

**RESIDUAL ANTIBIOTICS IN MARKETABLE MILK IN  
KHARTOUM STATE**

**By**

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# **DITICATION**

**To my parents,**

**My brothers & sisters**

## **PREFACE**

This study has been done in the Department of Preventive Medicine and Public

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## ABSTRACT

Milk spoilage remains a problem in many countries especially those in the tropics with absence of preservation practices such as pasteurization. This has tempted milk dealers to use antimicrobial substances to combat the problem of spoilage with consequent health hazards. Khartoum State may not be an exception as field observations suggested the use of antibiotics to preserve milk under the high ambient temperature of the day. Therefore the current study has been carried out in Khartoum State to investigate this possibility through culturing a strain of *Bacillus subtilis* (strain ATCC 6633) of no previously reported exposure to antibiotics in Nutrient Agar media. Wells were punched in the Nutrient Agar. Then, these wells were filled with individual milk samples. Two hundred and sixty milk samples were collected randomly from three provinces of Khartoum State. These samples were collected from two different sources, the first source was collected from 41 farms, the second source was collected from market milk and were collected both from the morning and the evening milking.

The result indicated that antimicrobial substances, possibly antibiotics, are widely used to preserve milk sold in Khartoum State. Percentages of 27.1%, 21.2% and 25% of the total milk samples collected from Khartoum, Khartoum North and Omdurman were respectively positive for antimicrobial residues.

Further, higher percentage of samples collected from Khartoum markets was positive (30.9%) compared with those collected directly from producer farms (24.3%). Also higher percentage of samples collected in the morning (38.9%) compared with those collected in the evening (16.8%) suggesting an attempt to protect milk from the higher possibility of spoilage due to the high day temperature.

The study has therefore recommended measures to combat the practice of adding antimicrobial substances to milk through random sampling and legislating regulations and rules, including penalties, to control the practice. Also milk producers should be encouraged to form cooperatives that should be provided with pasteurization, bottling and distribution facilities. In addition, door to door dispersion of milk should only be restricted to pasteurized and bottled milk.

## ملخص الأطروحة

يشكل فساد اللبن مشكلة في كثير من الأقطار خاصة في المناطق الحارة مع غياب استخدام طرق الحفظ كالبيسترة، هذه المشكلة دعت الكثير من متداولي اللبن لاستخدام مواد مثبتة للجراثيم، ذلك للتغلب على مشكلة فساد اللبن مما يتبع ذلك مشاكل صحية لمستهلكي هذه الألبان. ولاية الخرطوم ليست بمستثنية عن ما تشير إليه الملاحظات الحقلية من استخدام المضادات الحيوية لحفظ اللبن تحت درجة الحرارة العالية السائدة خلال اليوم. لهذا فإن هذه الدراسة قد هدفت إلى تفصي هذا الاحتمال في ولاية الخرطوم وذلك من خلال تزييع عترة من البكتريا العسوية الساتليزية 6633 التي لم يسجل تعرضها لمضادات حيوية من قبل في وسط آجار غذائي . ثم صنعت حفر على سطح الآجار الغذائي، ثم ملئ هذه الحفر بعينات اللبن الفردية والتي كانت في مجملها 260 عينة لبن جمعت عشوائياً من ولاية الخرطوم، هذه العينات جمعت من مصدرين مختلفين، المصدر الأول كان من 41 مزرعة والمصدر الثاني جمع من أسواق اللبن صباحاً ومساءً. أوضحت النتائج التي تم التحصل عليها في البحث أن مواد مثبتة للجراثيم، وربما مضادات حيوية يسود استعمالها لحفظ اللبن الذي يباع للمستهلكين في ولاية الخرطوم إذ أن النسب تتراوح من 27.1% 21.2% 25% من مجمل عينات اللبن التي جمعت من أسواق الألبان من الخرطوم ، الخرطوم بحري وأمدردمان كانت موجبة على التوالي لبقايا هذه المواد المثبتة للجراثيم إضافة إلى ذلك فهناك نسب مئوية عالية من العينات التي جمعت من أسواق الألبان (30.9%) مقارنة بـ (24.3%) من عينات اللبن التي جمعت مباشرة من المزارع المنتجة. كما أن هناك نسبة عالية تم تسجيلها في عينات اللبن التي جمعت في الصباح (38.9%) مقارنة مع تلك التي جمعت مساء (16.8%) مما يشير إلى محاولة لحماية اللبن من الاحتمال الكبير للتلف نتيجة لدرجة الحرارة العالية خلال اليوم.

خلصت الدراسة إلى توصيات تهدف إلى التغلب على مشكلة إضافة المواد المثبتة للجراثيم وذلك من خلال أخذ عينات عشوائية ووضع قوانين وأسس وضوابط بما في ذلك عقوبات للحد من هذه الممارسة كما أوصى هذا البحث أيضاً لتشجيع منتجي الألبان لتكوين تعاونيات وأن تمت هذه التعاونيات بإمكانيات البيسترة والتعبئة والتوزيع. إضافة إلى ذلك فقد أوصت الدراسة إلى أن يكون توزيع اللبن إلى المنازل حصراً على اللبن المبستر والمعبأ منه.

## INTRODUCTION

Milk has played a major contribution to the human nutrition in many different countries across the world. It is the neonatal food of animals and man, and provides practically all the ingredients necessary to promote and maintain life.

Cow's milk represents about 90.8% of the world milk production with buffalo, sheep, and goats producing 6%, 1.7% and 1.5% respectively (Harding & Ditton, 1995).

Antibiotics are applied as one of the most important chemical agents in the dairy farms as therapeutics, controlling infections, and as growth promoter.

Some countries report that more than 50% of their total output of antimicrobial compounds is used in agriculture (WHO, 1997).

These advantages of antibiotics usage have been associated with significant contamination from a wide variety of antibiotics residues introduced via treatment of the cow; thus rendering milk and meat unfit for human consumption, and it should be protected against direct or indirect contact with any source of contamination.

People all over the world's major dairy producing countries are concerned with the quality and safety of milk. These concerns are generated by pressure from consumers, processors, and the international market. Hence, consumers are increasingly demanding high quality foods that are

wholesome, nutritious, and safe. Processors are aware that antibiotic residues may result in economical and industrial problems in manufacturing produced milk product. As the international trade in dairy products increases, governments need to know the quality and safety of imported products that meets or exceeds their internal requirements.

Due to the consumers demand worldwide for improved food safety, the OIE is working with other relevant organizations in reducing food-borne risks to human health including hazards that involve chemical residues in food with the potential harmful health effect in humans (OIE, 2005).

In 1969 the US Food and drug administration issued a warning that the use of antibiotics in food-producing animals should be regulated more closely owing to the potential health hazard from residues in milk, meat, and eggs (WHO,1970).

However, in many countries especially those in the tropics such as Sudan, milk spoilage remains a problem in absence of preservation practices such as pasteurization. This has tempted milk dealers to use antimicrobial substances to combat the problem of spoilage with the consequent health hazards. Sudan may not be an exception as field observations suggested the use of antibiotics to preserve milk under the high ambient temperate of the day. Therefore the current study has been carried out to investigate this possibility through culturing a strain of *Bacillus subtilis* of no previously

reported exposure to antibiotics in nutrient agar media.

Objective of the study:

As based on the forgoing introduction the aim of this research is:-

- 1- To detect the presence of antibiotics residues in raw milk in Khartoum State market and dairy farms in order ascertain whether it is safe for human consumption or not .
- 2- The effect of temperature on the frequency of antibiotics in milk.

# **CHAPTER ONE**

## **LITERATURE REVIEW**

### **1.1 Definitions**

#### **1.1.1 Residues**

Residues are biologically active chemicals that deposited in animal tissues and may be excreted in milk resulting from the uses of chemical products in agricultural and livestock production to treat and protect against diseases, infestations and resulting production losses, and to improve productivity. Residues of these chemicals may remain in meat and milk and considered as food contaminants (FAO, 1993).

WHO (1970) reported that residues in animal products include antibiotics, pesticides, radionuclides, mycotoxins, plants toxins, and other chemical agents.

#### **1.1.2 Maximum residues limit (MRLs):**

MRLs is the level of the contaminant which should not be exceeded (Harding and Ditton, 1995).

FAO/WHO (2004) reported that MRLs is the maximum concentration of residues resulting from the use of a veterinary drug (expressed in mg/kg) that is accepted in or on food and considered to be without toxicological hazard for human health.

### **1.1.3 Marker residues**

Marker residues are the compound that used to monitor the depletion of total residues in a food-animal tissues and to determine the target tissue FAO/WHO (2004).

### **1.1.4 Acceptable daily intake (ADI):**

FAO/WHO (2004) reported that (ADI) is an estimate of the amount of substance in food or drinking-water, expressed on a body-weight basis, that can be ingested daily over a lifetime without appreciable risk ,and listed in units of mg per kg of body weight .

ADI is an ultra-safe maximum limit in that it sets the standard against which the chemical can be consumed for an entire lifetime without risk to the health of the consumer (Harding and Ditton, 1995).

### **1.1.5 Withholding time**

Withholding time is the length of time during which the product continues to be excreted in the milk after the last day of administration (WHO, 1970).

### **1.1.6 Antibiotics**

The word "*Antibiotic*" is derived from the term antibiosis, which literally mean "against life" (anti-against, bios life) (Robbers and Tyler, 1996).

The formal concept that is most widely accepted by scientific specialists defines antibiotic as a chemical substance, produced by a microorganism, that has the capacity, in low concentration, to inhibit or kill, selectively,

other microorganism (Robbers and Tyler,1996). Essentially all definitions limit antibiotics to compounds that exert their action in low concentration. This definition excludes compound such as ethanol that are active primary by physical action on the microorganisms (antiseptics) (Robbers and Tyler, 1996)

In (1942) Waksman proposed the widely cited definition that “ an antibiotic is a substance produced by microorganisms ,which has the capacity of inhibiting the growth and even of destroying other microorganisms.” (Delgado and Remers, 1998)

With the advances made by medicinal chemists to modify naturally occurring antibiotics and to prepare synthetic analogues, it has become necessary to permit the inclusion of semisynthetic and synthetic derivatives in the definition. Therefore, a substance is classified as an antibiotic if the following conditions are met ( Delgado and Remers, 1998):-

- 1- It is a product of metabolism (although it may be duplicated or even have been anticipated by chemical synthesis).
- 2- It is a synthetic product produced as structural analogue of a naturally occurring antibiotic.
- 3- It antagonizes the growth or survival of one or more species of microorganism.
- 4- It is effective in low concentrations.

### **1.1.6.1 Historical Background of antibiotics**

The search for antibiotics began in the late 1800s, reports, some dating back 2500 years, indicated that various ancient and primitive peoples applied moldy bread and other materials to boils and wounds liable to infection. This can be considered as a folk medicine that uses antibiotic therapy (Robbers and Tyler, 1996).

As early as 500 to 600 B.C, molded curd of soybean was used in Chinese folk medicine to treat boils and carbuncles. Moldy cheese had also been employed for centuries by Chinese and Ukrainian peasants to treat infected wounds (Delgado and Remers, 1998).

Robbers and Tyler (1996) stated that, during 1880s attempts were made to utilize antagonism to achieve an ecologic control of human microbial flora by introducing selected non pathogenic organism.

In 1877, Louis Pasteur showed that the anthrax bacilli, were killed when grown in culture in the presence of certain bacteria (Delgado and Remers, 1998). Again, in 1887, Rudolf Emmerich showed that the intestinal infection cholera was prevented in animals that had been previously infected with the streptococcus bacterium and then injected with the cholera bacillus.

While these scientists showed that certain bacteria could treat disease, it was not until a year later, in 1888, that the German scientist E. de

Freudenreich isolated an actual product from a bacterium that had antibacterial properties. Experimental results showed that pyocyanase, the crude mixture of metabolites extracted from *Pseudomonas aeruginosa*, first natural antibiotic discovered and could not be developed into an effective drug but became available around the turn of the century and could be considered the first commercial antibiotic (Robbers and Tyler, 1996).

In the early 1920s, the British scientist Alexander Fleming reported that a product in human tears could lyse bacterial cells. Fleming's finding, which he called lysozyme, was the first example of an antibacterial agent found in humans. Like pyocyanase, lysozyme would also prove to be a dead end in the search for an efficacious antibiotic, since it typically destroyed nonpathogenic bacterial cells. However, earlier and in 1896, the French medical student Ernest Duchesne has originally discovered the antibiotic properties of *Penicillium*, but failed to report a connection between the fungus and a substance that had antibacterial properties, and *Penicillium* was forgotten in the scientific community until Fleming's rediscovery ([www.helios.bto.ed.uk/bto/microbes.penicillin.htm](http://www.helios.bto.ed.uk/bto/microbes.penicillin.htm)).

Alexander Fleming in 1929, noted that a fungal colony of *penicillium nonatum* had grown as a contaminant on an agar plate streaked with the bacterium *Staphylococcus aureus*, and that the bacterial colonies around

the fungus were lysed (Robbers and Tyler, 1996).

Fleming has produced the antibiotics crude preparations of this substance, from culture filtrates, to control eye infections ([www.helios.bto.ed.uk/bto/microbes.peni\\_cillin.htm](http://www.helios.bto.ed.uk/bto/microbes.peni_cillin.htm)).

Later D.C Hodgking used x-rays crystallography to find the structural layouts of atoms and the overall molecular shape of over 100 molecules including: penicillin. Dorothy's discovery of the molecular layout of penicillin helped to lead scientists to develop other antibiotics ([www.helios.bto.ed.uk/bto/microbes.peni\\_cillin.htm](http://www.helios.bto.ed.uk/bto/microbes.peni_cillin.htm)).

Cross-continent cooperation in the early 1940s resulted in the increased scale of penicillin production and by 1946, the drug had become widespread for clinical use .

As early as 1945, Fleming warned that the misuse of penicillin could lead to selection of resistant forms of bacteria but, Penicillin was not available orally to the public without prescription until the mid 1950s ([www.helios.bto.ed.uk/bto/microbes.peni\\_cillin.htm](http://www.helios.bto.ed.uk/bto/microbes.peni_cillin.htm)).

By 1946, one hospital reported that 14% of the strains of staphylococci isolated from sick patients were penicillin resistant. By the end of the decade, the same hospital reported that resistance had been conferred to 59% of the strains of staph studied.

([www.helios.bto.ed.uk/bto/microbes.peni\\_cillin.htm](http://www.helios.bto.ed.uk/bto/microbes.peni_cillin.htm)).

A compilation of the microbial sources of antibiotic discovered in the United State and Japan between 1953- 1970 revealed that approximately 85% are produced by actinomycets , 11% by fungi, and 4% produced by bacteria (Robbers and Tyler, 1996).

penicillin was the first antibiotic discovered ,it is noteworthy that it has yet to be superseded as the most useful and least dangerous of all antibiotics, however,during The period of the Second World War (1939-1945) two other British scientists, Florey and Chain, has managed to produce the active ingredient of this antibiotic on an industrial scale for widespread use .Accordingly all three scientists shared the Nobel Prize for this work ([www.helios.bto.ed.uk/bto/microbes.peni\\_cillin.htm](http://www.helios.bto.ed.uk/bto/microbes.peni_cillin.htm) ) .

#### **1.1.6.2 Chemical Classification of antibiotics**

The chemistry of antibiotics is so varied that a chemical classification is of limited value. However ,some similarities can be found, including that some antibiotics may be the products of similar mechanisms and that these structurally similar products may exert their activities in a similar manner( Delgado & Remers, 1998).

Antibiotics that contain the  $\beta$ -Lactam (A four-membered cyclic amide) ring structure which include penicillins (penicillin G or benzyl penicillin, penicillin V or phenoxymethyl penicillin) and cephalosporins as well as the modifications of both of them. Cephalosporins is the product of

cephalosporium spp. that possess a similar mode of action as it binds to protein situated at the bacterial cell wall. (Delgado and Remers, 1998).

Antibiotics also include tetracyclines which are a group of drugs with a common 4-ring structure, hence the name and they include their effect via inhibition of bacterial protein (Smith and Reynard, 1995).

The term "macrolide" comes from the large lactone ring that contains fourteen atoms and two sugars attached to the ring. They interfere with the translocation of mRNA on bacterial ribosomes (Smith and Reynard, 1995).

Macrolides antibiotics have three common chemical characteristics: (1) a large lactone ring (2) a ketone group (3) a glycosidically linked amino sugar (Delgado & Remers, 1998).

Antibiotics also include aminoglycosides are so named because their structure consists of amino sugars linked glycosidically, the group includes streptomycin, neomycin, gentamicin, tobramycin and paromomycin and act by inhibiting the formation of the ribosomal inhibition complex resulting in mRNA misreading (Delgado and Remers, 1998).

Delgado and Remers (1998) reported that the term "Sulfonamide" is commonly used to refer to antibacterials that are:

1- aniline-substituted sulfonamides (sulfanilamides).

2- prodrugs that produce sulfanilamides (sulfasalazine).

3- non aniline sulfonamide (mafenide). They are analogues of p. amino benzoic acid producing folic acid formulation thus inhibiting nucleic acid synthesis by bacteria

Chloramphenicol is concerned as unclassified antibiotics, and act by blocking the growth of peptide chains in bacteria (Delgado and Remers, 1998).

#### **1.1.6.3 Absorption of antibiotic**

Tetracyclines are inactivated by iron, milk products and antacids (Davidson and Plumb, 2003). Best absorption is obtained when caution is used in scheduling the administration of tetracyclines to avoid interference from heavy metal ions on absorption (Robbers and Tyler, 1996).

Erythromycins (a macrolide) which is unstable in gastric acidity when taken orally and oral form must be used as acid resistant through its administration as a stearate salt (Brander and Puch, 1982).

Gentamycin is rapidly absorbed on intramuscular administration and readily distributed into various body tissues which peak serum levels are often achieved in less than 1 hour (Robbers and Tyler, 1996).

Sulphonamides include a systemic sulfonamide (e.g. Sulphadimidine and Sulfadiazine ) which are well absorbed from intestine, and "Gut active" Sulfonamides (e.g. Sulphaquanidie) which are poorly absorbed from intestine ; (Brander and Puch, 1982).

#### **1.1.6.4 Modes and Mechanism of action of antibiotics**

Antibiotics can be categorized to number of different classification schemes which could be used to the selective toxicity of antibiotics for susceptible microorganisms as the following :--

##### **a- Inhibition of microbial cell -wall formation.**

Penicillin and Cephalosporin prevent the cross-linking of small peptide chains in peptidoglycan, the main wall polymer of gram-positive bacteria cell's (Smith and Reynarnd, 1995)..

##### **b- Inhibition of protein synthesis .**

The selective toxicity of protein synthesis inhibition result from the fact that human or host cell have 80S ribosomes which bacteria cell have 70S ribosomes (50S and 30S subunit). Antibiotics that are used are selective for 70S ribosomes (Robber and Tyler, 1996) .

Chloramphenicol and Macrolides such as Erythromycin, act by binding to 50S subunit of microbial ribosomes and disrupts translation step of protein synthesis of susceptible organisms , Chloramphenicol has low affinity for 80S (mammal's cells ) ribosomes although it is thought that to inhibit mitochondrial 70S ribosomes resulting in bone marrow suppression (Robber and Tyler, 1996).

Tetracycline and aminoglycosides bind with the 30S subunits (Robber and Tyler, 1996).

**c- Inhibition of synthesis of some essential metabolite (antimetabolites).**

Smith & Reynard (1995) reported that sulphanomides are structurally similar to Para-Aminobenzoic Acid (PABA), the substrate for the synthesis of folic acid in many bacteria ;so it considered as antimetabolites in that they are competitive analogs of the folic acid.

#### **1.1.6.5 The interaction of antibiotics:-**

**a- Synergistic** :- is said to be present when the combined effect of two or more drugs exceeds the algebraic sum of the effects produced by the drugs acting separately (Bogan and Yoxall , 1983) such an effect is overt in using  $\beta$ -Lactam and aminoglycoside combination ( $\beta$  -Lactam allows better penetration of aminoglycoside) (Robbers and Tyler, 1996).

**b- Antagonism:** - may be defined as the circumstance in which the total effort of a combination of drugs is less than the algebraic sum of the effects of the individual drug in the combination (Bogan and Yoxall, 1983).

#### **1.1.6.6 Antibiotic Spectrum**

**a- A narrow spectrum** : A narrow spectrum antibiotic is one in which the antibacterial effect is restricted to a relatively small number of organisms e.g. penicillin , which is active mainly against Gram-positive organisms ,and polymyxin B ,which is active mainly against Gram-negative organism (Brander and Puch, 1982).

**b- A broad spectrum** : It is an antibiotic which is effective against a wide variety of organisms both Gram-positive and Gram –negative bacteria ,possibly rickettsiae, the larger viruses ,and even protozoa and helminthes ;chloramphenicol ,chlortetracycline hydrochloride ,oxytetracycline HCL and ampicillin are examples (Brander and Puch, 1982).

Blood and Hendderson (1983) reported that Tetracycline is effective against richettsia , protozoa, Mycoplasma , and larger viruses .

Macrolides are effective against richettsia, protozoa, and Mycoplasma (Brander and Puch, 1982).

Smith and Reynarnd (1995) stated that Chloramphenicol is effective against numbers of organisms which may be variably resistant to other antibacterial agents.

#### **1.1.6.7 Elimination and excretion of antibiotics**

##### **a- Renal excretion**

(Bogan and Yoxall,1983) described the elimination of antibiotics which includes both biotransformation and excretion .In all species of animals, the majority of antibiotics are eliminated mainly by renal excretion .The penicillins, cephalospoins, aminoglycosides ,oxytetracycline and tylosin are excreted unchanged in the urine.

Liver is the principal organ of elimination of erythromycin and chloramphenicol.Erythromycin excreted slowly unchanged in the bile whereas chloramphenicol inactivated by metabolism ,the metabolites

being excreted in urine and bile (Bogan and Yoxall,1983).

Trimethoprim and Sulphadoxine eliminated by both biotransformation and renal excretion ,Trimethoprium is removed only by hepatic metabolism (oxidative processes followed by glucuronide conjugation) .For Sulphadoxine renal excretion (glomerular filtration, carrier-mediated tubular secretion, and passive diffusion) is the principal process of elimination (Bogan and Yoxall,1983).

#### **b- Intramammary excretion**

Drugs can also be excreted in varying but significant amount via the mammary gland in lactating animals. A knowledge of the extent of such excretion is extremely important in considering appropriate mastitis therapy, and also in considering which drugs used for systemic treatment are likely to cause significant contamination of milk and render it unfit for human consumption (Bogan and Yoxall,1983).

Brander and Puch (1982) reported that, most antibiotics diffused into the mammary gland tissues and may be excreted in the milk in detectable and effective quantities .Diffusion of Penicillin into the milk in therapeutic concentrations is achieved only by massive doses given intravenously. Injection of streptomycin parenterally as well as locally in treatment of severe mastitis results in excretion of streptomycin in the milk.

Tylosin excretion in the milk persists for 96 hour after the last treatment while

Chlorotetracycline diffused into the milk in concentrations equal to or higher than those in the blood. Combined intravenous and local treatment when recommended in acute mastitis, the excretion by the udder persists for 48 hr and the observation is made that the udder probably provides one of the main means of excretion (Brander and Puch, 1982).

Chloramphenicol is also excreted in the milk and Oxytetracycline is readily diffusible into the milk and therapeutic milk levels can be achieved by single intravenous dose (Brander and Puch, 1982).

Withdrawal time of macrolides is from 7-28 days and milk discard time is 36 hours to 4 days (Abdrahaman, 2002).

#### **1.1.6.8 The uses of antibiotic in livestock**

The uses of antibiotics in livestock include for treatment, disease prevention and growth promotion (WHO, 1997).

##### **a- Therapeutic uses**

Antibiotics have been used in the therapy of animal disease since 1950 (Brander and Puch, 1982) and in some countries report that 50 percent of their total output of antimicrobial compounds is used in agriculture (WHO, 1997).

Antibiotics are used as empirical treatment of suspected infections or for the treatment of a known pathogen (Smith and Reynard, 1995). In livestock, they are predominantly used in the treatment of acute and peracute cases of mastitis. Intramammary infusion is commonly used in

cattle; approximately 10-15 million antibiotic intramammary tubes are used annually in the UK. (Bogan and Yoxall, 1983).

There are four members of the tetracyclines group commonly used in large animal practice. These are tetracycline, oxytetracycline, chlorotetracycline and rolitetracycline which are also active against disease associated with babesia, and anaplasma (Blood and Henderson, 1983)

The Macrolides that are commonly used in livestock include Erythromycin, carbamycin, spiramycin, oleandomycin and tylosin (Brander & Puch, 1982). Among these Erythromycin and spiramycin are mainly used to treat animals which are allergic to penicillins, or to treat infections by penicillin resistant strains (www.eruresidue.nl/ER\_IV/Key%20lectures/schepdael%20122-130.pdf).

Erythromycin is active against most gram positive, some gram negative, and protozoa as well as many rickettsiae. It can be used against penicillin resistant staphylococci as intramammary formulations. It is excreted mainly via the kidney (Brander and Puch, 1982). Erythromycin may be used in oral dosage and for topical administration. It can be used for treatment of upper respiratory and soft tissues infections (Delgado and Remers, 1998)

Tylosin is used in the treatment and prevention of chronic respiratory disease (CRD) in chickens, foot rot, pneumonia, and metritis in cattle. On

the other hand, Streptomycin is the most effective against Mycobacteria, Brucellosis, Tularemia and Yersinia infection (Delgado and Remers,1998), while Neomycin is mostly useful in the treatment of Gastrointestinal and dermatologic infections (Delgado and Remers, 1998) .

Gentamycin has a high degree of activity against systemic and tract infections caused by *Pseudomona aeruginosa* and other gram-negative Bacilli and skin infections for which a topical ointment may be used (Delgado and Remers,1998).

Chloramphenicol was active against a number of organisms which may be variably resistant to other antibacterial agent .It is one of the few antibiotics to penetrate into Central nervous System fluid and ocular fluid in significant amounts (Blood and Henderson, 1983).

Chloramphenicol is recommended specifically for the treatment of infections caused by strains of gram-positive and gram-negative bacteria that have developed resistance to penicillin G and ampicillin ,such as *Salmonella typhi* ,*H.influenzae*, *Streptococcus pneumoniae* (Delgado and Remers, 1998).

#### **b- Growth promotion**

The use of antibiotics such as monensin , virginiamycin, flavophospholipol and spiramycin at growth promoting levels influences the metabolism of the enteric flora producing a nutrient-sparing effect

which makes more of the dietary feed nutrients available for absorption by the host animal (Bogan and Yoxall, 1983).

### **c- Milk preservatives**

Unfortunately El Khawli (1999) stated that antibiotics may also be used by some producer as milk preservative substances. The substances which have been used in as milk preservative include boric acid, formaldehyde, hydrogen peroxide, hydrochloride and antibiotics, and if milk is of dubious microbiological quality there might be a temptation to use an antimicrobial agent to act as a preservative (Harding and Ditton, 1995).

In some countries the use of antibiotics in milk for improving keeping qualities has been suggested (Saratwell, 1977).

#### **1.1.6.9 Problems caused by Antibiotics & Antibiotic residues**

1. Short term allergic reactions amongst sensitive persons has been reported (Varnam and Sutherland, 1994).

These include skin, mucous rashes, and anaphylaxis. Estimates place the prevalence of hypersensitivity to Penicillin G throughout the world at between 1% and 10% of the population .In the United State and other industrial countries ,it is nearer to the higher figure ,ranking Penicillin as the most common cause of drug-induced allergy . (Delgado and Remers, 1998).

2. Alteration in the ecologic balance of the intestinal flora lead to gastrointestinal disturbances which is greatest problem with the use of

broad-spectrum antibiotics (Robbers and Tyler, 1996)

Alteration of gastrointestinal flora can cause overgrowth of *Clostridium difficile* which produce toxin that cause diarrhea. Overgrowth of candida can also happen in mouth ,vagina and gastrointestinal tract( Samuel, 1996).

3. There is also possible carcinogenicity if exposure to antibiotic is prolonged (Varnam and Sutherland,1994). Chloramphenicol has been evaluated several times by an internationally recognized scientific committee, the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which concluded that the compound is genotoxic, which means it could cause genetic damages and possibly lead to cancer (FAO, 2002).

4. Hematologic reactions can range from the life-threatening blood dyscrasia that occur in 1 in 60,000 individuals who receive chloramphenicol to hemolytic anemia due to sulfonamides in individuals who lack the enzyme glucose-6phosphate dehydrogenase. Depression of blood platelet activity has also occurred with many agents (Samuel,1996 ). Chloramphenicolc can also cause aplastic anemia (FAO, 2002) .

5. Regular ingestion of antibiotics may encourage the development of antibiotic-resistant strains of organisms in man and lead to failure to respond to therapeutic doses of antibiotic at a later stage (Harding and Ditton, 1995).

Following the introduction of fluoroquinolones in food-producing animals, the emergence of Salmonella serotypes with reduced susceptibility in human has become a cause for particular concern (WHO, 1997).

Bacteria and genes including antibiotics-resistance genes, can pass between human and other ecosystem (WHO, 1997).

Ebrahimi and Falian (2005) had found that *Staph aureus* and 77.77% of E.coli were resistant to one or more antibiotics. As exemplified by that Sweih, *et.al* (2005) had found that 0.7% and 1.7% of Streptococcus agalactiae were resistant to erythromycin.

**6.** Toxicity to patient undergoing treatment may be include :-

**a-** Damage to liver can occur in case of antibiotics that metabolized in liver e.g. isoniazid, which used to treat tuberculosis (Samuel, 1996).

**b-** Damage to kidney and auditory or vestibular apparatus can follow the use of aminoglycosides if the agent is not closely monitored (Samuel, 1996).

**7.** Failure to achieve the finest grade in the test of bacterial quality of milk due to the presence of antibiotics residues in milk (Harding and Ditton, 1995).

Inhibition of starter bacteria in the dairy industry by antibiotic in milk (Bogan and Yoxall, 1983).

8. Antibiotic residues has recently been implicated international trade interventions leading to loss of revenue for some exporting countries (FAO/WHO, 2004).

9. Chloramphenicol is very toxic to some humans in that even very small amount may cause death in 1 in 10,000 people exposed and because of this, its use is forbidden in animals that will be used for human food (Davidson and Plumb, 2003).

#### **1.1.6.10 Antibiotic Residues Test Methods**

There are different methods used for detection of antimicrobial agent .They depend on the type of residues and type of food to be analyzed (review by Patal and Bond, 1996).

##### **a- Antimicrobial inhibitor test**

These methods involve growth inhibition of specific test organisms such as *Bacillus stearothermophilus* in antibiotic is present in milk. The organism is cultured in small well in the presence of nutrients and an indicator dye which is changed from blue to yellow colour due to growth of the organism .If an antibiotic is present the culture is killed and the dye remains blue .The tests tend to be lengthy (3 hours) (Harding and Ditton, 1995). In addition this method was found not to identify the compound concerned (Patal and Bond, 1996).

##### **b. Enzyme linked immunosorbant assay (ELISA)**

Patal *and* Bond. (1996) stated that (ELISA) was a rapid ,highly specific,

and easy test that could provide better identification for antibiotic residues than microbial inhibition test but cross reaction with metabolites and compound with similar structure prevented precise identification. So confirmation test with mass spectroscopy or high performance liquid chromatography (HPLC) were needed.

Jackman (1994) detected Chlorotetracycline and oxytetracycline and tetracycline residues in milk using antitetracycline polyclonal sheep antibodies and a tetracycline-horse radish peroxidase conjugate.

### **c. Chemicals methods**

These methods include high performance liquid chromatography (HPLC) , mass spectroscopy and thin layer chromatography (TLC) .They can differentiate between different antibiotics (Patal and Bond, 1996).

### **d. Electrophoresis**

High voltage electrophoresis bioautography was used for identification of sulphamethazine and penicillin in milk (Loit and Vaughan, 1985).The antibiotics are extracted using acteonitrite and then electrophoresis is carried out using agar medium seeded with the microorganism.

## **1.2 Milk**

### **1.2.1 Milk definition**

Milk is a complex biological fluid that is produced by the mammary gland; the composition and physical characteristics of which vary from

species to species ,reflecting the dietary needs of the young animals (Varnam and Sutherland, 1994).

Milk is defined in the Milk Ordinance and Code recommended by the United State Public Health Service as “the lacteal secretion, practically free from colostrums, obtained by the complete milking of one or more healthy cows ,which contains not less than 8.25% of milk-solid-non fat and not less than 3.25% of milk fat” (Webb and Johnson,1987).

Cow’s milk has been defined as an amphoteric liquid designed by nature for feeding the calf from birth until it is weaned .It is opalescent, white or creamy and possesses a sweetish,palatable taste (Harvey and Hill,1967).

### **1.2.2 Milk composition.**

Milk principle constituent is water, and it also contains, fats ,sugars, salts nitrogenous compounds, enzymes and vitamins (Harvey and Hill, 1967).

Milk comprises 87.4% water and 12.6 % solids (Harvey and Hill, 1995).Milk contain less than 8.25 % of milk-solid-non fat and not less than 3.25% of milk fat (Webb and Johnson, 1987).

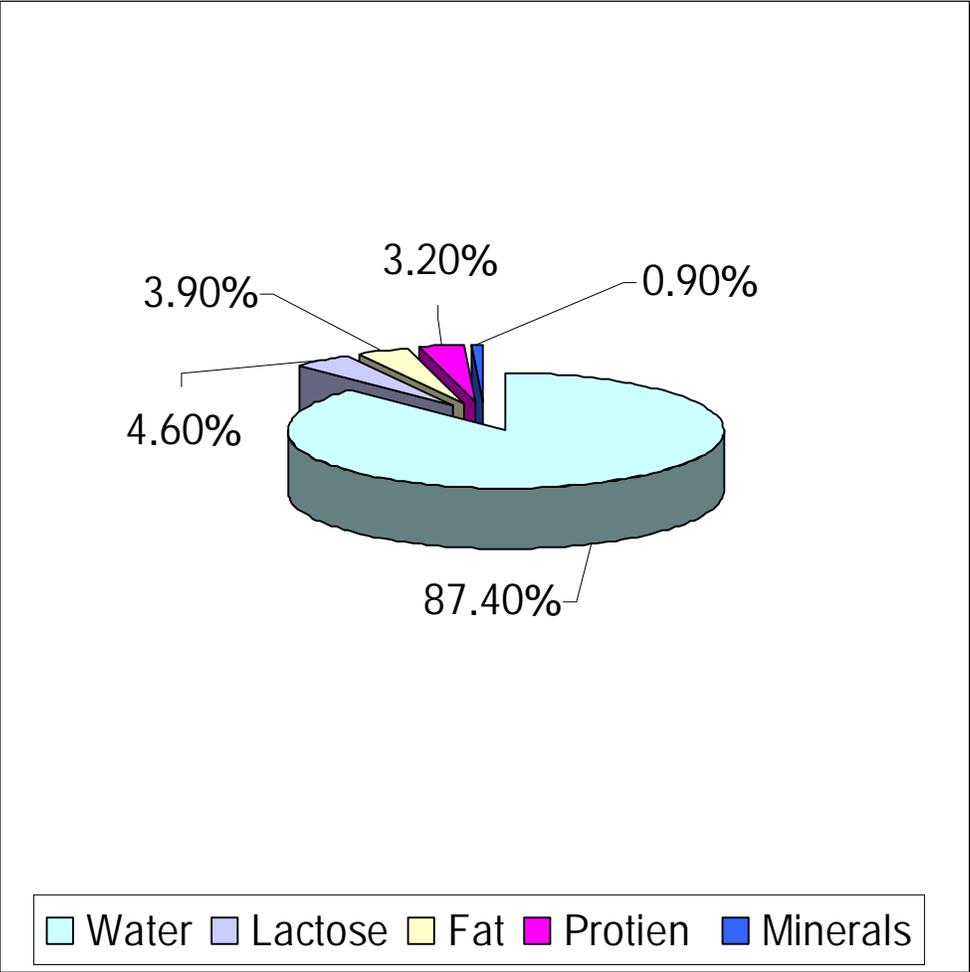
The milk composition from different mammals varies enormously .In order to provide high energy to their offspring , whales and porpoises produce milk with a fat content in excess of 40% ,elephants produce milk of about 20% fat and reindeer milk of over 15% fat while cow like humans produce milk of lower fat content-close to 4% (Harvey and Hill, 1967).

Camel milk with 2.9 % - 5.38 % fat, while the water content of camel milk fluctuates from 84% to 90% (FAO, 1982).

The following table (1.1) shows the typical composition of cow's , sheep's, and goat's milk (Harding and Ditton, 1995):-

	Fat%	Protein%	Lactose%	Ash	Total solid%
Cow	3.9	3.2	4.6	0.72	12.6
Sheep	7.1	5.7	4.6	0.93	18.2
Goat	3.6	3.3	4.6	0.80	12.1

Figure (1.2) shows cow's milk compositions.



**Figure (1.2) cow's milk composition.**

### **1.2.3 Biosynthesis of milk**

Milk is the product of the mammary gland and originated in the secretory tissue (Varnam and Sutherland, 1994). The alveolus may be considered as the smallest complete unit of milk production and is approximately spherical in shape with a central storage lumen surrounded by a single layer of secretory epithelial cells that are oriented adjacent to the lumen, while the basal end is separated by a basement membrane from blood and lymph (Varnam and Sutherland, 1994). Metabolites enter the secretory cell from the blood stream via the basement membrane and are utilized in milk synthesis by the rough endoplasmic reticulum, which empty the product into the Golgi apparatus which transport the aqueous phase milk components into the lumen (Varnam and Sutherland, 1994). The lipids phase is also synthesized in the endoplasmic reticulum and collects as droplets on the cytoplasmic side of the membrane (Varnam and Sutherland, 1994). The droplet pass into the lumen by the pinocytosis, and in this process the surface acquires a coating of plasma membrane (Varnam and Sutherland, 1994).

Synthesis is completed in the alveolar lumen where lactose is synthesized and proteins glycosylated and phosphorylated while casein molecules appear both in the Golgi vesicles and in the lumen (Varnam and Sutherland, 1994).

The secretory epithelial cells are surrounded by a layer of myoepithelial cells and blood capillaries, and when the circulating pituitary hormone, oxytocin is bound to the myoepithelial cells, the alveolus contracts and the milk is expelled from the lumen into the duct system (Varnam and Sutherland, 1994).

#### **1.2.4 Temperature**

It is generally accepted that bacterial growth is sufficiently restricted at temperatures below 7 c° but this becomes rapid at temperatures above 10 c° (Mark and David, 1998). Nada (2000) reported that the temperature of market milk collected from Khartoum State was between 31-39 c° .

#### **1.2.5 Basic principles of milk production applicable in developing countries:-**

FAO (1970) reported three different applicable systems in developing countries:-

- a- A system based on large dairy farms owned and operated by the government or some other public authority .The farms have access to milk .Processing plants and they can organize animal husbandry.
- b- A system based on large numbers of small privately owned dairy herds operating without a communal organization for collection, processing and distributing the milk. The farmers sell their own raw milk either directly to customers or through street venders or milkmen .

c- A system practiced by nomadic tribes who keep flocks of milk animals.

The second and third systems present a great number of difficult organizational and hygienic problems. Since the countries concerned cannot afford to disrupt the life and economy of small dairy farms and nomadic communities, improvements must be introduced by means of an evolutionary rather than a revolutionary process (FAO, 1970).

In the warm countries, the bulk processing of milk by modern methods of pasteurization and sterilization is still limited to a few urban areas. Much of the milk passes from the small producer to the home directly or through an intermediary dealer. Under such conditions many traditional practices, such as the direct milking of the animal into the consumer's clean receptacles, should continue, as should the boiling of raw milk in the home (FAO, 1970).

The extent of the antibiotics residues problem in many of the developing countries is unknown, and this information must be obtained before remedial measures can be undertaken. However, the problem in developing countries is apparently becoming more acute (FAO, 1970).

FAO/WHO (2004) reported that there is an attempt toward, increased consumption of dairy products and meat in developing countries, it is estimated that the consumption in developing countries of livestock products could rise by as much as 44% in the year 2030. To meet this

demand, an increasing share of livestock production is by commercial enterprises and intensive or semi-intensive peri-urban production systems rather than traditional mixed farming systems, with a consequent increase in use of compounds such as antibiotics (FAO, 1970).

In order to create the basis of the progressive development of individual private dairy and because milk should be protected against direct or indirect contact with any source of external contamination, a system of milk collecting centers should be established, (FAO, 1970).

#### **1.2.6 Milk production in Khartoum State**

The main source of milk in Khartoum state is from cows which are kept in traditional fences either in separate farms, in fences of different sizes, or grouped at periphery of the main domestic areas .In 1997 animal census there were 15160 cows in the state; 44.4% of them were in Khartoum North, 35.25 % in Khartoum and about 20% in Omdurman ( Suliman, 2005) .

The total amount of raw milk produced in the State as estimated by Ministry of Agriculture and Animal Resources of Khartoum in 1997 was 238,726 thousand tons of milk 50% of which was produced in Khartoum North region .According to the same report this amount will be doubled at 2004; some 95% of this milk is distributed as raw milk to the consumer ( Suliman, 2005) .

### **1.2.7 Adulteration**

Adulteration of milk is defined by the US Department of Health, Education and Welfare (1953) as " any milk to which water has been added or any milk which contains any unwholesome substance, or which is defined in this ordinance does not conform with its definition, shall be deemed to be adulterated" (Siegenthaler and Schulthess, 1977).

The problems of adulteration in developing countries, which have modern and well-equipped laboratories for checking and regulating product quality, is not under complete control. Therefore, in developing countries where there is lack of testing facilities and of proper food legislation the situation will be far worse (Siegenthaler and Schulthess, 1977).The methods of adulteration are :- addition of water ,extraction of butter fat in the form of creame, addition of preservatives and addition of colouring matter (Siegenthaler and Schulthess, 1977).

Because of the possible harmful effect to highly sensitive individuals who may consume low concentration of antibiotics, the Food and Drug Administration has ruled that milk containing antibiotics is an adulterated milk (Sartwell, 1977).

### **1.2.8 Causes of Antibiotic Residues in Milk**

Milk is at risk of being contaminated from a wide variety of chemical residues Figure (1.2) shows residues and contaminants introduced via treatment of the cow, feed, the milking environment and processing plant (Hardiny and Ditton, 1995).

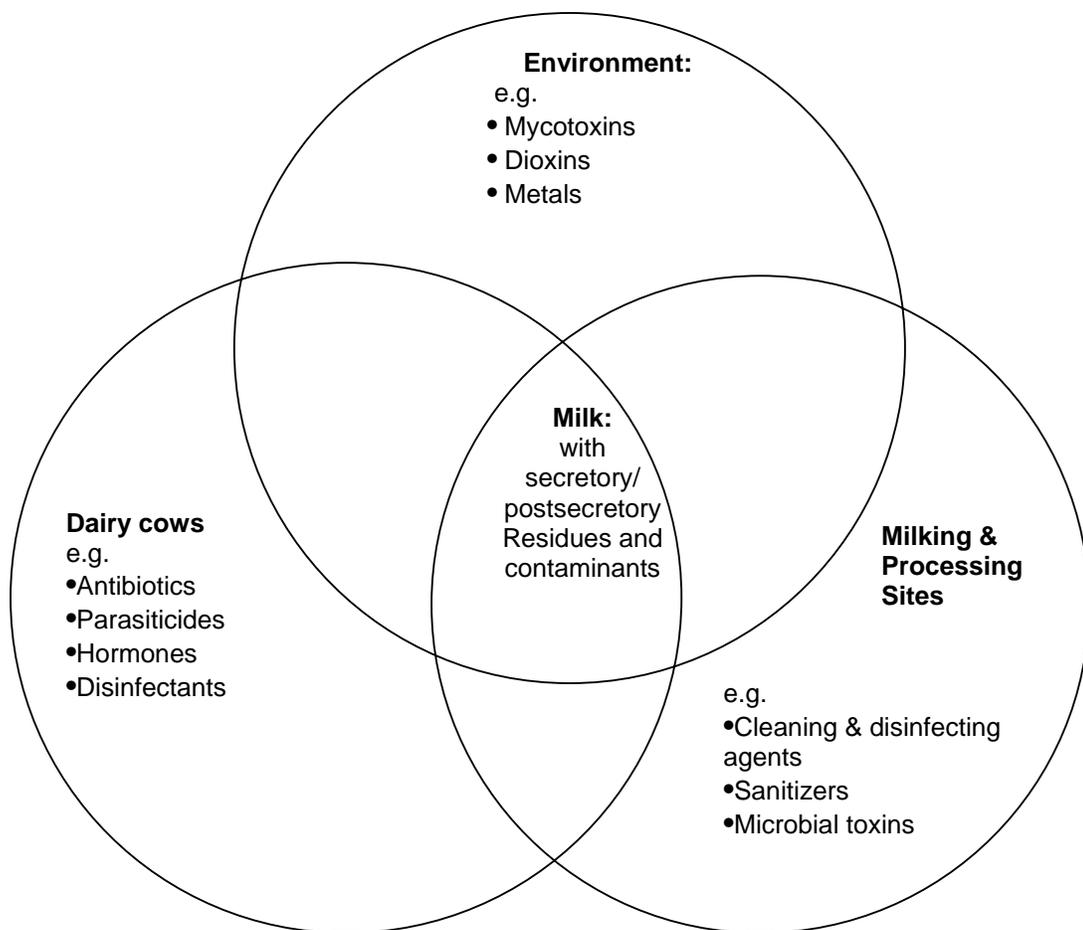
The following points were considered by WHO/FAO (2004) as possible causes of antibiotic residues :-

- 1- No quality control of the antibiotics supplied and concentrations.
- 2- Dosage rates may be incorrectly recorded.
- 3- Lack of veterinary advice regarding withdrawal periods and this may be compounded by illiteracy, reading labeling and printed instructions for drugs of limited use.
- 4- Drugs are frequently administered by unqualified farmers.
- 5- Extending usage or excessive or multiple dosage of the compound.
- 6- Treatment records are frequently poorly maintained or non-existent.
- 7- Individual animals identification and traceability is often impossible.
- 8- Treatment records are frequently poorly maintained or non-existent.

Jones (1999) stated that the improper use of drugs in the control of mastitis is the major source of residues found in milk supply, and the suspected causes include:-

- 1- Early calving or short treated quarters only.
- 2- Use of dry cow therapy to lactating cows.
- 3- Withholding milk from treated quarters only.
- 4- Contaminated milking equipment.

Intra uterine infusions with tetracycline, Dihydrostreptomycin, Benzyle penicillin, Sulphamethazines, and Streptomycin can cause detectable residues in milk for 24 – 48 hours after Intra uterine infusion (Sundlof *et al*, 1990).



**Figure (1.2) Residues and contaminants in milk and milk products (Hardiny and Ditton, 1995).**

### **1.2.9 Control of Antibiotic Residues in Milk**

It is generally accepted that milk should be free from antibiotic residues and

regular testing for antibiotics is therefore practiced ,with severe penalties being applied when positive results are obtained (Harding and Ditton, 1995)

The following points are considered as methods for controlling antibiotic residues in milk :-

#### **1. Keep antibiotics out of milk**

Dairy farmers are usually well aware of the reasons why it is necessary to keep antibiotics out of milk (Harding and Ditton, 1995):-

#### **2. Correct and clear identification of treated cows**

Cow should be either marked clearly or segregated for milking last (Castle and Watkins, 1984).It is the law in some countries that all treatments of the cow should be recorded as a permanent written record that should include identity of the cow, the person giving the antibiotic treatment ,type of treatment, dosage given, dead time (or milking at which the prescribed withholding time ends and the milk can safely be included for sale (Harding and Ditton, 1995).

### **3- Withholding time**

The length of time during which the antibiotic continues to be excreted in the milk after the last day of administration should be known by the producers (WHO, 1970).

### **4- Dry cow therapy**

All animals should be routinely treated at the time of drying off with an antibiotic which has been specifically formulated for the purpose of mastitis control. This simple routine measure does not contaminate saleable milk with antibiotic (Castle and Watkins, 1984).

### **5- Milking plant contamination**

The only safe course is to milk treated cow last when the rest of the herd has been milked or preferably to use separate bucket and cluster (Harding and Ditton, 1995).

### **6- Monitoring the incoming milk**

Test incoming milk for antibiotics before acceptance (Varnam and Sutherland, 1994).

Milk from producer-retailers will require to be checked by Food and Drug Authorities as the Milk Marketing Board's Scheme makes provision only for wholesale milk (Harvey and Hill, 1967).

### **7- Antibiotics administration**

Antibiotics should only be administered under veterinary control (Varnam and Sutherland, 1994).

## CHAPTER TWO

### MATERIALS AND METHODS

#### 2.1 Materials:-

##### 2.1.1 Test medium

**a- Nutrient Broth (Oxoid U.K)** : Provided as dehydrated form and was prepared by dissolving 25 g of nutrient broth in 1000 ml distilled water , mixed well and autoclaved at 121 C° to 15 lb/sq inch for 15 minutes, dispatched in bijou bottles and was used for original isolation and preservation of stock culture.

**b- Nutrient agar (Oxoid U.K)** : Supplied in dehydrated powder form and was prepared by dissolving 28 g of nutrient agar in 1000 ml distil water, dispatched into 90 mm Petri dishes, and was used for isolation of primary cultures. Wells had been made into the nutrient agar and were used for individual milk samples.

##### 2.1.2 Solutions

###### 2.1.2.1 Distilled water

It was obtained from Faculty of Agriculture, U. of K.

###### 2.1.2.2 Normal saline

Was prepared by dissolving 9 g of sodium chloride in 1000 ml distilled water and sterilized at 121 C° to 15 lb/sq inch for 15 minutes, and cooled.

### **2.1.3 Test organism**

*Bacillus subtilis* (strain ATCC 6633) : The organism is of no recorded prior exposure to antibiotic (Dr.Tawfig, personal communication).

Check for contaminants : dispensed media were left at room temperature for 24 hour before use or storage to check their freedom from contaminants.

### **2.2 Sterilization**

Petri dishes and flasks were sterilized by heating in hot air oven at 160 C° for one hour.

Media, solutions, micropipette's tips and containers with rubber lining (bijou and universal bottles) were sterilized in the autoclave at 121 c° equivalent to 15 pounds pressure for 15 minutes.

Inoculating wire loop was sterilized by flaming.

### **2.3 Collection of the samples**

Two hundred and sixty raw milk samples were collected randomly from three provinces of Khartoum state during the year 2005 (April, May). The samples were collected from two different sources without the knowledge of antibiotics treatment.

#### **2.3.1 Source of the samples :-**

Forty one samples were collected from Khartoum and Khartoum North farms where as 219 samples were collected from the main milk markets (Market) groceries and small venders (Vender) .

### **2.3.1.1 Market milk :-**

Milk collected from the following main milk markets in Khartoum state included :-

#### **2.3.1.1.1 Khartoum area**

Milk for the study was collected from Kalakla market, Sajana markets, Lafat Soba markets, Riad , Arkaweet and Gerafe. In total 67 market milk sample were collected , 58 of which were collected at the morning ,and 9 sample were collected from evening milking.

#### **2.3.1.1.2 Khartoum North area**

Milk was collected from the following Kuku Elsoug, Id Babikir, Al Kadaro, Shambat, Al Droshab, Kafuri ,Haj Usif (Mahlab 7). A total of 72 samples were collected from the morning milking and 20 samples were collected from evening milking.

#### **2.3.1.1.3 Omdurman area**

Milk samples were collected from El sheikh Abu Zeid, Marzoug markets, El Thawra, El hiejra . In total, 80 market milk were collected, 40 of which were collected from the morning milking where as 40 were collected from evening milking.

### **2.3.1.2 Farm samples**

Forty one milk samples were collected from Khartoum and Khartoum North farm's bulk milk and were tested to detect the antibiotic residues

without knowing the prior treatment with antibiotics .In order to draw a comparison between farms and markets samples.

#### **2.4 Samples processing**

All samples were collected in sterile disposal containers , immediately closed to avoid contamination, and placed in thermoflask to the lab. Of dept. of Preventive Medicine and Public Health Faculty of Veterinary Medicine, University of Khartoum.

#### **2.5 Methods**

The microbiological inhibition test method was used to detect antibiotic residues in the susceptible milk samples.

##### **2.5.1 Preparation of test medium:**

Colonies of *Bacillus subtilis* were transferred from Nutrient Agar plate using sterilized wire loops and inoculated into 100 ml of sterilized nutrient broth which was previously prepared in bijou bottle, and then incubated at 37 °C for overnight. Ten fold dilutions of the broth culture were prepared in normal saline.

##### **2.5.2 Preparation of culture media**

One ml from the diluted culture of *Bacillus subtilis* was added into the empty sterile Petri dish. Nutrient agar sterilized in an autoclave and was allowed to cool up to 50°-55 C°, twenty ml of this media was poured into the Petri dishes containing 1 ml of standard organisms. Then both the media and the bacterial culture was mixed and the

media was left for 10 minutes to solidify. With a sterile tip of a micropipette, wells were punched in the solidifying agar. Then, these wells were filled with individual milk samples by micropipette .

The plates were then incubated at 35 C° until the growth is apparent visible (within 18- 24 hours).

Positive result of the susceptible milk samples was indicated by the appearance of an inhibition zone of the growth of *Bacillus subtilis* around the wells, while the negative one was indicated by the absence of the inhibition zone around the wells.

## **CHAPTER THREE**

### **RESULTS**

First group of results includes milk samples which were collected randomly from two sources. The first source was from bulk milk tanks, and were conducted from 41 farms. The second source of samples were collected randomly from markets (raw milk) present in Khartoum and Khartoum North provinces.

The second groups of samples were collected at different times, and from the surveillances which were conducted in markets State of Khartoum. Samples were collected both from the morning and evening milking.

The result reveals that 30.9% of the market samples which were collected from Khartoum and Khartoum North market are positive; 32.8% and 29.1% respectively. In contrast, 24.3% of the farms samples were positive.

38% of the total market positive samples were those collected in the morning while 16.8% of the positive samples were from the samples collected from evening milking. Strikingly, 42.5% of the positive Omdurman's samples were those collected from the morning milking where as 7.5% of the positive samples were from the evening milking. In Khartoum samples, however, 36.2% of the market positive samples were those collected in the morning while, 11.1% of the positive

samples were from the evening milking. Considering the samples collected in Khartoum North markets, 40% of the positive samples were from those collected from the morning milking while 25% of the positive samples were from those collected from evening milking.

The result reveals that the samples which were collected from the morning milking are with the high percentage of antibiotic residues (38.9%) comparing with the other samples which were collected from evening milking (16.8%).

The percentage of antibiotics residues in milk samples which were collected from markets are with high percentage of antibiotic residues (30.9%) comparing with the percentages of positive samples which were collected from farms (24.3%).

Khartoum is the most area with antibiotic residues (27.1%), comparing with Khartoum North area (21.2%), and Omdurman area (25%).

Omdurman market sample which were collected from the morning milking is the most market area with high percentage of positive antibiotic residues (42.5%) comparing with the morning samples which were collected from Khartoum's and Khartoum North's market.

The following tables and figures show the percentages of these results.

**Table no. (3.1) Results of market's and farm's milk Samples for the three localities of Khartoum state using microbial inhibition test :-**

Site of sampling	Number of samples	Positive samples	percentage
Khartoum	81	22	27.1%
Khartoum North	99	21	21.2%
Omdurman	80	20	25%
<b>Total</b>	<b>260</b>	<b>63</b>	<b>24.2%</b>

**Table (3.2) Results of market's milk samples during the morning and the evening for Khartoum area using microbial inhibition test**

Khartoum	Morning Sampling	Evening sampling	Total
Samples no.	58	9	67
Positive samples	21	1	22
Percentage	36.2%	11.1%	32.8%

**Table (3.3) Results of market's milk samples during morning and evening in Khartoum North area using microbial inhibition test**

<b>Khartoum North</b>	<b>Morning Sampling</b>	<b>Evening sampling</b>	<b>Total</b>
<b>Samples no.</b>	20	52	72
<b>Positive samples</b>	8	13	21
	40%	25%	29.1%
<b>Negative</b>	12	39	51
	60%	75%	70.9%

**Table (3.4) Results of market's milk samples during morning and evening for Omdurman area using microbial inhibition test**

<b>Omdurman</b>	<b>Morning Sampling</b>	<b>Evening sampling</b>	<b>Total</b>
<b>Samples</b>	40	40	80
<b>Positive</b>	17	3	20
	42.5%	7.5%	25%
<b>Negative</b>	23	37	60
	57.5%	92.5%	75%

**Table (3.5) Results of milk samples for Khartoum's markets during the morning using microbial inhibition test**

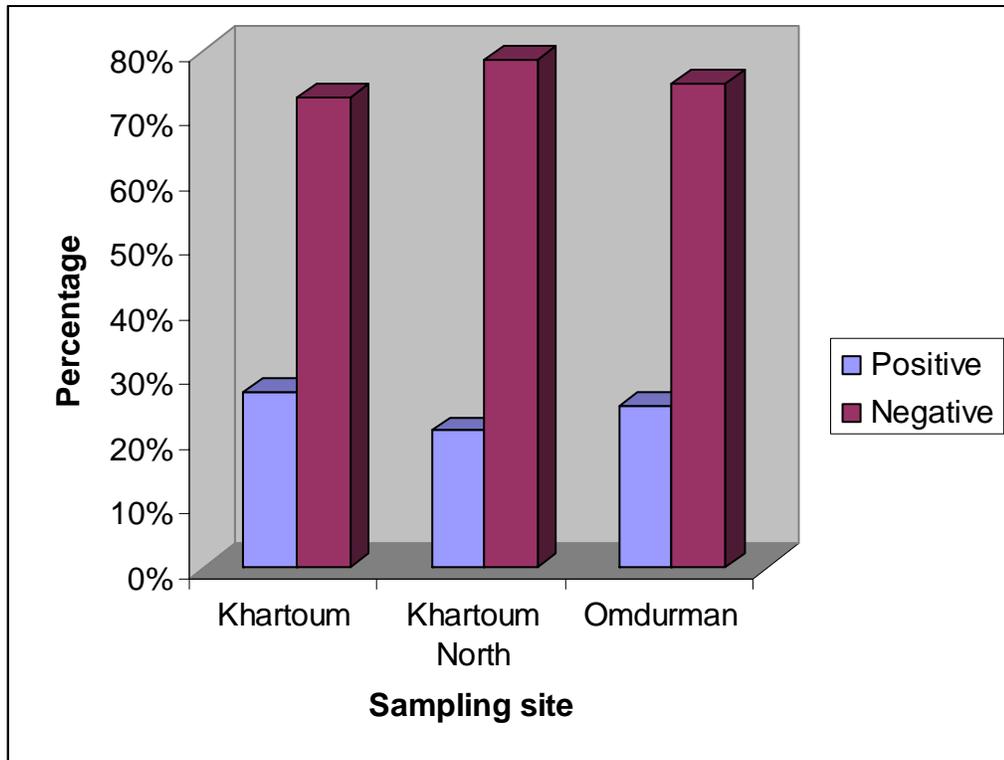
	<b>Khartoum</b>	<b>Khartoum north</b>	<b>Omdurman</b>
<b>Samples</b>	58	20	40
<b>Positive</b>	21	8	17
<b>percentage</b>	36.2%	40%	42.5%

**Table (3.6) Results of milk samples for the three Khartoum's markets localities during the evening using microbial inhibition test**

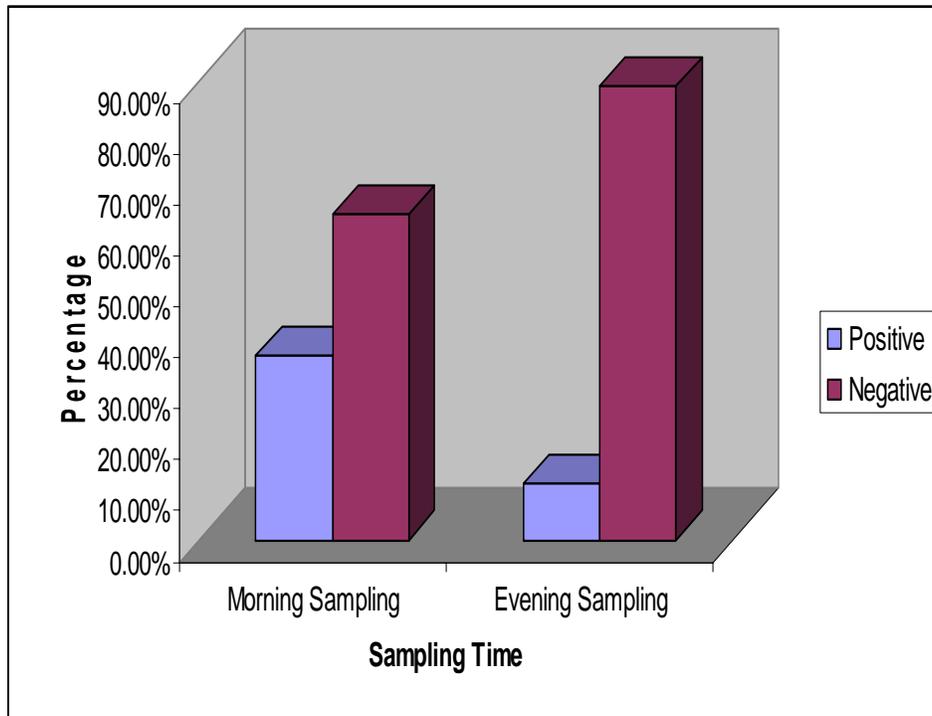
	<b>Khartoum</b>	<b>Khartoum north</b>	<b>Omdurman</b>
<b>Samples no.</b>	9	52	40
<b>Positive</b>	1	13	3
<b>percentage</b>	11.1%	25%	7.5%

**Table no. (3.7) Results of farm's and market's milk samples for Khartoum and Khartoum North localities using microbial inhibition test.**

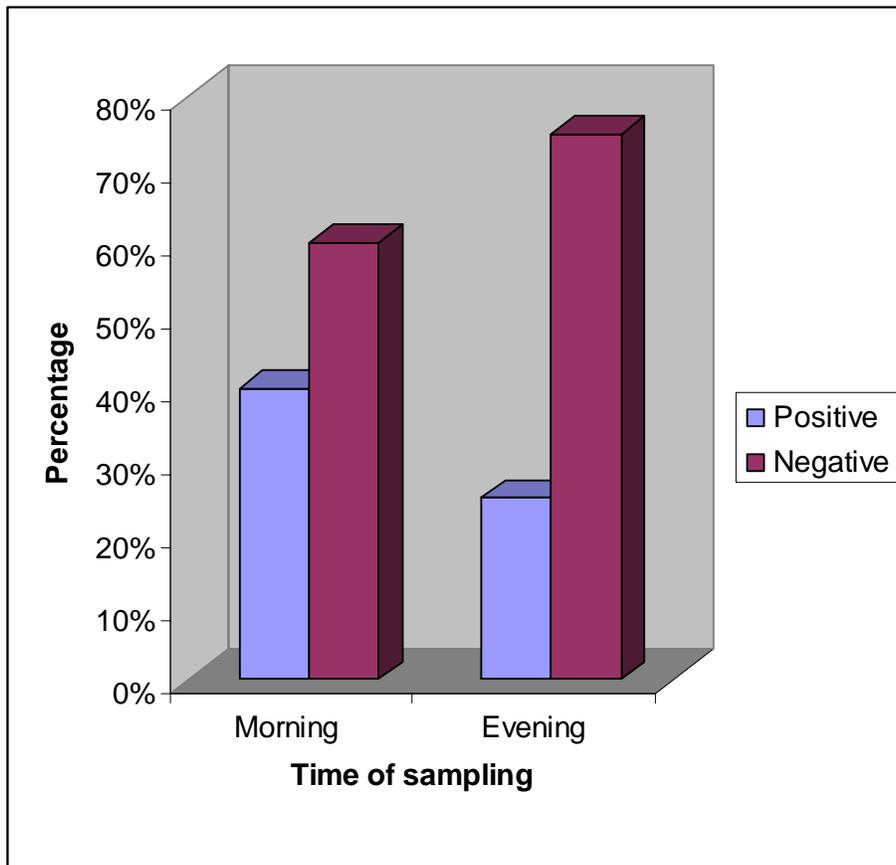
	<b>Numbers of farm Samples</b>	<b>Positive Samples no.</b>	<b>Numbers of market samples</b>	<b>Positive Samples no.</b>
<b>Khartoum</b>	14	5	67	22
<b>Khartoum North</b>	27	5	72	21
<b>Total</b>	41	10	139	43
<b>percentage</b>		24.3%		30.9%



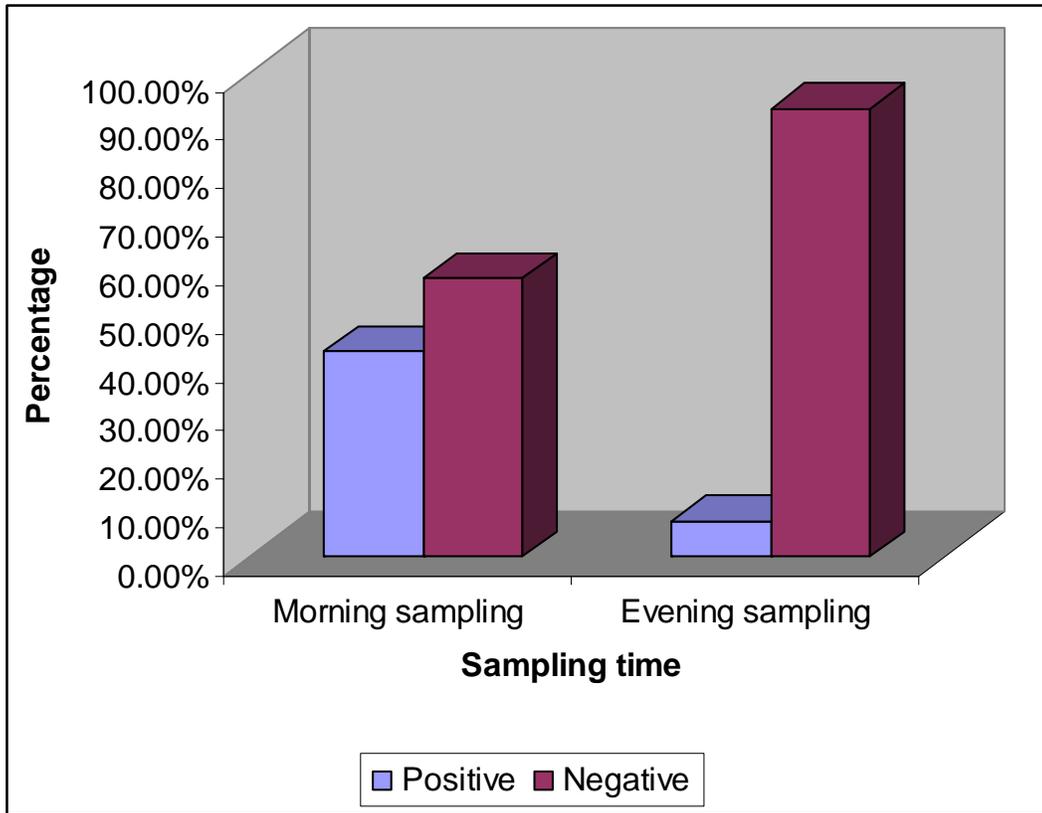
**Figure (3.1) Result of microbial inhibition test conducted on market's and farm's milk samples from the three localities of Khartoum State.**



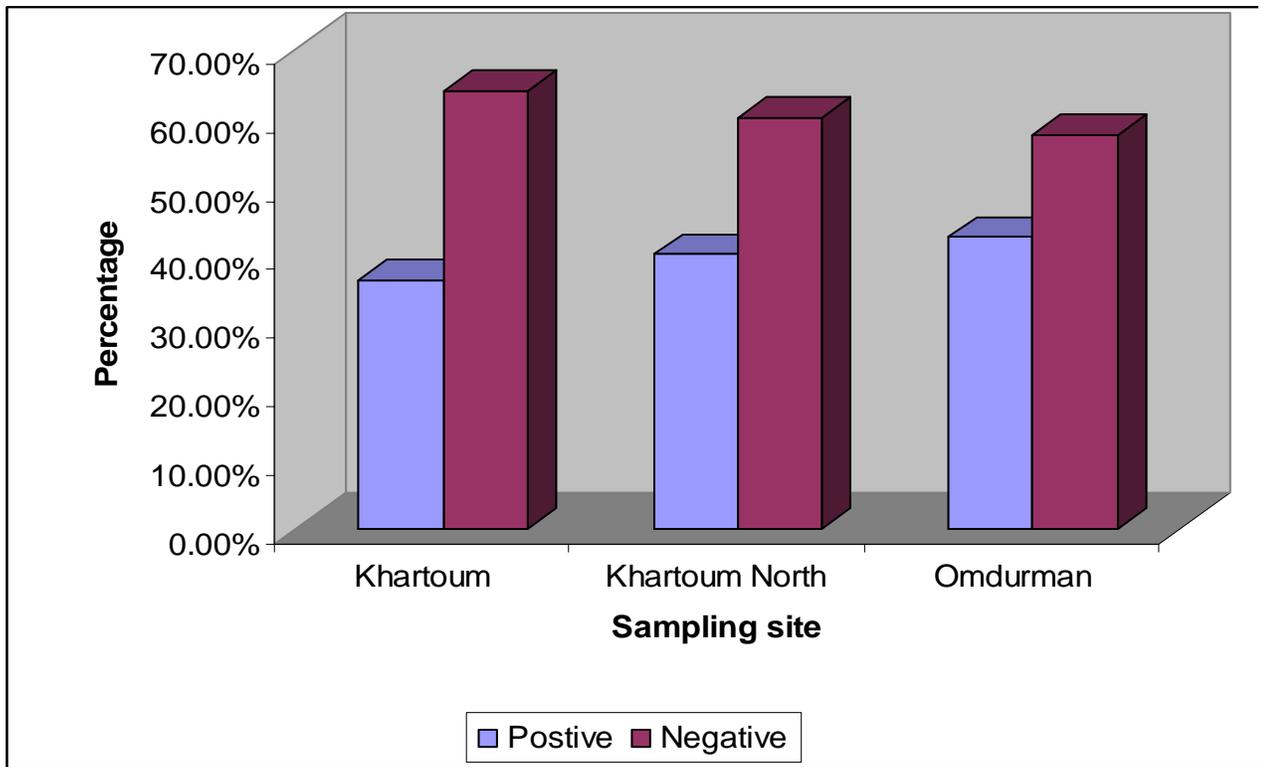
**Figure (3.2) Result of microbial inhibition test conducted on market's milk samples collected in the morning and evening from Khartoum area .**



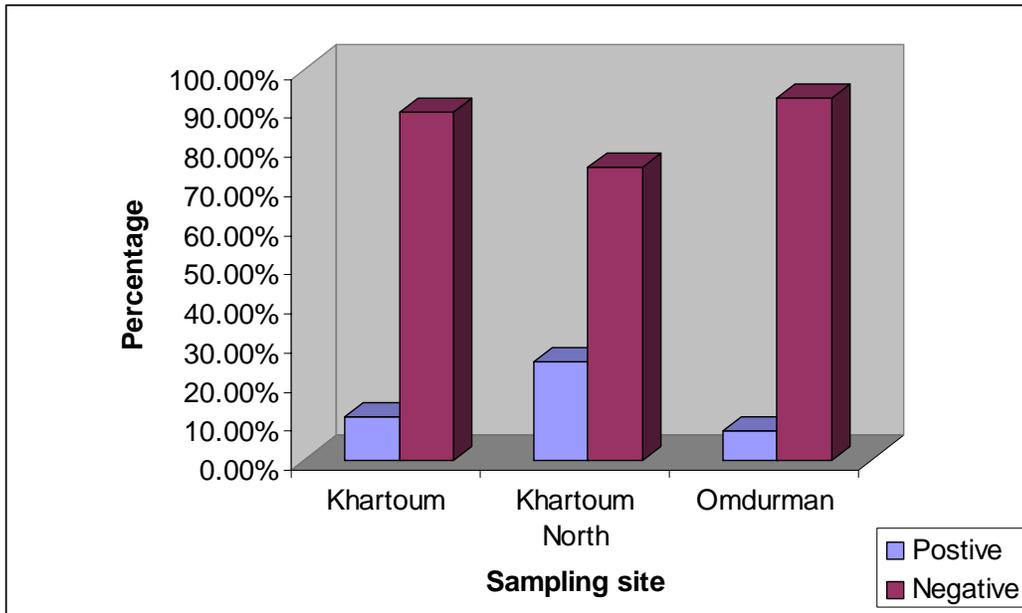
**Figure (3.3) Result of microbial inhibition test conducted on market's milk samples were collected in the morning and evening for Khartoum North area.**



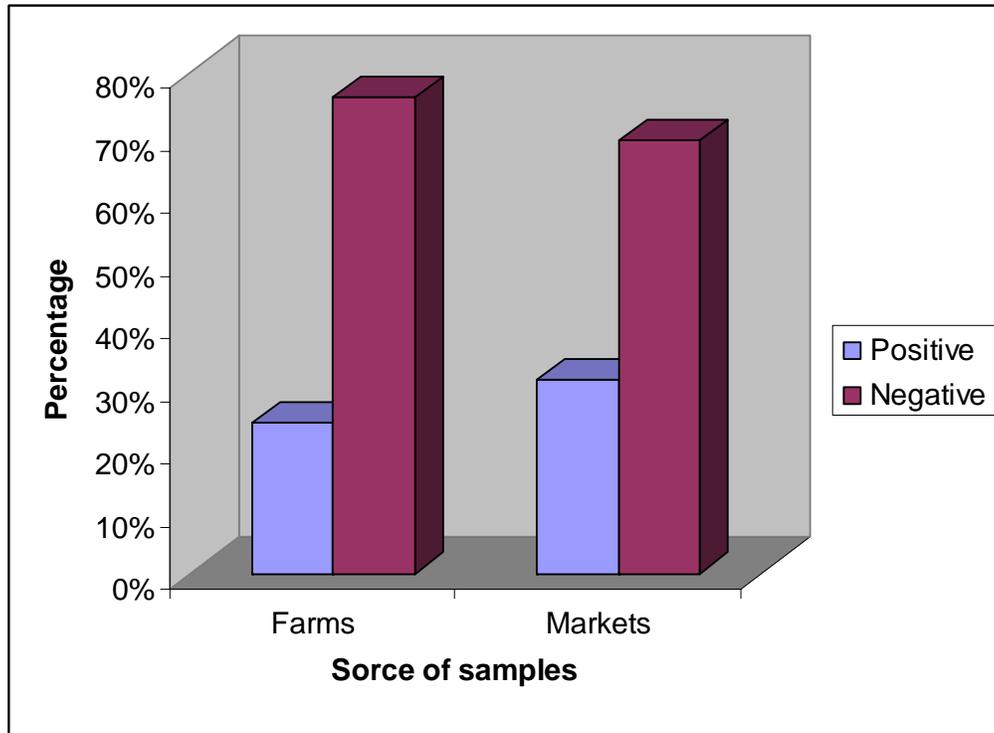
**Figure (3.4) Result of microbial inhibition test conducted on market's milk samples collected in the morning and evening from Omdurman area .**



**Figure (3.5) Results of microbial inhibition test of market's milk samples collected in the morning from the three localities of Khartoum State.**



**Figure (3.6) Results of microbial inhibition test of market's milk samples collected in the evening from the three localities of Khartoum State's markets.**



**Figure (3.7) Results of microbial inhibition test of market milk samples from Khartoum's and Khartoum North's farms and Markets.**

## **CHAPTER FOUR**

### **DISCOSSION**

Antibiotic residues in milk are of concern for two reasons. Firstly, from the medical view they are dangerous for human consumption, and may be associated with significant health problems. Secondly, from milk producer's point of view, they may result in industrial problems in the manufacturing cultured milk product; this is in addition to the economical problems such as international trade interventions leading to loss of revenue for exporting countries in dairy products.

It is generally accepted that bacterial growth is sufficiently restricted at temperatures below 7 C° but promoted at temperatures above 10 C° (Mark and David, 1998). Taking this into consideration the high ambient temperatures in Khartoum state, we have to protect milk against spoilage by cooling, pasteurization, sterilization, or by adding some chemicals as preservatives. Recently antibiotics has been used for this purpose as milk preservatives.

In the warm countries such as Sudan most milk sold and maintained for long time without cooling and despite of the high temperature both at collection and transit, the bulk of this milk from the small producer to the consumer's homes and much of the milk passes from the small producer to the home directly or through an intermediary dealer. Thus spoilage may be the expected.

Under such conditions many traditional practices, could be preferable

for the preservation, such as the direct milking of the animal into the consumer's clean receptacles, such milk will now be boiled by the consumer at home.

The main market milk supply is the milk markets , groceries and small vender. Many pickup cars distribute milk either to small venders or directly to the supermarkets.

Because of the long distance from the farms, the milk containers usually covered by sacks soaked in water to protect milk from direct sun light and dust and to keep milk cold. Some of the shops place the milk containers after received in deep freezers or refrigerators to keep it cold.

All these combined conditions of transportation and distribution, and the high ambient temperature has attempted the use of antibiotics as milk preservatives, and this was indicated by the percentages of antibiotic residues in markets milk was higher than those in the farms (fresh milk which were collected direct from bulk milk of the farms). The highest percentages of antibiotics residues was recorded in milk collected in the morning ( 38.9%) compared to that collected in the evening (16.8%). This could be to protect milk from spoilage due to the high day temperature.

Further samples collected from milk markets showed higher percentage of antibiotic residues (30.9%) to those collected from

farms (24.3%) for the same obvious reason i.e. spoilage control. Sample from Khartoum markets are higher antibiotic residues percentage (27.1%) while those of Khartoum North markets and Omdurman localities were of 21.2%, 25% positive respectively.

This may be attributed to the fact that in Khartoum milk is susceptible for contamination due to the vast distances desired to cover to reach the customers placing through crowded area and traffic jam. Therefore, to protect milk from spoilage, antibiotics are added as a preservative

Similarly, Omdurman market samples which were collected from the morning milking showed a higher percentage of positive antibiotic residues (42.5%) compared with samples which were collected from Khartoum and Khartoum North markets which were from the morning milking. This is because Omdurman is the most popular among the three concerned cities with the expected long distance the milk has to cover.

The high positive result of antibiotic residues in marketable milk agreed with Manal (2004) who detect 35%, 20% and 18% in Khartoum locality, Khartoum North and Omdurman localities respectively .Barbosa (1991) who examined 2248 samples of consumer milk in 1981 to1985 in Lisbon, 30 % of them contain inhibitory substances .

The positive result of antibiotic residues in milk agreed with Barakat (1995) who found that 8.75 % gave positive result in 80 milk samples. The results also agreed with Paern and Kind (1995) who examine 47 raw milk samples sold in Tartu and the residues were detected in 8.5 % of the samples. Also, in Zimbabwe, 73 samples of raw milk from three main dairy marketing board collection centers, were tested for the presence of microbial growth inhibitory substances .4.4 % of the samples were found to contain antibiotic residues (Chagonda & Ndikumera; 1989).

The positive result of the samples which were collected from farms (bulk milk) agreed with Salam et al. (1991) who examined 66 milk samples from three small holder dairy farms for the presence of antibiotic residues. The residues were detected in three milk samples . All these findings support the presence of antibiotic residues in the milk consumed in Khartoum state. Although there are difference in the level, the results support the fact that antibiotics are used as milk preservatives. However, the impact of this practice in human health has to be further clarified.

## **RECOMMENDATIONS**

As based on this study and in order to provide inhabitants of the modern cities of Khartoum State will safe and well preserved milk :-

- 1- The practice of adding antibiotics to milk should be discouraged by the local Government and rules governing marketable milk including penalties should be installed.
- 2- The production and marketing of milk should be encouraged to form co-operatives or unions that should be provided with facilities of milk production such as pasteurization, bottling and dispensing vehicles either solely or under Government aid programme.
- 3- Only pasteurized and bottled milk should be accessible for door to door distribution.
- 4- Bulk milk tanks should be manufactured of a stainless steel container surrounded by an elaborately insulated jacket, the space between the two being maintained at a correct temperature by chilled water to 5 C° from an ice bank. The tank should be fitted with a thermometer from which the cowman and the driver can check the temperature of the milk and the tanks can be emptied by suction .
- 5- Establishment of a number of collecting system centers which include milk chilling directly after milking and tanker collection from the farm gate.
- 6- The public should not have access to veterinary antibiotics, whose distribution should be regulated.

- 7- An established networks of scientists and regulators to implement a residue control programme.
- 8- Drug screening tests on-farm use.
- 9- There should be a clear identification of the source of antibiotic residues in milk to make suitable solutions.
- 10-Permission should be withdrawn for the use of chlortetracycline, oxytetracycline, tylosin, sulphanomides and these drugs should only be issued on prescription.

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