THE UNIVERSITY OF NAIROBI.
COLLEGE OF AGRICULTURE AND VETERINARY SCIENCES.
FACULTY OF VETERINARY MEDICINE.

PROJECT TITLE: DETERMINATION OF THE LEVELS OF ANTIMICROBIALS IN MILK SAMPLES FROM UTHIRU MARKET, KIAMBU COUNTY.

A PROJECT REPORT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTFOR THE AWARD OF BACHELOR OF VETERINARY MEDICINE DEGREE OF THE UNIVERSITY OF NAIROBI.

INVESTIGATOR

J30/2079/2010

WAITHAKA JOHN WAWERU

DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY

FACULTY OF VETERINARY MEDICINE, UNIVERSITY OF NAIROBI

SUPERVISOR

DR. GATHURA PETER BAARO, BVM, MSc. Ph.D. (UNIVERSITY OF NAIROBI)

DEPARTMENT OF PUBLIC HEALTH PHARMACOLOGY AND TOXICOLOGY,

FACULTY OF VETERINARY MEDICINE (UNIVERSITY OF NAIROBI.)

DECLARATION

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

Signatureé é é é é é é é é é é é é Dateé é é é é é é é é é é é é ..

J30/2079/2010

WAITHAKA JOHN WAWERU

This report has been submitted for examination with my approval as the University supervisor

Signatureé é é é é é é é é é é é é Dateé é é é é é é é é é é é é é

Dr. P. B. GATHURA (BVM, MSc., PhD.)

Senior Lecturer

Department of Public Health,Pharmacology and Toxicology, University of Nairobi

DEDICATION

This project work is dedicated to my dear parents, Mr. and Mrs. Sammy Waithaka, my brothers, sisters, auntie and to all my fellow students in the Faculty of Veterinary Medicine.

ACKNOWLEDGEMENT

I take this opportunity to sincerely appreciateall those who contributed in one way or another in the success of my project. My efforts could not have yielded any fruits without your involvement, guidance, provision of the necessary equipments and materials, reading and interpretation of results, encouragement, advice, corrections. My success was simply from team work. Special regards to;

- My supervisor Dr. P.B. Gathura (Senior lecturer, University of Nairobi)
- The Chief Technologist, Department of Public Health, Pharmacology and Toxicology, Mr.NduhiuGitahi (lecturer, University of Nairobi)
- The laboratory technologists, Department of Public Health, Pharmacology and Toxicology, University of Nairobi.
- My parents, Mr. and Mrs. Sammy Waithaka and my auntie for encouragement and financial assistance.

TABLE OF CONTENTS

CHAPTER ONE

1.0 Introduction

1.1 Milk

Milk is the white substance produced by the mammary glands of mammals. Milk is valuable food, readily digested and absorbed; it consists of nutrients which are needed for proper growth and maintenance of body. Milk and milk products form a significant part of the diet and a substantial amount of food expenditure goes to milk and other dairy products.

Milk is transported from the point of production to consumers and processing plants by middlemen. They do not maintain proper hygienic conditions during this transport, which leads to increase in the total viable bacterial count.

They also adulterate milk to increase their profit margins by adding several chemicals like urea, starch, flour, cane sugar, vegetable oils, detergents e.t.c. Various preservatives like formalin and some antibiotics are also added in milk to increase its shelf life. This addition decreases the nutritive value of milk.

Milk adulteration is an act of intentionally debasing the quality of milk offered for sale either by admixture or substitution of inferior substances or by the removal of some valuable ingredients (Food & Drug Administration, 1995). Adulterated food is dangerous for health as it may contain various toxic chemicals, it may be deprived of nutrients required for proper growth and development of human body (Marcus 1979). Milk used by the people for consumption is adulterated to such an extent that there is little nutritive value and may also be toxic to the public. Antibiotics in milk are a concern due to the risk of allergic reactions and the development of antibiotic resistant pathogens.

1.2 OBJECTIVES

1.2.1 GENERAL OBJECTIVE

- To enhance milk hygiene and human health.
- To assess the mode of preservation of milk by the vendors in Uthiru shopping centre and its environs.
- To determine the levels of growth inhibition of microorganisms from various milk samples.

1.2.2 SPECIFIC OBJECTIVE

- To detect the presence of antimicrobial residues in market milk sold in Uthiru marketing centre and its environs.

1.3 JUSTIFICATION

Antimicrobial drugs are used for treatment of diseases in both human beings and animals. Presence of antibiotic residues in the milk for human consumption can pose a great health risk to the consumers. Some of the risks posed to the consumers include, development of resistant bacteria to drugs due to mutation of bacteria, some of which are pathogenic bacteria and development of imbalance in gastrointestinal micro-flora. Other individuals are allergic to antibiotics such as penicillin which can lead to serious anaphylactic shock which can eventually lead to death. My research will help in analysis of market milk for the presence of antibiotics and hence create public health awareness.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 MILK

Milk is an important source of nutrient required for growth in infants and children and for maintenance of health in adults. Milk is a perfect food, readily digested and absorbed. It is a sole natural food for infants and children. It is chiefly a valuable source of good quality protein, fat, carbohydrates, vitamins and minerals. Protein in diet supply the amino acids required for growth of infants and children. It is also required for maintenance of tissues in adults.

The adulterants/preservatives assume the proportion of health hazards for end consumers particularly infants (*Tipu et al.2007*). Suppliers of milk appear to have found three ways to increase their margin from the sale of milk: (i) Dilution. (ii) Extraction of valuable components, i.e. milk fat removed as cream. (iii) Addition of cheap (and sometimes potentially harmful) bulking additives, such as low quality flour, to bring the total solids to a level which is acceptable to consumers. Some of the chemicals, adulterants and malpractices results in public health concern and malnutrition.

Normally, the adulteration in food is done either for financial gain or lack of proper hygienic conditions of processing, storing, transportation and marketing. This ultimately leads to the stage that the consumer is either cheated or often becomes victim of diseases. Such types of adulteration are quite common in developing countries. It is equally important for the consumer to know the common adulterants and their effects on health.

These adulterants, preservatives and drugs in milk cause very serious health related problems (*Afzal et al. 2011*). The extensive consumption of milk and dairy products makes these foodstuffs targets for potential adulteration with financial gains for unscrupulous producers (*Nicolaou et al. 2011*). Therefore, it is important to protect the consumer by ensuring that adequate control measures are in place, and that the food analyst has suitable methods for the detection of milk adulteration. Labeling and authenticity regulations may differ from country to country and contribute towards the need for analytical tests to enforce such legislation (Dennis, 1998).

In the treatment of bovine mastitis, antibiotics are widely used, and improper application can lead to the contamination of milk at farm level. Nowadays beta lactam(penicillin G

etc.),aminoglycosides(streptomycin,neomycin,etc)and tetracycline (oxytetracycline etc.)antibiotics are the most frequently used antimicrobials in treatment of mastitis in dairy cows and consequently the most commonly found type of residues in milk.(Seyda ERGIN KAYA, Ayhan FILAZI 2010)

When treatments are made, the milk from the treated animals is not supposed to be used for human consumption for at least 72 hours following the last treatment. If such milk is added to milk from untreated cows before 72 hours period is completed, residual antibiotics may be detected in the entire supply. These antibiotics may enter the milk directly as intramammary treatments for mastitis or indirectly through the blood stream of an animal which has been intravenously injected with an antibiotic preparation for some pathological conditions. Penicillin apparently has greater influence on public health than other antibiotics in common use. (HENRY V. Atherton, J.A Newlander, 2003). Table 1.1 shows the withholding periods of various antibiotics.

Table 1:A table showing the withholding periods of various antibiotics.

Antibiotic	Withholding period in hours
Amoxicillin	60
Cloxacillin	48
Erythromycin	48
Novobiocin	72
Penicillin	84
Sulfadimethozine	60

Some of the following measures should be put in place to prevent occurrence of antibiotics residues in milk;

Prevention of disease to minimize the use of antibiotics through the maintenance of good herd health programs, good animal husbandly practices, observe herd bio-security at the farm level, proper diagnosis and treatment of diseases and strict observation of milking withdrawal periods for the treated animals.

Follow directions on drugs leaflets and labels. Follow instructions for treatment dosage, frequency and route of administration. Adhere to recommended milk-withholding time. Follow label directions for medicated feed, and never use feed intended for other livestock. Note whether drug is approved for lactating cows. Test all dry-treated cows, especially those that freshen early, before putting them back into the milking line. Dry-cow treatments last longer.

Proper record keeping of the treatment program in the farm, the records should entail the animalsô bio data, dates and time of treatment, the antibiotic used, its dosage, frequency and route of administration, the milking withdrawal period of the antibiotics used and regular monitoring of the treatment records.

It is also important to keep milk from cows that are receiving oral, intramuscular, udder infusion or intrauterine antibiotic treatments out of the milking line. Milk all treated animals last and discard milk from all the four quarters of the udder, even if only one quarter was treated. Test all purchased herd replacements before introducing them into the milking herd (http://www.idexx.ca/view/xhtml/en_ca/dairy/training/residue-prevention.jsf)

CHAPTER THREE

3.0 MATERIALS AND METHODOLOGY

3. 1 Materials

- Sterile cotton swabs
- Autoclave
- Analytical balance
- Cork borer
- Normal saline
- Test tubes
- Rubber gloves
- Incubator
- Distilled water
- Beakers
- Micropipettes
- Bunsen flame
- Sterile petri dishes
- Glass spreaders
- Vanier calipers
- 70% alcohol
- McFarland tubes
- Nutrient agar
- *Micrococcus lutea*

3.2 Methodology

3.2.1 Study area

The study was carried out in Uthiru shopping center and its environs where random sampling of non ï processed raw milk from the milk vendors was done during the morning hours between 7.30 am to 11.30 am. A total of 30 samples were collected. Most of the milk sold in Uthiru area and its environs comes from the vast KiambuCounty. Uthiru is a shopping center located about 15 kilometers west of the Kenyaôs capital city, Nairobi, along Waiyaki way.

3.2.2 Sampling and sample size

The procedure comprised of collection of samples in the morning hours between 7.30 am and 11.30 am in which a total of 30 samples were collected. The milk was put in packages the vendors use (clear polythene bags). Each sample comprised of quarter a litre of milk from each milk vendor. The mode of storage to increase shelf life by each milk vendor was noted against each sample collected. A total of 15 samples were collected from milk vendors using deep freezers as their mode of storage of milk and the remaining 15 samples were collected from milk vendors using a bucket of cold water into which the milk holding container was dipped into, to increase shelf life. The samples were transported to the Departmental Public Health laboratories of the University of Nairobi in a cool box. The samples were stored in a refrigerator at 4^0c awaiting sensitivity tests.

3.2.3 Screening the milk samples for antibiotic residues

3.2.3.1 Microbial growth inhibition

This method is based on measurement and evaluation of diameters of inhibited bacterial growth on the media. Microbiological methods detect inhibitory substances diffusing from the sample placed in the well to the agar media in the plate. The diffused substance will inhibit

growth causing a clear zone to form around the well in case the substances have microbial growth inhibiting characteristics. If there are no microbial inhibiting substances the growth of the microorganisms will be up to the periphery of the well. (AndrejKirbis, 2007). There are natural inhibitors that are present in milk such as lysozyme or lactoferin. These can cause false positive results therefore it is crucial to destroy them when testing for antibiotic residues by this method. This is done by subjecting the milk sample to thermal inactivation for five minutes at 80^0C. (STN570531).

3.2.3.2 Preparation of media

Nutrient agar was weighed and dissolved in the appropriate amount of water according to the manufacturerôs instructions. This media was then autoclaved at a temperature of 121^0C for 15 minutes and it was allowed to cool to about 55^0Cin a warm water bath. This media was poured in sterile petri dishes in an airflow meter to avoid its contamination from the environment. The media was then allowed to solidify after which the petri dishes were labeled and stored in a refrigerator at 4^0C. Consequently, 100mls of normal saline, cotton swabs and the cork borers were prepared and they were sterilized by autoclaving at 121^0C for 15 minutes. These were then stored in a sterile condition to avoid contamination.

A stock culture of *Micrococcus lutea* in cooked meat medium, which had been stored for a long period in a refrigerator at 4^0C, was revived by inoculating it on one plate of Nutrient agar. This plate was then incubated at 37^0C overnight. This is the organism that was used to test for the sensitivity of the antimicrobial residues in the milk samples. Its choice was because it is highly sensitive to most of the antimicrobial agents used.The following day when the microorganism had grown, its colonies were suspended in 10mls of normal saline, mixed using a vortex machine andits turbidity was compared with the McFarlandôs set of tubes, to achieve a turbidity

equivalent to 10^6 McFarland standards. This was the standard inoculum that was used to inoculate the plates.

The Nutrient agar plates were removed from the refrigerator and they were dried in the incubator for 20 minutes. Using the sterile cotton swabs, the standard inoculum was swabbed on the surface of the plate so that a uniform growth of the microorganisms on the plate covered the entire surface. After inoculation, the plates were allowed to dry for ten minutes after which the wells were dug in the media. Using a sterile cork borer three wells were dug strategically through the media on each plate leaving enough allowance of about 2cm from the edges of the plates. Using a mark pen each well was labeled in numbers according to the sample to be put in that well. Using a micropipette and a micropipette tip, 100 µl of each milk sample were dispensed into the wells taking care not to spill the milk sample beyond the well margin and also changing the micropipette tubes from one milk sample to the next to avoid antibiotic transfer. These plates were left for some time on a sterile bench to allow for diffusion of the milk samples in the media. Afterwards the plates were incubated at 37ºC for 24 hours in an upright position after which the results were read.

3.3 Standard Controls

The tests were accompanied by positive and negative controls. A positive control was made by dissolving 1 ml of penicillin in 10mls of milk and stored in a refrigerator at 4^0C. As the tests were conducted, 1ml of this solution was dissolved in 100mls of distilled water to dilute it ten folds and then it would be dispensed in the wells as described above. For a negative control, a known milk sample, free from antibiotic residues was used.

CHAPTER FOUR

4.0 THE RESULTS

After the incubation of the samples for 24 hours, the results were read out. Positive results were indicated by presence of an inhibition zone around the well. Negative results were indicated by presence of the bacterial growth up to the margins of the well. For the positive results, where there was inhibition in the growth of the *Micrococcus lutea*around the wells, the diameter of inhibition zone was measured using a Vanier caliper, which was then placed on a ruler and the measurements recorded in millimeters in the table below.

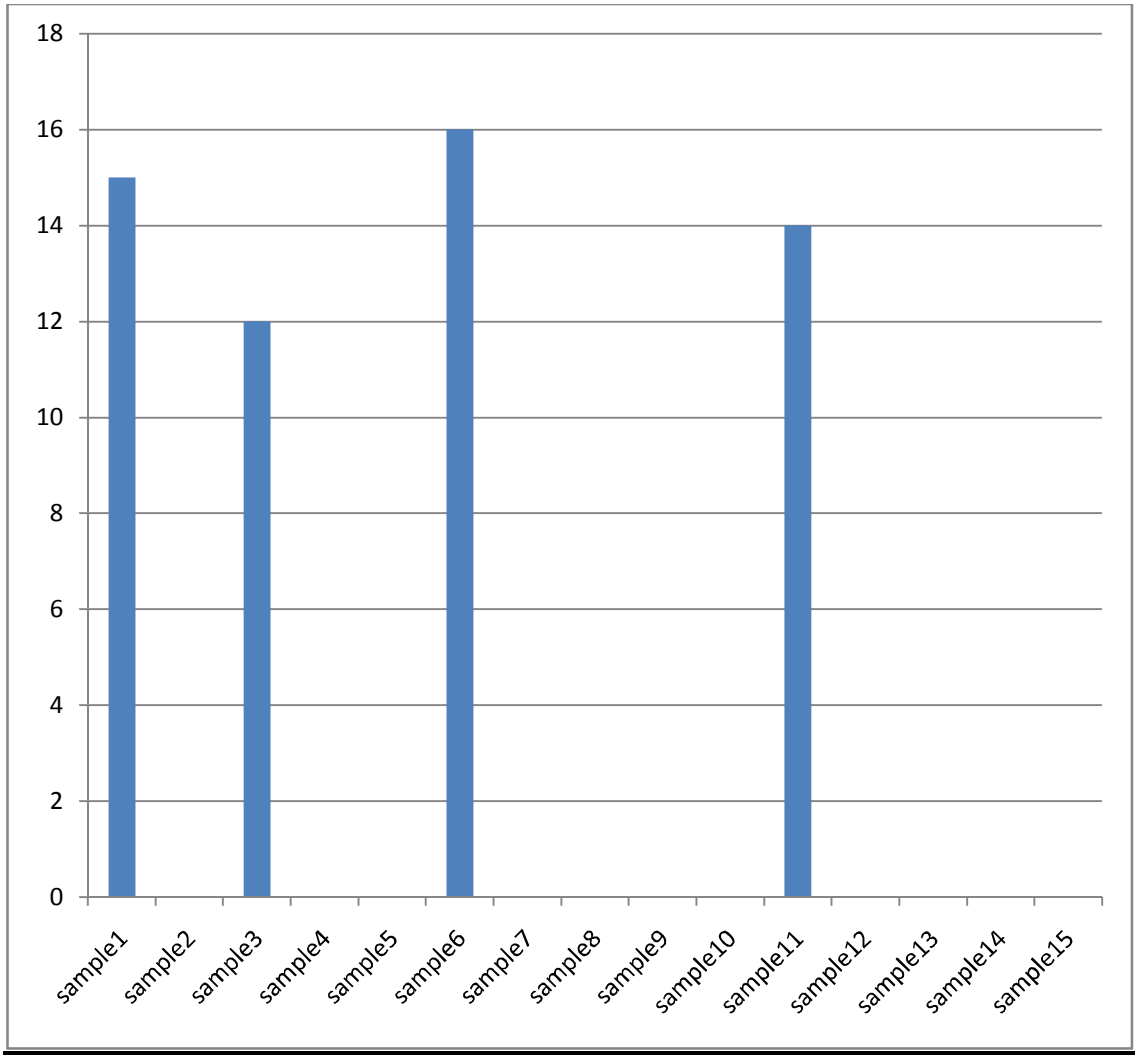
Table 2:A table showing the milk sample number, mode of storage by the vendors and the inhibition diameter(millimeters)

Sample number	Mode of storage by vendors	Diameter of inhibition in mm
1	Deep freezer	15.0
2	Deep freezer	0.0
3	Deep freezer	12.0
4	Deep freezer	0.0
5	Deep freezer	0.0
6	Deep freezer	16.0
7	Deep freezer	0.0
8	Deep freezer	0.0
9	Deep freezer	0.0
10	Deep freezer	0.0
11	Deep freezer	14.0
12	Deep freezer	0.0
13	Deep freezer	0.0
14	Deep freezer	0.0

15	Deep freezer	0.0
16	Bucket of cold water	0.0
17	Bucket of cold water	19.0
18	Bucket of cold water	12.0
19	Bucket of cold water	11.0
20	Bucket of cold water	0.0
21	Bucket of cold water	13.0
22	Bucket of cold water	12.0
23	Bucket of cold water	0.0
24	Bucket of cold water	0.0
25	Bucket of cold water	19.0
26	Bucket of cold water	15.0
27	Bucket of cold water	13.0
28	Bucket of cold water	12.0
29	Bucket of cold water	0.0
30	Bucket of cold water	0.0

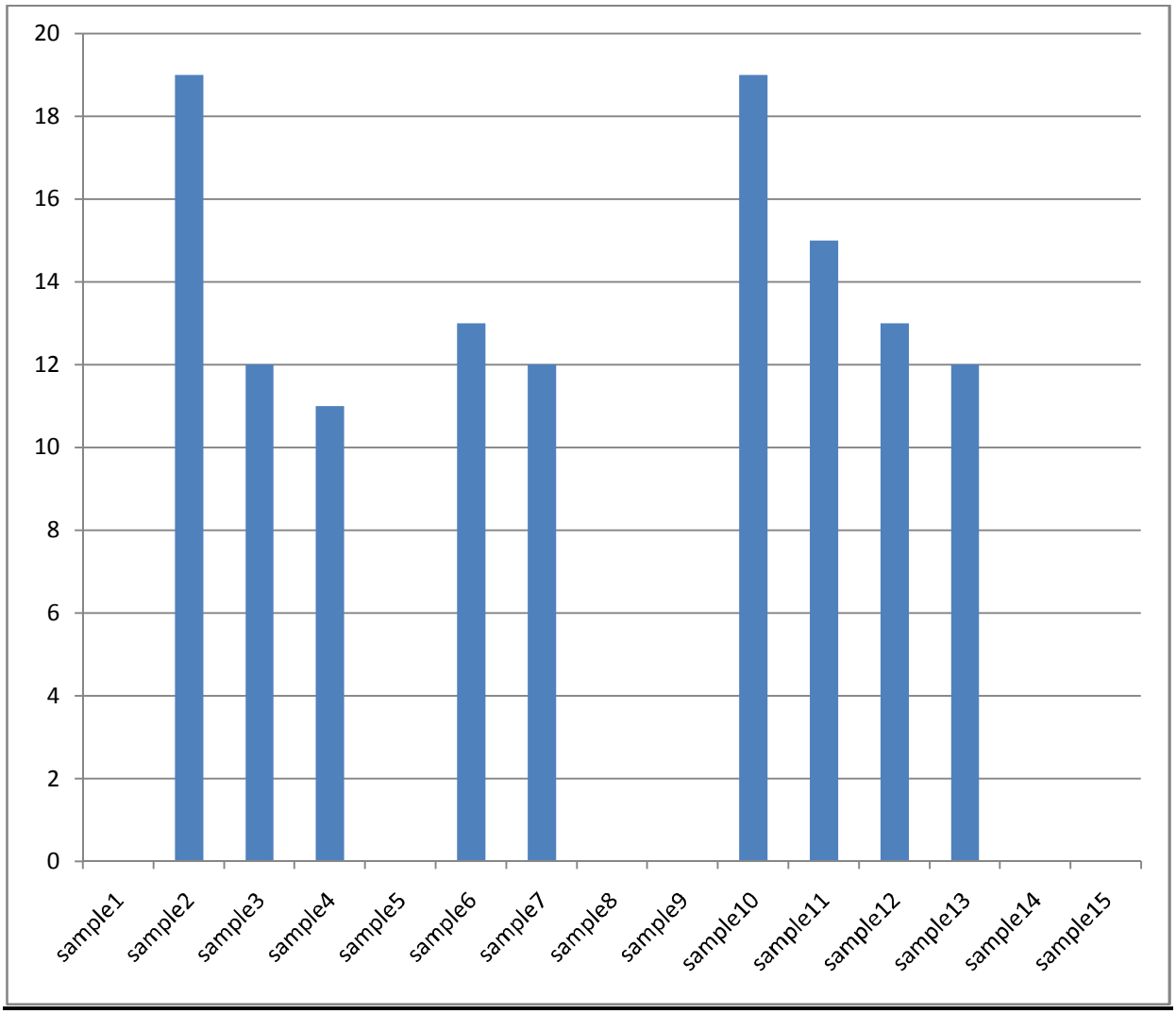
Graph 1 : graphical representation of the results obtained from milk vendors using deep freezer as their mode of storage.

Figure 1 : Inhibition diameter in millimeter (y-axis) against sample number (x-axis)



Graph 2: graphical representation of the results obtained from milk vendors using a bucket of cold water at room temperature as their mode of storage.

Figure 2 : Inhibition diameter in millimeter (y-axis) against sample number (x-axis



CHAPTER FIVE

5.0 DISCUSSION OF THE RESULTS.

The survey conducted in Uthiru shopping centre and its environs showed the presence of antibiotic residues in the milk sold to the consumers in the region. A total of 30 milk samples were collected and out of this, 13 milk samples tested positive for presence of antibiotic residues which inhibited microbial growth around the wells. On further analysis of the milk samples, it was observed that samples collected from milk vendors using deep freezers in storage of the milk awaiting buying by the consumers, the positive samples were fewer compared to those immersing milk in a bucket of cold water at room temperature. A representative of 15 milk samples were collected from vendors using deep freezers to store milk, 4 milk samples tested positive .these was a representative value of 26.67 %. On the other hand those using a bucket of cold water, out of the representative value of 15 milk samples 9 milk samples (60%) tested positive for presence of antibiotic residues.

The above results, when interpreted in percentage form, a representative value of 43.33 % of the milk consumed in Uthiru and its environs contains antibiotic residues. This could probably be as a result of milk adulteration by unscrupulous traders whose aim is to maximize on milk profits through extension of shelf life of the milk by intentional addition of antibiotics agents. Their origin can be traced back to the farm or maybe addition to the milk by unscrupulous vendors who fear making loses through milk spoilage(Aboge G.O, 2002). Another probable sourceof antibiotics in the milk couldbefrom cows treated with antibiotics against various diseases e.g. mastitis, if the owners fail to observe milking withdrawal periods of various antibiotics and hence their presence in the market milk. On the other hand milk contains natural inhibitors of microbial growth such as lysozyme and lactoferin. The natural inhibitors can pose as false positive for presence of microbial growth inhibiting agents(STN 570581). However these microbial growth inhibitors can be destroyed by subjecting the milk sample to heat for a period of five minutes as they are heat labile. The persistent microbial growth inhibitors after heating are thermal stable and these could only be antibiotic residues present in milk

The representative 15 samples collected from milk vendors (unlicensed)using a bucket of cold water asthere mode of storage, 9 samples tested positive, which could probably be as a result of intentional addition of antibiotic residues such as hydrogen peroxide, penicillin, tetracyclineto extend the shelf life of the milk as they had no reliable mode of storage and to a small extent, presence of heat labile microbial growth inhibitors.

5.1 CONCLUSION

The study conducted on the analysis of milk quality was a clear manifestation that the milk sold to consumers in Uthiruand its environshad a probability of close to 43.33% of the milk vendors selling milk that has microbial growth inhibitors. It was evident too that milk retailed by vendors (unlicensed) who had no proper storage facilities, had increased chances of having microbial growth inhibitors/antibiotic residuescompared to the licensed milk vendors. Some of the contributing factors could possibly be;

The presence of antibiotic residues could simply be traced back from the farms where the milk is sourced. This is as a result of the cows being treated against various diseases using antibiotics and the farmers failing to observe the milking withdrawal periods as directed by the clinicians and the milk ending up in the market. This can be controlled at the farm level by screening milk for antibiotics residues and discarding the milk testing positive for the presence of antibiotics.

Lack of proper mode of storage of milk by the unlicensed milk vendors, increases the chances of adulteration of milk through the use of antibiotics such as penicillin to extend the milk shelf life, as opposed to the licensed milk vendors, who uses deep freezers to store milk, therefore they donôt need to use antibiotics to extend milk shelf life.

Regular inspection of the licensed milk bars and dairies by the public health officers also contributes to the decreased use of antibiotics by the milk vendors as compared to higher chances

of use of antibiotic agents by the unlicensed milk vendors who donôt have to worry about the inspection by the public health officers.

5.2 RECOMMENDATIONS

The main objectives of carrying my project was to investigate on the milk quality retailed to consumers in Uthiru area and its environs. The objectives were well covered through the investigation of modes of storage of the milk by the milk vendors and the screening of milk samples collected as representatives for antibiotic residue sensitivity. The goal of the above investigation was to sensitize and create awareness to the members of the public and to the government authorities on the presence of antibiotic residues in milk. This was supposed to be an indicator to the members of the public to be cautious on the milk vendors from which to purchase milk for their daily consumption as milk bought from licensed milk vendors was safe compared to milk bought from unlicensed milk vendors. The responsible government authorities dealing with issues pertaining to public health should also impose strict laws and regulations to control and prevent milk adulteration as well as make follow ups to arrest the unlicensed milk vendors. These measures if seriously taken into consideration by both the consumers and the government authorities, they will help in curbing the trend.

REFERENCES

1.Aboge G.O. 92002): Antimicrobial residues detected in marketed milk in urban and rural areas in Kenya. Masterôs thesis, University of Nairobi.68pp

2.**Nina Bilandzic, Bozikasolomun, Kolanovic, Ivanaveranina, ZoricaJurkovic:** Concentrations of Veterinary Drug Residues in Milk from Individual Farms in Croatia. Journal of veterinary drugs in milk 61(3) 260-267 (2011)

3.Dairy Technology in the Tropics and Subtropics - by J.C.T. van den BergPudocWagenigen 1988 ISBN 90 220 0927 0Netherlands

4. Milk and Milk Products - Technology, chemistry and microbiologyVol. 1. !st Ed. 1994

byAlan H. Varman& Jane P. SutherlandChapman & Hall, London.

5.Standard methods for the Examination of dairy Product - 16th Ed. 1992 by: Robert T. Marshall (Ed), APHA, ISBN 0-87553-210-1

6.Modern Food Microbiology-By James Jay ï 5th edition

7.Milk hygiene: hygiene in milk production, processing and distribution ï by Abdussalam, M. 1962

http://www.milk.hygiene/antimicrobial .residues.html.Revised2012

Milk,Research article,16(suppl-A)S31-S35,2010.

8. Andrej Kirbis (2007): Microbiological Screening Method for Detection of Aminoglycosides,ß-Lactams, Macrolides, Tetracycline and Quinolones in Meat Samples, Original Research Paper,Slov Vet Res 2007; 44 (1/2): 11-8

9. Anon (1998): Milk and milk products ï Guidance for standardized description of microbial inhibitors tests.

10. APVMA (2000): Definition of Residues for Purpose of Setting an MRI. Residue guideline No.6.

11. Cogan, T. M. (1972): Susceptibility of Cheese and Yoghurt Starter Bacteria to AntibioticsApp.Micro. 23:960-965.

12. HENRY V. Atherton,J.ANewlander (2003): CHEMISTRY AND TESTING OF DAIRY PRODUCTS, Prahald Street, 24 Ansari Road, Daryagand, New Delhi-110002 India.

13. J.C.T. Van den Berg (1998): strategy For Dairy Development in the Tropics and Subtropics, Wageningen: PUDOC-111.

14. Kanwar Pal Singh Sangwan(2008): Technology of Dairy Plants Operation, agro-bios, India, Jodhpur.

15.Mitchell, J.M.M.W.Griffiths, S.A.McEwen, W.B. McNab, and A.J.Yee.
(1998). Antimicrobial drug residues in milk and meat: causes, concerns, prevalence, regulations, tests, and test performance. J. Food Prot. 61:742-756.