuru and Narok, Kenya, where a prevalence of 11.1% amongst milk vendors and 16% amongst farmers was observed. The higher prevalence of antimicrobial residues observed in our study was attributed to lower levels of awareness of withdrawal periods amongst farmers [**28**].

Antimicrobial residue occurrence in milk has been reported globally. However, in countries with effective quality assurance systems, reports of residues in foods destined for the market are minimal or non-existent [**8**]. For example, in Brazil, a study to assess hazards in unpasteurized marketed milk at farm level found a prevalence of antimicrobial residues of 11.5% [**29**]. A study in the peri-urban areas of Accra and Kumasi cities in Ghana [**14**]

found that 35.5% of samples of raw marketed milk, collected from different marketing agents including farmers, processors, wholesalers and retailers, were contaminated with antimicrobial residues. In Tanzania, a prevalence of 36% was observed in a study to investigate the risk of exposure to antimicrobial residues present in marketed raw milk in Mwanza and Dar es Salaam [**30**]. With such high prevalence observations in various countries, there is need to intensify safety assurance efforts both at farm and market levels, promote prudent use of antibiotics and observance of drug withdrawal period.

This study noted the existence of a non-antibiotic heat-sensitive inhibitor [**16**] in milk from Witu and Mpeketoni towns. From the interview findings, locals acknowledged to be using a herbal substance they called *mpingo* to preserve milk. This practice of using natural antimicrobials in milk preservation has been reported elsewhere [**31-33**]. Such substances are likely to affect growth of starter cultures in the milk industry, if the milk is not properly heated before start of processing. Little is known about the *mpingo* herb, which could have side effects to consumers.

In interpreting the findings of this study, it should be noted that sampling of farmers was independent from sampling of vendors. This study could not follow milk along the market value chain, that is, from individual farms to individual vendors, to determine the source or point of adulteration or the antimicrobial residues in the milk.

Conclusion

This study identified the occurrence of antimicrobial residues above the set limits (EU MRLs) and adulteration of marketed raw cow milk through addition of water in Lamu West Sub-county. The antibiotics detected in the milk pose a health risk to the consumers by eliciting harmful effects. There is need to routinely test marketed milk, intensify public health education regarding milking and good milk handling practices, train farmers on strict adherence to antimicrobial use and withdrawal periods and impose stiffer penalties on those adulterating milk.

What is known about this topic

- The compositional quality and prevalence of antimicrobial residues in central Kenya and areas surrounding Nairobi city are well known courtesy of several studies (2002, 2005), regular checks by the regulatory body, Kenya Dairy Board and multiple milk processing companies.

What this study adds

- This study quantifies the extent of compositional quality and prevalence of antimicrobial residues in raw marketed cow milk in the coastal Kenya region, particularly Lamu West Sub County where there are no records of previous studies done in this region on the same topic;
- This study epitomizes the importance of raising awareness on good milking and milk handling practices amongst farmers and vendors, for good quality and safe milk.

Competing interests

The authors declare no competing interests.

Authors' contributions

GKO conceived the study, drafted the proposal, carried out data collection, laboratory examination, data analysis, interpretation of the results and ultimately finalized write up of the manuscript. JNO, MO, ZG, JG, AB and JKG assisted in proposal preparation, in-process consultation and review of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

I would like to acknowledge and give thanks to the following persons for their much appreciated support and encouragement during the study: The Kenya Field Epidemiology and Laboratory Training Programme (K-FELTP) for the scholarship opportunity and financial sponsorship of the study; Dr. Victor Tole, Medical Officer of Health, Lamu County Hospital, for accepting and allowing me to use his facility to do the study; Mr Jamleck Safari (the Hospital Laboratory Manager), Mr David Gitau and Mr Ignatius Kamau (Laboratory Technicians) for their assistance during sample processing and analysis; Voi Milk Analysis Station for equipment support; Ms Doris Marwanga for the much valued assistance during designing of the map of the study site; supervisor, Dr Charles Gichohi for providing ample time to enable me complete this study; Dr Peter Wasswa for assistance during data interpretation and review of this work; Mr Waqo Boru for much valued inputs during proposal development and Dr. Sara Lowther for scientific review of this manuscript. I thank my wife, Nyaboke and my beloved children, Valarie and Jesse for being so patient with me while I was away from home. To all my workmates, relatives and friends, I wish to say thank you and may our good Lord richly bless you

Tables and figure

Table 1: socio-demographic characteristics of study participants in Lamu West Sub County, 2015

Table 1: socio-demographic characteristics of study participants in Lamu West Sub County, 2015		
Characteristics	**Total Farmers (N=207) n (%)**	**Total Vendors (N=152) n (%)**
Selected Market Centres		
Amu	27 (13.0)	46 (30.3)
Mokowe	40 (19.3)	39 (25.7)
Mpeketoni	52 (25.1)	44 (28.9)
Witu	88 (42.6)	23 (15.1)
Sex		
Female	44 (21.3)	101 (67.1)
Male	163 (78.7)	51 (33.5)
Age groups (years)		
<45 (19-44)	93 (44.9)	102 (67.1)
≥ 45 (45-80)	114 (55.1)	50 (32.9)
Livestock production System		

Pastoralism	157 (75.9)	-
Non pastoralism	50 (24.1)	-
Level of education		
< Secondary Education	130 (62.8)	114 (75.0)
≥ Secondary Education	77(37.2)	38 (25.0)
Type of vendor		
Kiosk-Shop	-	15 (9.9)
Hawker	-	137 (90.1)
Who treats their livestock		
Self	93 (44.9)	-
Veterinarian	114 (55.1)	-

Table 2: compositional quality of informally marketed raw cow milk, Lamu West, 2015

Table 2: compositional quality of informally marketed raw cow milk, Lamu West, 2015					
	Farmers, N=207		**Vendors, N=152**		
Milk Component	**Median (Range)**	**No. out of normal range**	**Median (Range)**	**No. out of normal range**	**Normal Range**
Butter Fat %	5.21 (2.02-9.47)	15/207	5.25 (2.26-9.34)	12/152	3.3-7.0
SNF	9.32 (5.86-12.1)	36/207	9.29 (5.45-12.4)	38/152	> 8.5-12.0
Specific Gravity kg/l	1.030 (1.018-1.037)	36/207	1.029 (1.018-1.037)	38/152	1.026-1.036
Added water %	14.28 (0.37-27.90)	36/207	14.04 (0.48-27.6)	38/152	0.00
Freezing point (0C)	-0.597 (-0.401- -0.733)	36/207	-0.595 (-0.402 –0.649)	38/152	-0.525 - -0.565
Protein %	3.53 (2.26-5.27)	5/207	3.52 (2.30- 4.68)	3/152	2.9-5.0

Table 3: prevalence of antimicrobial residues in informally marketed raw cow milk per urban centre from farmers and vendors, Lamu West Sub-County, 2015

Table 3: prevalence of antimicrobial residues in informally marketed raw cow milk per urban centre from farmers and vendors, Lamu West Sub-County, 2015					
Urban centre	**No. of milk samples**		**Antimicrobial residues**		
	From farmers	**From vendors**	**Positive milk samples from farmers n (%)**	**Positive milk samples from vendors n (%)**	**p-value**
Amu	27	46	6(22.2)	8(17.4)	0.615
Mokowe	40	39	7(17.5)	5(12.8)	0.560
Mpeketoni	52	44	8(15.3)	8(18.2)	0.704
Witu	88	23	11(12.5)	7(30.4)	**0.038**
Total	**207**	**152**	**32(15.5)**	**28(18.4)**	0.467

Table 4: prevalence of poor compositional quality of informally marketed raw cow milk per urban centre from farmers and vendors, Lamu West Sub-County, 2015

Table 4: prevalence of poor compositional quality of informally marketed raw cow milk per urban centre from farmers and vendors, Lamu West Sub-County, 2015					
Urban centre	**No. of milk samples**		**Poor compositional quality**		
	From farmers	**From vendors**	**Poor compositional quality milk samples from farmers n (%)**	**Poor compositional quality milk samples from vendors n (%)**	**p-value**
Amu	27	46	2(7.4)	22(47.8)	**< 0.001**
Mokowe	40	39	11(27.5)	3(7.7)	**0.021**
Mpeketoni	52	44	10(19.2)	8 (18.2)	0.900
Witu	88	23	13(14.8)	5(21.7)	0.424
Total	**207**	**152**	**36(17.4)**	**38(25.0)**	0.786

Table 5: factors associated with presence of antimicrobial residues in informally marketed raw cow milk by farmers in Lamu West Sub County, 2015

Table 5: factors associated with presence of antimicrobial residues in informally marketed raw cow milk by farmers in Lamu West Sub County, 2015						
Characteristics	**Residues Positive n (%)**	**Residues Negative n (%)**	**Crude OR (95%CI)**	**p-value**	**Adjusted OR (95%CI)**	**p-value**
Age Group						
≥ 45 years (45-80)	20 (17.5)	94 (82.5)	1.43 (0.66-3.12)	0.440	-	-
< 45 years (19-44)	12 (12.9)	181(87.1)				
Level of education						
< Secondary Education	26 (20.0)	104(80.0)	**2.98 (1.16-7.56)**	**0.027**	1.49(0.52-4.31)	0.461
≥ Secondary Education	6 (7.8)	71 (92.2)	1.00			
Who treats your sick livestock						
Veterinarian	14 (12.3)	100 (87.7)	0.58 (0.27-1.25)	0.180	-	-
Other	18 (19.4)	75 (80.6)	1.00			
Sex						
Male	22(13.5)	141(86.5)	0.53(0.23-1.22)	0.158	-	-
Female	10(22.7)	34(77.3)	1.00			
Aware of danger of the residues						
Aware of danger	5 (5.6)	84(94.4)	**0.20(0.07-0.55)**	**<0.001**	**0.20(0.07-0.55)**	**0.002**
Not aware of danger	27 (22.9)	91(77.1)	1.00			
Livestock Production System						
Pastoralist	23 (14.6)	134 (85.4)	0.78 (0.33-1.82)	0.653	-	-
Non-Pastoralist	9 (18)	41 (82)	1.00			
Training on good milking practices						
Trained	4 (6.9)	54 (93.1)	**0.32(0.11-0.96)**	**0.033**	**0.33(0.11-0.99)**	**0.048**
Not trained	9 (18.8)	121 (81.2)	1.00			

Table 6: factors associated with poor compositional quality of informally marketed raw cow milk by farmers in Lamu West Sub County, 2015

Table 6: factors associated with poor compositional quality of informally marketed raw cow milk by farmers in Lamu West Sub County, 2015						
Characteristics	**Poor Quality n (%)**	**Good Quality n (%)**	**Crude OR (95%CI)**	**p-value**	**Adjusted OR (95%)**	**p-value**
Age Group						
≥ 45 years (45-80)	18 (19.4)	75 (80.6)	1.28 (0.62-2.63)	0.581	-	-
< 45 years (19-44)	18 (15.8)	96 (84.2)	1.00			
Level of education						
≥ Secondary Education	21 (27.3)	56 (56)	**2.88 (1.38-5.99)**	**0.007**	**3.03 (1.44-6.39)**	**0.004**
< Secondary Education	15 (11.5)	115 (88.5)	1.0	-	-	-
Sex						
Male	27 (16.6)	136 (83.4)	0.77 (0.33-1.79)	0.511	-	-
Female	9 (20.5)	35(79.5)	1.00			
Training on good milking practices						
Trained	10 (17.2)	48 (82.8)	0.84 (0.40-1.99)	0.842	-	-
Not trained	26 (19.0)	111 (81.0)	1.00			
Livestock Production System						
Pastoralist	32 (20.4)	125(79.6)	2.94 (0.99-8.78)	**0.053**	**3.20 (1.05-9.71)**	**0.040**
Non-Pastoralist	4(8)	46 (92)				

Table 7: factors associated with poor compositional quality of informally marketed raw cow milk by vendors in Lamu West Sub County, 2015

Table 7: factors associated with poor compositional quality of informally marketed raw cow milk by vendors in Lamu West Sub County, 2015						
Characteristics	**Poor Quality n (%)**	**Good Quality n (%)**	**Crude OR (95%CI)**	**p-value**	**Adjusted OR (95%CI)**	**p-value**
Age Group						
≥ 45 years	23 (22.6)	79 (77.4)	0.68 (0.32-1.46)	0.326	-	-
< 45 years	15 (30.0)	35 (70.0)				
Level of education			1.00			
< Secondary Education	27 (23.7)	87 (76.3)	0.76 (0.33-1.74)	0.522	-	-
≥ Secondary Education	11 (30.0)	27 (70.0)	1.00			
Sex						
Male	21 (41.2)	30 (58.8)	**3.46 (1.61-7.47)**	**0.002**	**2.73 (1.22-6.08)**	**0.014**
Female	17 (16.8)	84 (83.2)	1.00			
Trained on good milk handling practices						
Trained	5 (83.3)	1 (16.7)	**17.12 (1.93-151.7)**	**0.004**	9.05 (0.97-84.3)	0.053
Not trained	33 (22.6)	113 (77.4)	1.00			
Type of trade						
Kiosk/shop	0 (0)	8 (100)	0.00	-	-	-
Hawker	38(26.4)	106 (73.6)				

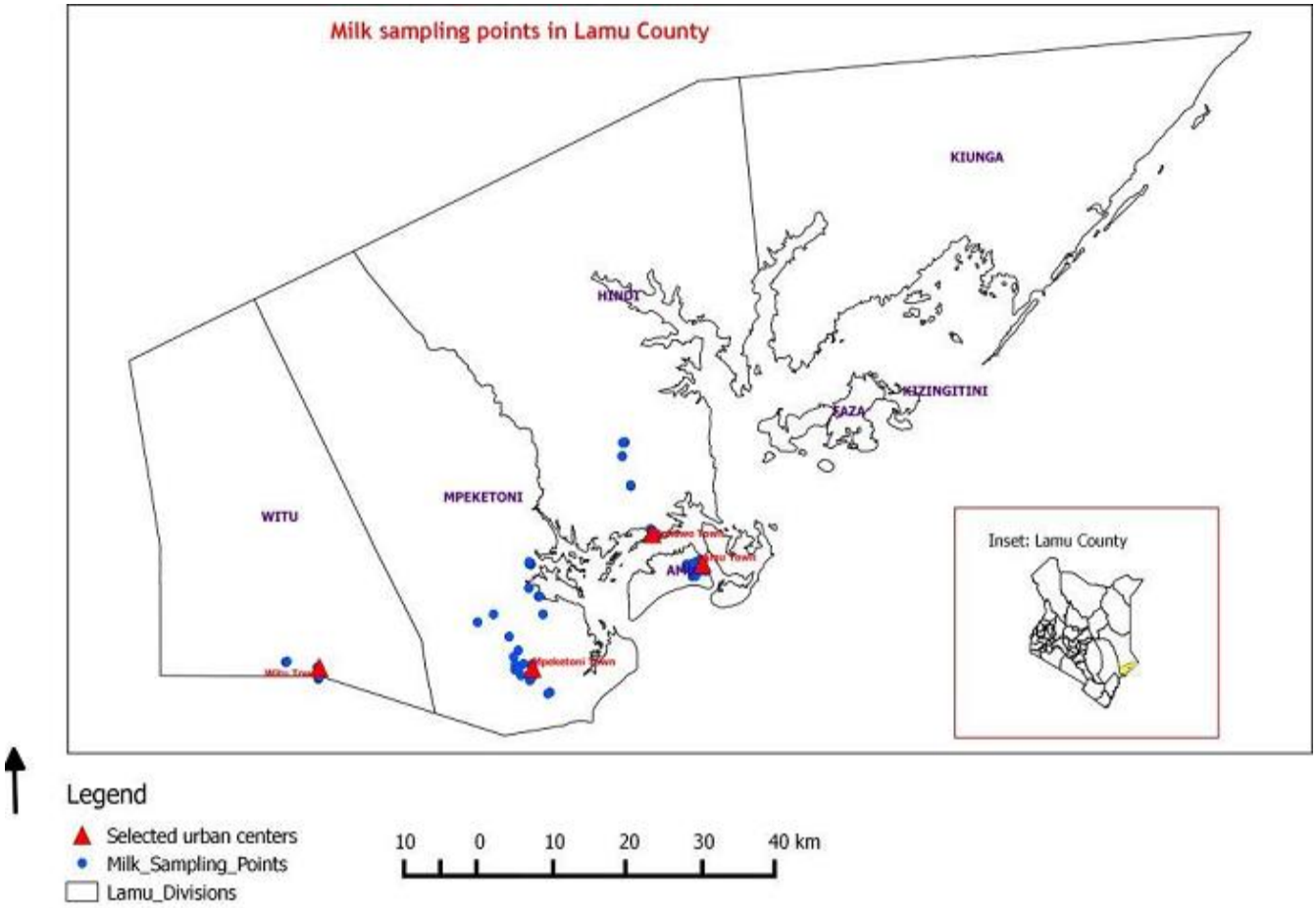

Figure 1

a map showing the study site, Lamu West Sub-County, the selected urban centres

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