T.C.

YEDITEPE UNIVERSITY

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DEPARTMENT OF NUTRITION AND DIETETICS

INVESTIGATION OF ANTIBIOTIC RESIDUES IN PACKED AND UNPACKED YOGHURTS

MASTER THESIS

ESMA ARZU MENEKŞE

İstanbul, 2019

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Supervisor

Assist. Prof. Dr. İskender KARALTI

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ONAY

Bu tez Yeditepe Üniversitesi Lisansüstü Eğitim-Öğretim ve Sınav Yönetmeliğinin ilgili maddeleri uyarınca yukarıdaki jüri tarafından uygun görülmüş ve Enstitü Yönetim Kurulu'nun 06/09/2019 tarih ve 2019/14-13 sayılı kararı ile onaylanmıştır.

Prof. Dr. Bayram YILMAZ
Sağlık Bilimleri Enstitüsü Müdürü

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has bee$_{n}$ made in the text.

26.08.2019

ESMA ARZU MENEKŞE

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LIST OF SYMBOLS AND ABBREVIATIONS

°C: Celcius

CFU: Colony Forming Units

EU: European Union

FAO: Food and Agriculture Organizaton

FDA: Food and Drug Administration

g: Grams

GMP: Good Manufacturing Practice

HPLC: High-performance Liquid Chromatography Method

IGF-1: Insulin-like Growth Factor-1

IU: International Unit

LAT: Latex Agglutination Test

µ: Micro

MFG: Milk Fat Globules

Min: Minutes

ml: Mililiter

MRLs: Maximum Residue Limits

OMPs: Outer Membrane Proteins

PGs*:* Prostaglandins

ppb: Parts Per Billion

RABV: Rabbies virüs

SNF: Solid Non Fat

WHO: World Health Organization

ABSTRACT

Menekşe, EA. (2019). Investigation of Antibiotic Residues in Packed and Unpacked Yoghurts. Yeditepe University, Institue of Health Science, Department of Nutrition and Dietetics, MSc thesis, İstanbul.

Milk is a main component of the healthy diet for 6 billion people. In addition to milk, many dairy products such as cream, yogurt, butter, cheese and kefir have been manufactured and consumed in all around the world. For this reason, there are many investigations and studies about the effect of milk and dairy products on human health. In modern farming system for dairy, antimicrobial drugs are used for both prophylactic and therapeutic purposes. Pencillins, aminoglycosides, tetracyclines and sulphonamides were frequently used in lactating animals, which led to presence of their residues in milk. Drugs are generally used to support the animal health, for treatment of the infection and to make a better production. Mastitis is one of the main problems that leads to use antibiotics in livestock.

In our study, totally 80 yoghurt samples were studied. 40 of them were opened and other 40 were packaged. These samples were studied as qualitative analysis by MiRA test; Beta lactam ELISA test kit also performed quantitative antibiotic residue tests. As a result, all samples were found negative (%100). In despite of the antibiotic residue problem in milk and dairy products still continues in worldwide, the beta lactam levels in the yoghurts we studied were under the acceptable limits. However, these studies should be extended with more examples and dairy products should be routinely analyzed in terms of hygiene criteria. Because not only antibiotic residues, but also food substances such as hormones, mycotoxins, pesticides, heavy metals must be examined for various pollutants.

Keywords: Yoghurt, Antibiotic, Beta-Lactam, Elisa, Dairy

ÖZET

Menekşe, EA. (2019) Paketli ve Açık Yoğurtlarda Antibiyotik Kalıntılarının Araştırılması. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Beslenme ve Diyetetik Anabilim Dalı, Yüksek Lisans Tezi, İstanbul.

Süt, dünyadaki 6 milyar insan için ana besin maddelerinden biridir. Süte ek olarak; krema, yoğurt, tereyağı, peynir ve kefir gibi süt ürünleri de üretilir ve dünyanın her yerinde tüketilir. Bu sebeple, süt tüketiminin insan sağlığı üzerinde etkileri üzerine; süt ürünleri ve bileşenleri ile yapılan birçok çalışma vardır. Modern tarım ve hayvancılıkta ilaçlar, hastalıkları önleme ve iyileştirme amaçlı olarak sıkça kullanılmaktadır. Sütte kalıntıya neden olabilecek; penisilinler, aminoglikozitler, tetrasiklinler ve sulfinomidler emziren hayvanlarda sıkça kullanılır. İlaçlar genellikle; hayvan sağlığını desteklemek, enfeksiyonu kontrol etmek ve iyileştirmek ve üretimi hızlandırmak için kullanılır. Ek olarak; mastitis, hayvancılıkta antibiyotik kullanımının en temel sebeplerinden biridir.

Bizim çalışmamızda 40 açık, 40 paketli yoğurt örneği olmak üzere toplam 80 yoğurt örneği çalışıldı. Bu örnekler kalitatif açıdan MiRA Test ile, kantitatif açıdan da Beta Laktam ELISA Test kiti ile değerlendirildi. Sonuç olarak; bütün yoğurt örnekleri negatif bulundu (%100). Dünya çapında süt ürünlerinde antibiyotik kalıntı problemi devam etmesine rağmen, bizim çalışmamızda bütün yoğurt örneklerinde beta laktam düzeyleri kabul edilebilir limitlerin altında bulundu. Yine de bu gibi çalışmalar daha fazla örnek ile genişletilmeli ve hijyenik kriterler de değerlendirilerek analizler sıklaştırılmalıdır. Çünkü antibiyotiklere ek olarak; hormonlar, mikotoksinler, pestisitler ve ağır metaller de süt ürünlerinde bulaşmaya neden olabilecek faktörlerdir.

Anahtar Kelimeler: Yoğurt, Antibiyotik, Beta-Lactam, Elisa, Süt ürünleri

I. INTRODUCTION AND PURPOSE

Milk is a main component in the nutrition of around 6 billion people. 730 million tons of milk produced in the world in every year. Eventhough, mammals produce milk to feed their babies, humans continue to consume milk during their life in many areas of the world.

In addition to milk there are many milk products that are consumed by people all around the world such as; kefir, cream, yoghurt, butter and cheese. For this reason, there are many investigations and studies about the effect of milk and dairy products on human health (1).

Scientific studies have evidenced several chemical residues in milk and their side effects on human health. The most common residues in milk and dairy products include veterinary drugs such as pesticides, antibiotics, hormones, mycotoxins and dioxins. The presence of these chemical residues in milk is a potential threat for public health since milk products are widely consumed by adults and children worldwide. For this reason, many countries have controlled the limits for the level of chemical residues in milk and dairy products (2).

According to National Statistics Institue's database; in our country 11.107.896 ton of cow milk, 789.877 ton of sheep milk and 253.759 ton of goat milk is producing per year. %20-25 of this milk is used for fermented products like yoghurt, kefir etc. In Turkey; cow, goat and sheep milk or mixture of these milks are used for making both industrial and homemade yoghurt.

In modern farming system for dairy, antimicrobial drugs are used for both prophylactic and therapeutic purposes. Pencillins, aminoglycosides, tetracyclines and sulphonamides were frequently used in lactating animals, which led to presence of their residues in milk. Drugs are generally used to support the animal health, for treatment of the infection and to make a better production. Mastitis is one of the main problems that leads to use antibiotics in livestock (3).

Antibiotic residues in milk is a serious public health problem. In order to protect human health, the obligation to leave compulsory deadlines (breakthrough time) between the application of the drugs allowed for use and the cut of the animal, the lowest levels of residues levels in foodstuffs were accepted by law.

In our study, it was aimed to investigate the antibiotic residue levels in yoghurts in the open and sterilized by pasteurized (4).

II.LITERATURE REWIEW

II.1. Milk

Milk and it's ingredients are also used as component of many other food products. Excellent nutritive value, desirable texture, natural image, and unique flavor will occur with their helps.

Milk can be defined in many ways. Milk's main components are; lactose, fat, water, whey proteins, minerals and casein. Their amounts can differ to the species of the mammals that produce the milk (5).

Most of the milk fat is composed of complex triglycerides. The chain length of the fatty acids in the structure and the rate of unsaturation and saturation vary. Other lipid components are cholesterol, mono and diglycerides, free fatty acids, phospholipids. 80% of the proteins are made up from the casein. Casein kinds are; αS1, αS2-, β-, and κ-casein, and they are phosphorous compounds. The other 20% of the proteins form are serum proteins (β-Ig). Milk contains also a large number of minor proteins and enzymes. Lactose is a single carbon hydrate, a disaccharide composed of galactose and glucose. Lactose is found only in milk.

Milk has many minerals in it such as; Cl, K, Ca, Na, phosphate and Mg. There are also many trace elements. Milk salts, citrates, organic acids are also presents in milk. In addition to these there are many other compounds in trace amounts.

In milk there are water and dry matter. Everything except water is called dry matter. This dy matter includes mostly fat. The nutritional value significantly affected by the chemical composition of the milk. Theremight be also some microorganisms in milk and theese microorganisms affect the quality and the chemical reactions (4).

II.1.1 Yoghurt

FAO/WHO defines the yoghurt as a fermented milk product that produced by the fermentation with *Streptococcus salivarius* subsp. *thermophilus, Lactobacillus*

delbrueckii subsp. *bulgaricus.* At the end of the reaction, lactic acid occured in the milk and leads to coagulation. This coagulated milk is called yoghurt (6).

Yoghurts is also defined as a product that produced from milk with some addition such as; whey concentrates, cream, milk powder, caseinates that leads to coagulation with a lactic acid bacteria reaction in milk. Additionally, these bacteria must be abundant and viable while consumption (7).

According to the Codex standard for fermented milk (Codex standard 243–2003), yoghurt has to contain a minimum of 2.7% milk protein and less than 15% fat. Concentrated fermented milk is a fermented milk where the protein has been increased prior to or after fermentation to a minimum of 5.6%. High-protein yoghurt is not defined for the standardization legally. Besides, the concentrated fermented milk term may involve high-protein yoghurt. Based on the Codex standards definition of concentrated fermented milk is asserted that high-protein yoghurt has to contains at least 5.6% protein, and less than 15% fat. The protein content can be obtained prior to fermentation by fortification with milk powder, mechanical separation or membrane filtration, evaporation or after fermentation by straining (draining) or membrane filtration. The term high-protein yoghurt includes yoghurt that processed by increasing the protein content either after or before fermentation.

A plain yoghurt with a high consumer acceptance should in general have a texture spoonable, smooth, be free from lumps, uniform, visual whey separation, graininess and should have a typical yoghurt flavour. Lactic acid, diacetyl and acetaldehyde are considered as the main aroma components of yoghurt, but there are also other components, like acetoin, acetone, propanoic, acetic, butanoic and formic acids have been listed as additive to yoghurt flavour.

In a sensory evaluation of a wide range of commercially available plain yoghurts, strained “Greek-style yoghurts” with different fat levels were distinguished from the stirred or set-type yoghurt samples by having a firmer and thicker consistency. Full-fat (8.8 or 20%) “Greek-style yoghurts” differed from the low-fat (2%) and non-fat (<0.05%) “Greek-style yoghurts” by having a less chalky mouthfeel like dry, powdery sensation in the mouth. All “Greek-style yoghurts” had a relatively high degree of smoothness irrespective of fat content. In a consumer acceptance test a full-fat strained yoghurt received a higher impression score than non-fat and low-fat “Greek yoghurts”.

The full-fat yoghurt was characterised by the descriptive panel as having high sensory intensities of firmness, denseness, viscosity and milk fat flavor and moderate amounts of sweet and sour taste. Despite full-fat high-protein yoghurts have preferable sensory properties, the largest dairy companies offer a wide range of low-fat and non-fat high-protein yoghurts to supply consumer demands (8).

II.1.2. Classification of Yoghurt

There are many different types of yoghut products. Tamime and Deeth classified all types of yoghurts into four categories based on the physical characteristic of the products (Table 1). In addition to this, these products are subdivided into different groups based on the following conditions;

- fat content or chemical composition (skimmed, semi skimmed, whole fat,)
- Physical nature of the product (stirred, liquid etc.)
- Flavours (fruit flavoured, plain)
- Post fermentation processing (adding vitamin, fortification etc.) (9).

Table 1. Classification of yoghurt products

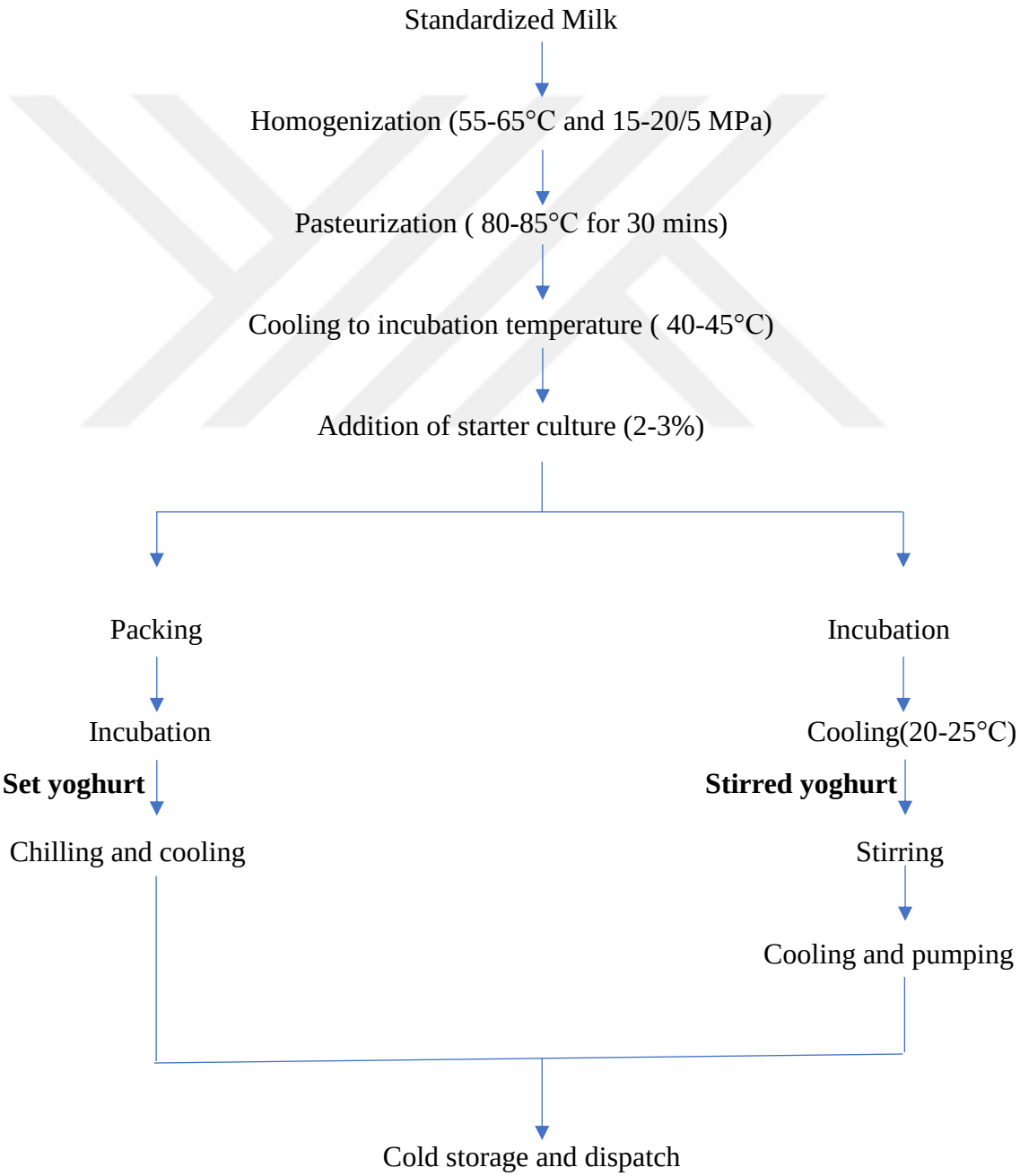
Category	Physical state	Yoghurt products
I	Liquid/viscous	Yoghurt
II	Semi-solid	Concentrated/ strained
III	Solid	Frozen
IV	Powder	Dried

Milk and it's ingredients are used as component of many food products. Unique flavor, desirable texture, natural image and excellent nutritive value will occur with their helps.

II.1.3. Production of Yoghurt

The composition and quality of yoghurt that applied bacterial cultures affects the quality of the yoghurt acquired as the result of the milk fermentation processes. There is a symbiotic relationship between the *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and this leads to more rapid acid development than in the single strain culture. (6).

Table 2. Production of Yoghurt



In dairy industry; to remove somatic cells and other solid pollution from the raw milk there is a centrifugal process. Later, there is a process called thermalization which is a mild heating process. This is applied at temperature range 60–69 °C for 20–30 s, that aims to kill many vegetative microorganisms and inactivate some enzymes. At the end of this process there is almost no other irreversible change in milk. Then, the milk is cooled to less than 5 °C or inoculated with lactic acid bacteria or other microfloras to control the growth of the psychrotrophic bacteria.

a) STANDARDIZATION

The standardization of the milk means the standardization of solid-non-fat content (SNF) and fat. The standardization process is very important because the characteristics of yogurt influenced by the fat content of the milk. Increasing the fat content of milk results high viscosity and consistency in yoghurt. Also, the milk fat content affects the maximum rate of pH decrease and pH lag phase during yogurt fermentation.

The SNF components of milk mainly consist of protein, minerals and lactose used for yogurt production is altered by producers in order to get the desired characteristics of the coagulum; the higher SNF level means higher viscosity and firmness in the latest product. It must be known that the fat and SNF content of milk has an impact on the fermentation process.

b) HOMOGENIZATION

Milk is a typical oil in water emulsion with milk fat globules (MFG) acting as the oil droplets and the milk fat globules membrane as the emulsifier. During the process of milk, the milk fat rises to the surface of the milk and thus creates the unwanted effect of separation. In order to prevent this effect, standardized milk undergoes homogenization.

Homogenization affects the characteristics of acidified milk gels, like yogurt. According to Cho et al., the smaller milk fat globules facilitate the incorporation of fat into the protein network, while their increased surface area favors the interactions

between fat and denatured whey, milk proteins and casein, during subsequent gel formation and acidification.

c) HEAT TREATMENT

To reduce the number of pathogenic microorganisms to acceptable safe limits in milk heat treatment is applied. The most common heat treatment methods are known as sterilization, low and high pasteurization, UHT (Ultra Heat Treatment) and thermalization.

Table 3. Heat treatments

Heat treatment	Heat	Time	Died bacteries	Condition
Low pasteurization	63-65 °C 72-75 °C	20 min 15-20 s	Most pathogens, vegetative bacteria, yeast, molds	Enzymes become inactive No serum proteins denatured, bacteriostatic properties remain virtually
High temperature pasteurization	85°C 90-95 °C	20-30 min 5 min	most vegetative microorganisms, except from spores	Most enzymes are deactivated Most whey proteins are denatured A clear "cooked" flavor is occured
Sterilization	110 °C 130 °C	30min 40s	all microbial content of milk, bacterial spores included	most milk enzymes are inactivated Maillard reaction leads to darken the milk color Weakening the flavor of the milk milk proteins are damaged
UHT	145 °C	1-2 s	all microbial content of milk, bacterial spores included	Minimal flavor loss several whey proteins are denaturated

d) FERMENTATION

Starter culture is the key aspect of the fermentation process. The starter culture acts through biochemical reactions and causes the development of flavor components and the formation of the curd. A fermented product should contains the two living bacteria which are *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in it, to be labeled as "yogurt".

These two species lead main differences in the milk environment such as metabolizing lactose into lactic acid and reducing the milk pH. The sensory and physicochemical characteristics of yogurt occurs with the growth of the symbiotic cultures. The starter cultures changes in the native components of the milk. During fermentation, microbial content, milk proteins and lactose, as well as several carbon compounds, meet major changes, even as minor changes occur for minerals and vitamins. Lactose is reduced by 30% and produces double the molar amount of lactic acid. Whey and casein proteins form groups, increasing the density of yogurt.

e) COOLING

To inhibit the growth and metabolic reaction of the starter culture and prevent the rise in acidity, the yoghurt is cooled around 5°C just after the pH of yogurt reaches the value of 4.7–4.3 (7).

II.2 Types of Yoghurt

a) Fermented Milk is a milk product made by fermentation of milk, that milk may have been manufactured from products obtained from milk with or without compositional modification, by the action of suitable microorganisms and resulting in reduction of pH with or without coagulation (iso-electric precipitation). These starter microorganisms shall be viable, active and abundant in the product to the date of minimum durability. If the product is heat treated after fermentation the requirement for viable microorganisms does not apply.

Fermented Milks are differ from each other and the starter cultures determine the type of the product as follows:

Table.4 Characterization of Milk Products

Yoghurt:	-	Symbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. bulgaricus.
Alternate Culture Yoghurt:	-	Cultures of *Streptococcus thermophilus*
	-	Any Lactobacillus species.
Acidophilus Milk:	-	*Lactobacillus acidophilus.*
Kefir:	-	*Lactobacillus* kefiri, species of the genera (*Leuconostoc, Lactococcus, Acetobacter)*
	-	Lactose fermenting yeasts (Kluyveromyces marxianus)
	-	Non-lactose-fermenting yeasts (*Saccharomyces unisporus, Saccharomyces cerevisiae, Saccharomyces exiguus*).
Kumys:	-	*Lactobacillus delbrueckii* subsp. (*Bulgaricus*, Kluyveromyces marxianus.)

b) Concentrated Fermented Milk is high protein fermented milk. Generally protein is increased to at least %5.6 before or after the fermentation. Stragisto which is strained yoghurt or Labneh can be an example for concentrated fermented milks.

c) Flavoured Fermented Milks are complex milk products, for the General Standard for the Use of Dairy Terms (CODEX STAN 206-1999) they should contain non dairy ingredients and it has to be maximum%50 of the product. These additives might be sweeteners, fruits or vegetables, pulpsi purees, juices, honey, nuts, cereals, chocolate, spices, coffee or other natural flavours. These ingredients and be added just before or after the fermentation.

d) Drinks based on Fermented Milk are composite milk products, in the General Standard for the Use of Dairy Terms (CODEX STAN 206-1999), produced by adding water or other additives like flavourings, whey, non dairy ingredients. These drinks have to contain at least %40 fermented milk. Also some microorganisms and starter cultures might be added. (36).

II.3. Unwanted Substances in Dairy Products

Milk is a basic food in the human diet, both in its original form and as various dairy products. As an excretion of the mammary gland, it can carry numerous xenobiotic substances (veterinary drugs, heavy metals, pesticides and various environmental contaminants). These chemical residues in food of animal origin lead to global public health and economic concerns. These agents can easily transported to milk and dairy products which are evaluated the most important contamination sources for the human health (11).

II.3.1. Antibiotics

Food Safety and Standards Act, 2006 defines veterinary drug residues as the parent compounds or their metabolites or both in any edible portion of any animal product and include residues of associated impurities of the veterinary drugs concerned. Presence of any drug or antibiotic residue in milk and dairy product is illegimate and also lead to economic losses to dairy industry (3). The presence of antibiotic residues in dairy products is an undesirable situation because of many reasons. For example, even the lowest concentrations of antibiotics have bacterisid or inhibitory effect on the activity of starter culture (13).

Nowadays, one of the most important problem in dairy industry is the antibiotics used in treatment of cows. As known, there are many kinds of antibiotics that used for mastitits and some of them kept by the cells and the rest of the antibiotics (%30-80) are remove out of the body with milk. Residues of the antibiotics might injected to the animals or added to the diet of the treated animals and excrete through milk. (14).

According to the evidences; both injection or feeding lead to antibiotic residue in milk (13). Pencillins, tetracyclines, β-lactams, chloramphenicol, aminoglycosides,

and sulphonamides were most frequently used in lactating animals, which led to occurrence of their residues in milk (3). Penicillin is one of the special concern because of its proven allergenicity even in low concentrations.

Having antibiotic residues with dairy products regularly might lead to antibiotic resistence and it effects human health directly (13). The residues of antibacterials may resent immunopathological, microbiological, pharmacological and toxicological health risks for humans. Possible acute and chronic adverse effects of antibiotic residues have been suggested like transfer of antibiotic resistant bacteria to the human nephropathy, autoimmunity, carcinogenicity, hepatotoxicity, mutagenicity, bone marrow toxicity, reproductive disorders, allergy like Penicillin (3).

The good quality of milk must contain no harmful or toxic residues, such as antimicrobial drugs (14).

II.3.2. Pesticides

As synthetic pyrethroid substances are effective for insects, they are widely used against many pests. Their main uses are protection of stored products. field-treatment of crops, stable premises, hygienic treatments in houses and on animals to control ecto- and endo-parasites. Possible contamination sources of pesticides to the milk and dairy products are:

- foodstuffs can contain high levels of pesticide residues because of the post-harvest treatment;
- foodstuffs that are exposed to pesticides to treat any spoilage while the plants were growing up
- use of the pesticides directly on the animal to treat the disease or fight with the factors
- use of insecticides against insects in stables and in milk processing factories (11).

In agriculture, crops like vegetables, fruits and cereals are treated with different types of synthetic chemicals are known as pesticides (15). Pesticides include

herbicides, insecticides, fungicides and rodenticides etc. These pesticides are applied post harvest, pre-harvest and storage stages. They have ability to transfer from lower plants and animals to the higher plants and animals among the food chain and can accumulate in the higher organisms (16). In addition to this, sometimes pesticides are directly sprayed to the animal accommodation to infest the pest (17).

Ultimately, both routes (animals and plants) lead to the bioaccumulation of pesticides in the animal products like fat, eggs, meat and milk. Pesticides source in dietary rout is main way of chronic exposure to these substances (18,19).

II.3.3. Heavy Metals

The toxicity of heavy metals to humans and animals is the result of exposure to long term and lower contamination in our environment, including in the air we breathe, water, food, and so on (20).

Heavy metals are referred to any metal element that has a relatively high density and low toxicity. It contains iron (Fe), lead (Pb), arsenic (As), mercury (Hg), zinc (Zn), chromium (Cr), copper (Cucadmium (Cd) and their actual volume is more than 6 grams per cubic meter (21). Due to the increase in industrial activity, the pollution from heavy metals has grown widely throughout the world since the late 19th and early 20th centuries (22).

Heavy metals can cause serious health problems therefore accurately determining their residues is concerned seriously. Heavy metals in the body may cause side effects such as renal failure, nervous system disorders, genetic mutations, neurological disorders, types of cancers, respiratory disorders, immune system weakening, cardiovascular diseases and infertility (23).

It is well known fact; lead and cadmium are toxic in children; because children are more sensitive to these heavy metals than adults. While zinc and copper are essential, they can be toxic in high doses (24).

Determination of the residual concentrations of heavy metals in milk can be both direct and indirect indicator for pollutions. It is direct effect to show the hygienic status of the milk and indirect effect to identfy the degree of pollution of the environment in which the milk is produced (25). Heavy metals content of milk products are variable due to factors such as geographical area, differences between species,

possible contamination from the equipment during the process and characteristics of the manufacturing practices (26).

II.3.4. Mycotoxins

Mycotoxins are produced by different genera of filamentous fungi and present serious health hazards such as mutagenicity and carcinogenicity. Under optimum growth conditions, toxigenic fungi produce mycotoxins which can contaminate the cow's feedstuff (29).

Milk and dairy products are the most sensitive group for the contamination of mycotoxins. Aflatoxin B1 and aflatoxin M1 are mycotoxins which are the major milk contaminants. Contamination takes place in 2 ways:

Direct contamination: Contamination of dairy products. In particular, aflatoxins which are both benefited from maturation of cheese and are the result of unwanted mold contamination of cheese. Certain strains of *Penicillium roqueforti* and *Penicillium camemberti* are used in mold-ripened cheeses. These types of cheeses include mycotoxins such as mycophenolic acid, rocfortine, cyclapiazonicacid. Also ocratoxin A secreted by *Penicilliumssp* kinds, penicillic acid and patulin are the other mycotoxins on the cheeses. Other than these, sterigatocystin, synthesized by *Aspercillus versicolor*, is also a contaminant found in hard cheeses. Food may also contain fumonisin B1 and B2 from fusarium toxins

Indirect contamination: Containment occurs with consumption food of dairy cattle. Aflatoxin B1 is synthesized by *Aspergillusflavus* and *Aspergillus paraciticus* in agricultural products (Oilseeds, cereals, dried coconut) which are used as feed and feed 13 additives under appropriate temperature water activity and nutrient conditions. In Milk, M1 is the 4th hydroxy derivative of B1. For this reason, restrictions have been introduced regarding aflatoxin levels in statutes and regulations (4)

II.3.5 Hormones

Milk contains many natural hormones like androgens, corticoids, oestrogens etc. which are produced by mammals. The presence of these natural hormones in milk shows difference due to animal's physiological characteristics, different periods of lactation and pregnancy (11).

The most important hormones that found in milk and other dairy products by using a variety of analytical methods consist of prolactin, corticoids, steroids including estrogens, progesterone, and androgens. Moreover, the existence of other hormones such as local hormones including prostaglandins (*PGs*) and insulin-like growth factor-1 (*IGF-1*) in dairy products has been reported. It has been assumed that most of the hormones are transferred into milk by diffusion (27).

The naturally occurring hormones in dairy foods have biological effects in humans and animals, which are ranging from growth promoting effects that related to sex steroids, to carcinogenic properties that associate to some active metabolites of oestrogens and IGF-1 (27).

According to Gandma D. et all Among the main concern about milk and dairy products, the milk that people drink is produced from lactating cows, in which progesterone and estrogen levels are significantly elevated. Because of this; milk consumption might be related with hormone based cancers; like ovary, uterine, breast cancers (28).

II.4. Analyse Methods For Residue in Dairy Products

There are many different residues that threat the human health in yoghurt and many analyzes are required for effective monitoring. For this reason, the use of screening assays is essential.

II.4.1. Lateks Agglutination Test

Latex agglutination test (LAT) is generally used to determine rabies virus (RABV) antibodies in human body and sera. The test is based on the capability of specific antibodies to agglutinate antigen- sensitized polystyrene latex beads. In this test inactivated whole RABV, vaccine or glycoprotein are used as antigen to determine RABV antibodies. (30).

II.4.2. Automated and Manual ELISA (Linked Immunosorbent Assay) Methods

Even though there are different kinds of methods to carry out the antigen antibody reaction, ELISA is the most common one in the latest years. In this method, antigen or antibody are marked with an enzyme and immunological reaction is measured as an enzymatic activity (31).

This technology is extremely sensitive because of the use of antibodies whic are developed according to the target molecule. Due to its high sensibility, the results of the analysis are reliable. It is possible to analyze large number of samples for different drug residues as soon as they contain easy sample preparation procedures (4).

II.4.3. High-performance Liquid Chromatography Method

HPLC is a very sensitive method in which the liquid phase soluble chemical substance mixture can be easily and rapidly separated into its components. Today, HPLC is widespread used in many areas. Chemical separation, concentration determination, purification and identification are its primery uses. (4).

II.4.4. Immunochromatographic Method

Immunochromatography is a combination of chromatography and immunochemical reactions. Chromotograpyhy is a separation of the components of the sample and is is based on the differences in the components movements. Immunochemical reactions can be applied in many different ways. Nowadays, the most common immunochromotographic method is test strip. This is an assembly of several plain porous carriers impregnated with immunoreagents. On contact with the test strip, a liquid sample flows along the carriers, and detectable immune complexes are formed in certain zones of the test strip.

Immunochromatographic analyses are easy to apply, rapid and simple also allows for point of care testing. Because of these adventages this method is one of the most succesful tecniques in medical diagnostics.

The usage of the immunochromatographic systems in foods for safety and quality control is new field. There is recent progress in this area and researches show

the same as well. Most of them described research related to the development of systems to detect a group of food contaminants or a particular compound, and discussed factors related to increasing the speed and the sensitivity of the proposed assays (32).

II.4.5. Immunomagnetic Separation Method

Immunomagnetic separation comprises coupling of biological macromolecules, for example specific antibodies, to superparamagnetic iron oxide (Fe_3O_4) particles. Superparamagnetic particles exhibit magnetic properties when placed within a magnetic field but have no residual magnetism when removed from the magnetic field.

The magnetic particles are added to a heterogeneous suspension to bind to the desired target (bacterial cells, viruses, proteins, nucleic acids, etc.) and form a complex composed of the magnetic particle and target. A magnet is used to immobilize the magnetic particles complexed with the target against the vessel wall, and the remainder of the material is removed (33).

II.4.6. Fast Scan Kits

Many fast screen kits are available for the detection of residues. MIRA test is one of them. Some antimicrobial agent groups, such as tetracyclines and beta-lactams are sensitive to heat; molecules belonging to these chemical classes are inactivated shortly at the growth temperature of thermophilic bacteria. The MIRA test involves a rapid pre-incubation step that allows growth and multiplication of Geobacillus stearothermophilus. Following this step, the interaction between the vegetative form of *G. stearothermophilus* and heat-sensitive antibiotics, if present in the sample, is carried out at room temperature. Finally, the test tubes are subjected to a final incubation and color change is observed (4).

II.5. Microbial Criters for Yoghurt

Yoghurt is a miscellaneous food as it is an important source of vitamin A, calcium, riboflavin, phosphorus, potassium, magnesium and protein. Yoghurt is a

fermented product; so it is a natural source of probiotics, that helps to maintain a healthy immune system and gut.

Because of the health benefits there is a significant increase in yoghurt consumption. There are many different types of yoghurt. Texture, fat, flavour, sugar and type of the fruit that has been added can change the types of yoghurts. Yoghurt can be kept at ambient temperature for 1-2 days and in the refrigerator for 1 week. Microbes in yoghurt may be gained from many different sources. The presence of microbes in daily products including yoghurt are undesirable, at these render the milk products of inferior quality. The spoilage of yoghurt generally because of the yeasts and molds. Low pH doesn't affect the yeast and molds. Microbiological specifications should be applied to some additive employed in the manufacture of yoghurt (34).

Table.5 Allowed patogen levels

Product types	**Test to be conduced**	**Limit- FSC or User Guide**	**DFSV minimum requirements Sampling**	**Frequency**
Yoghurt and other fermented milk products (e.g. sour cream)	*E.coli*/g	n = 5 c = 0 m = 0	1 sample (limit: <1/g)	Every 20 batches
Yoghurt and other fermented milk products with high-risk post-pasteurisation inclusions	Coagulase positive *Staphylococci*/g	n = 5 c = 2 m= 10 M = 100	1 sample (limit: <10/g)	Every 10 batches
	E. Coli /g	n = 5 c = 0 m = 0	1 sample (limit: <1/g)	Every 10 batches
	Salmonella/25g	n = 5 c = 0 not detected in 25g	5 samples composited (limit: ND/125/g)	Every 10 batches

According to the Food Standards Code, as a fermented milk product, yoghurt must have a pH 4.5 and it is classified as dairy desserts. These products have a higher risk for spoilage to the consumers. Testing for *Salmonella* and *S. Aureus* may not be

required if the related post-pasteurisation remains have been tested by an accredited laboratory (35).

According to good manufacturing practice (GMP); the yoghurt need to contain less than 10 yeast cells and have 3-4 weeks shelf life at refrigerator. Yoghurt having initial yeast counts more than 100 CFU/g tend to spoil quickly. The microbial quality standards of yoghurt can be improved with using hygienic practices while production process. Strict supervision and quality control standards are obligatory to avoid the microbial activity in the product, and ultimately reduce the microbial hazards (34).

II.6. Beta Lactam Group of Antibiotics

Beta-lactam antibiotics have been widely used in the treatment and prevention of a variety of bacterial infections. Although they can be classified into monobactam, penicillin, carbapenem and cephalosporin subclasses, all have a chemical structure called a beta lactam ring and carry out bacterial activity through binding to penicillin binding proteins and inhibiting synthesis of the bacterial peptidoglycan cell wall (39).

II.6.1 Penicillins

In Gram-positive infections such as *Spreptococcus pneumoniae, Staphylococcus* species, beta hemolytic strains of *Sptreptococcus* and *Enterococcus faecalis* penicillins are used for treatment. Ampicillin, Penicillin G, nafcillin, amoxicillin and Penicillin V are the most common penicillins that used for treatment.

These drugs may be used alone or in preperations that include beta-lactamase inhibitors, such as sulbactam, tazobactam and clavulanic acid for treatment of organisms that are penicillin resistant (37).

II.6.2 Cephalosporins

The cephalosporins are related ro penicllins an they are also B-lactam antibiotics too. Likewise penicillins, they also inhibit the cell wall sythesis and killbacteria. The cephalosproins are the most common used antibiotics in latest years (38). The cephalosporin antib,otics have wide spectrum against to Gram positive and Gram negative bacterias. That makes these kind of antibiotics more preferable (37).

II.6.3 Carbapenem

Carbapenems are one of the most important antibiotic groups. Of the many different β-lactams, carbapenems have the widest spectrum of activity against Gram-positive and Gram-negative bacteria.

In contrast to other beta lactams; carbapenems do not diffuse the bacteria cell wall easily. Carbapenems enter Gram-negative bacteria through the structures called porins or outer membrane proteins (OMPs)

The carbapenems isolated from *Streptomyces* were found to be chemically unstable and susceptible to hydrolysis by host enzymes (40).

II.6.4 Monobactam

Monobactams are a naturally occuring antibiotic isolated from *Chromobacterium spp* and have a unique beta lactam structure. In opposition to carbapenems, cephalosporins and penicillins; monobactams are not fuse an adjacent ring.

Aztreonam is the only available agent in this class. It has been modified chemically with side chains and demonstrates gram negative activity comparable with the third generation cephalosporins but without significant gram positive or anaerobic activity (41).

III. MATERIAL AND METHOD

III.1.Material

For our work, 80 different brand yoghurt samples were collected. 40 packed samples were bought from the market, while the other 40 open samples were bought from the dairy farm. Accepted samples were delivered to the laboratory in sterile conditions in the cold chain conditions and stored at 2-8 °C until the day of operation.

III.2. Method

Firstly, the antibiotic residues in the samples were qualitatively determined by MiRA Test and then beta lactam level is quantitated by ELISA.

III.2.1 Antibiotic Residue

First, the screening test was performed with Mira Test. It is a microbiological test containing *Geobacillus stearothermophilus* spores for the detection of antimicrobial substance residues in milk.

Some antimicrobial agent groups such as beta-lactams and tetacyclines are sensitive to heat. Molecules belonging to these chemical classes are inactivated shortly at the growth temperature of thermophilic bacteria. The MiRA test involves a rapid pre-incubation step that allows growth and multiplication of G. stearothermophilus. Following this step, the interaction between the vegetative form of G. stearothermophilus and the heat-sensitive antibiotics, if present in the sample, is carried out at room temperature. Finally, the test tubes are subjected to a final incubation. This incubation step of the MiRA test is a critical step in achieving extremely low detection limits. The work steps are described below:

1) Mira Test kit is removed out from the refrigerator and waited for the room temperature.

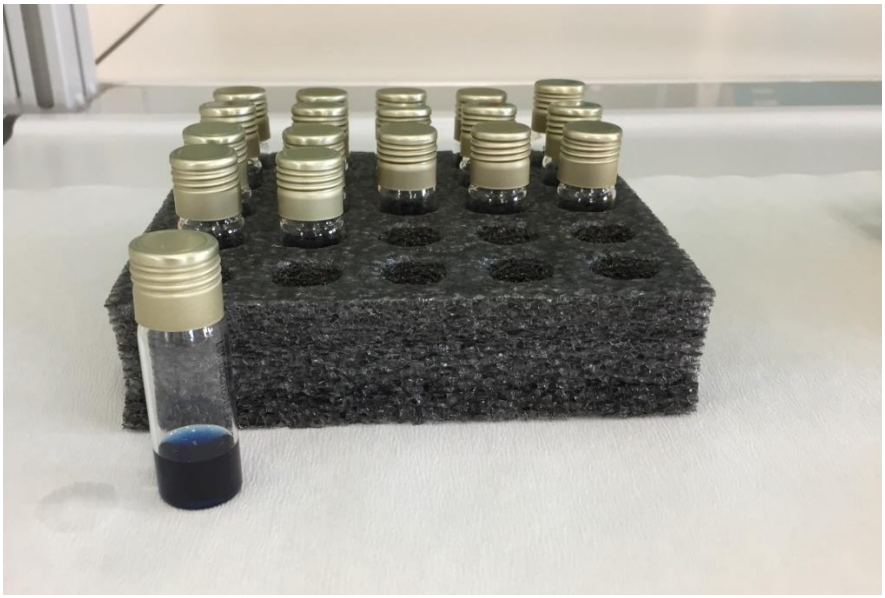


Figure.1: Mira Test kit

2) All empty tubes were numbered.

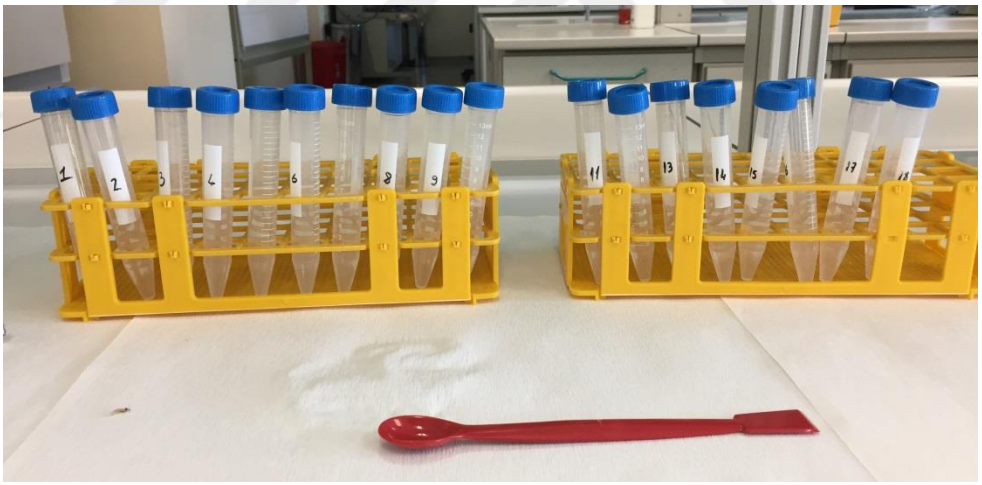


Figure.2: Empty tubes

3) Examples were taken from the yoghurt samples to be tested and 6 ml of distilled water was transferred to a 10 ml test tube (yoghurt: water ratio 1: 3)

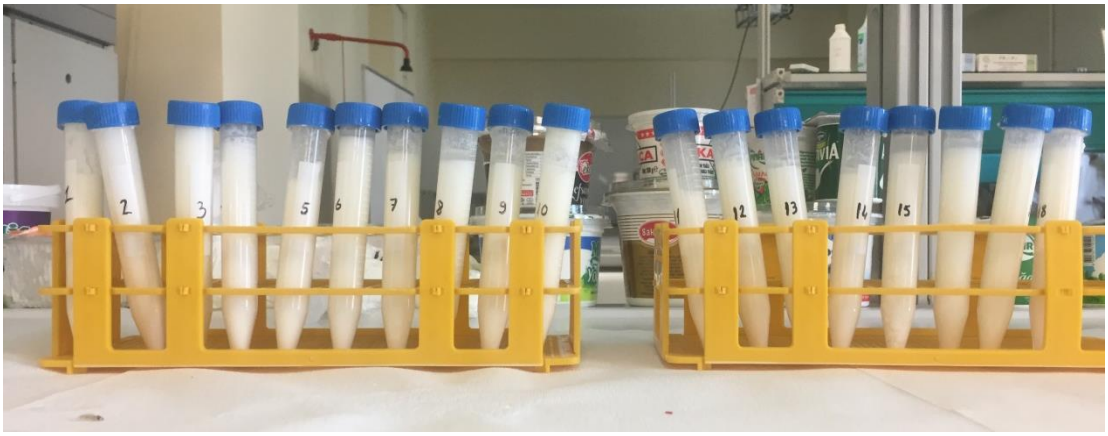
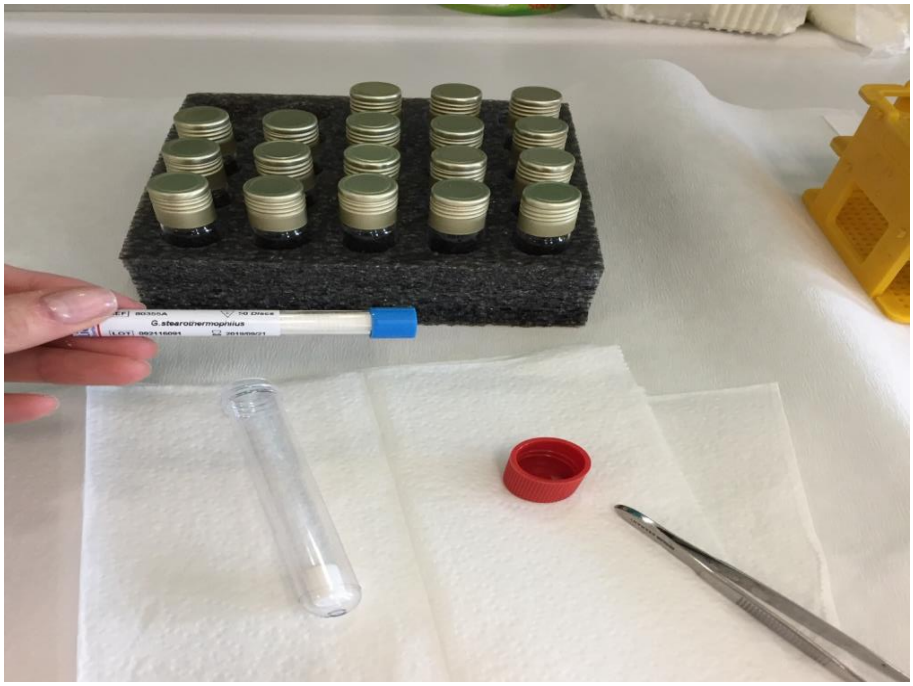


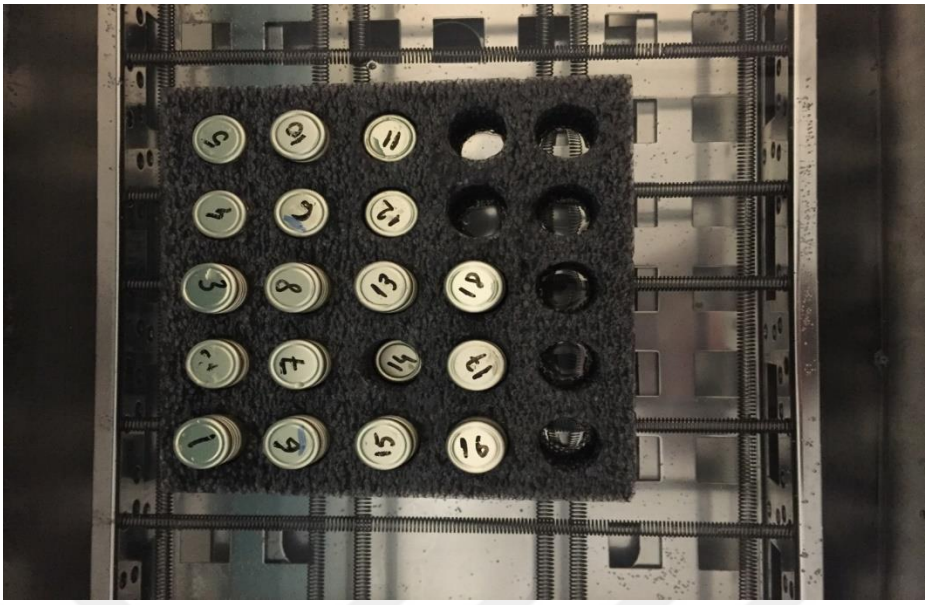
Figure.3: Examples were taken from the yoghurt samples

4) The samples were homogenized by shaking for 3-5 seconds.
5) One G. Stearothermophilus disc was added in every testing bottle



Figüre.4: G. Stearothermophilus disc

6) Pre-incubation of the sports disc in was performed with a 20 min solution at 64 °C



Figüre.5: Pre-incubation of the sports disc in tubes

7) After the incubated solution reached room temperature after incubation, 1ml of homogenized supernatant (test sample) was transferred into the incubated solution
8) The test sample was incubated for 3 to 3. 5 hours on a water bath or thermoblock at 64 ° C.
9) If there is no discoloration after observing the color change in the tube (Blue-Green Color): The concentration of the antimicrobial agent in the sample is accepted above the detection limits. If there is color change (Yellow Color): No antimicrobial agent or concentration is accepted below the detection limits.

III.2.2 Elisa Test

In addition of the Mira Test, the yoghurt samples were analyzed for the residue levels of beta lactam group of antibiotics.

III.2.2.1. Beta Lactam Elisa Test Working Method

Preparation of the samples: 50 ml of yoghurt samples were transferred to tubes and 20 ml of 50 mM succinic acid was added. Then the balcony tubes were shaken in a 15 minutes in the shaker incubator at room temperature. After that it was centrifuged for

15 minutes at 4000 g. The supernatant after centrifugation was diluted 1/10 (100 µl supernatant, 900 µl PBS-Phosphate buffered saline). 50 µl of this mixture was used in the experiment.

Preparation of Standards: Standards are concentrated first, they were diluted. Each standard (50 µl) was diluted with 450 µl sample buffer. Standards were prepared on the working day because they must be fresh.

Preparation of Elisa Test: The solution and plate in the kit were brought to room temperature before the operation and the following steps were followed step by step and the operation was completed.

1. The test sample was placed in the plate as much as the sample and standard number.
2. Standard and samples were pipetted into 50 µl wells respectively.
3. 50 µl anti-tetracycline antibody was pipetted into each wells. It was incubated for 1 hour at room temperature.
4. In the automatic elisa washer, 250 µl wash buffer was washed in each wash 3 times.
5. 100 µl of conjugate was added to each buffer with the help of a multi-channel pipette, shake, and incubated for 15 min at room temperature.
6. In an automatic elisa washer, 250 µl wash buffer was washed 3 times in each wash.
7. 100 µL of sucrose / chromogen was added to each wells, shaked, and incubated at room temperature for 15 minutes.
8. 100 µl stop solution was added and Elisa was read in reader using a 450 nm filter. A standard curve graph was drawn using the Rida Soft Win program Absorbance sample / zero Values of tetracycline values in ppb were calculated using the standard absorbancex100 formula.

IV. RESULTS

IV.1. Qualitative Antibiotic Residue Test Results

The MiRA test qualitatively detects antibiotic residues. Apart from the beta lactam group, this test kit can also detect traces of antibiotics, including tetracycline, and other antibiotic groups such as macrolides. These antibiotics and the limits of detection of this kit are given in table 6.

Table.6 : Maximum Acceptable Limits and MiRA Test Kit Detection Limits.

ANTIBIOTIC/SULPHAMIDE	*MRL* for milk [1] MAXIMUM RESIDUE LIMITS (µg/kg)	**MiRA Test** Sensitivity in 3h 30' DETECTION LIMITS (µg/kg)
BETA-LACTAMS		
Penicillin G	4	2-4
Ampicillin	4	2-4
Oxacillin	30	<10
Cloxacillin	30	<10
Dicloxacillin	30	<10
Amoxicillin	4	2-4
Benzylpenicillin	4	2-4
Nafcillin	30	15-30
Penethamate	4	2-4
Cefalexin	100	50-100
Cefazolin	50	25-50
Ceftiofur	100	50-100
Cephapirin	60	5-10
Cefquinome	20	10-20
TETRACYCLINES		
Tetracycline	100	50-100
Clorotetracycline	100	50-100
Oxytetracycline	100	50-100
MACROLIDES		
Erithromycin	40	<10
Tylosin	50	25-50
Tilmicosin	50	20-40
Spiramycin	200	100-200
LINCOSAMIDES		
Lincomycin	150	75-150
Pirlimycin	100	<50
AMINOGLYCOSIDES		
Gentamicin	100	50-100
Neomycin	1500	<100
Streptomycin	200	<100
Dihydrostreptomycin	200	<100
SULPHAMIDES		
Sulfadiazine	100	<150
SULFANILAMIDES		
Sulfadimidine	100	<200
BENZIL PIRIMIDINE		
Trimethoprim	50	25-50
QUINOLONES		
Flumequine	50	50-100
Enrofloxacin	100	50-100
NOVOBIOCIN		
Novobiocin	50	100-200

According to Mira Test results, All samples were negative which means; there are no antibiotic residues in the samples we worked on.

Table .7 : MiRA Test Results

Packed Yoghurt Sample No	Result	Unpacked Yoghurt Sample No	Result
1	Negative	**1**	Negative
2	Negative	**2**	Negative
3	Negative	**3**	Negative
4	Negative	**4**	Negative
5	Negative	**5**	Negative
6	Negative	**6**	Negative
7	Negative	**7**	Negative
8	Negative	**8**	Negative
9	Negative	**9**	Negative
10	Negative	**10**	Negative
11	Negative	**11**	Negative
12	Negative	**12**	Negative
13	Negative	**13**	Negative
14	Negative	**14**	Negative
15	Negative	**15**	Negative
16	Negative	**16**	Negative
17	Negative	**17**	Negative
18	Negative	**18**	Negative
19	Negative	**19**	Negative
20	Negative	**20**	Negative
21	Negative	**21**	Negative
22	Negative	**22**	Negative
23	Negative	**23**	Negative
24	Negative	**24**	Negative
25	Negative	**25**	Negative
26	Negative	**26**	Negative
27	Negative	**27**	Negative
28	Negative	**28**	Negative
29	Negative	**29**	Negative
30	Negative	**30**	Negative
31	Negative	**31**	Negative

32	Negative	**32**	Negative
33	Negative	**33**	Negative
34	Negative	**34**	Negative
35	Negative	**35**	Negative
36	Negative	**36**	Negative
37	Negative	**37**	Negative
38	Negative	**38**	Negative
39	Negative	**39**	Negative
40	Negative	**40**	Negative

The samples studied with this test kit are evaluated according to the color change. At the end of the incubation, yellow ones are considered as negative.

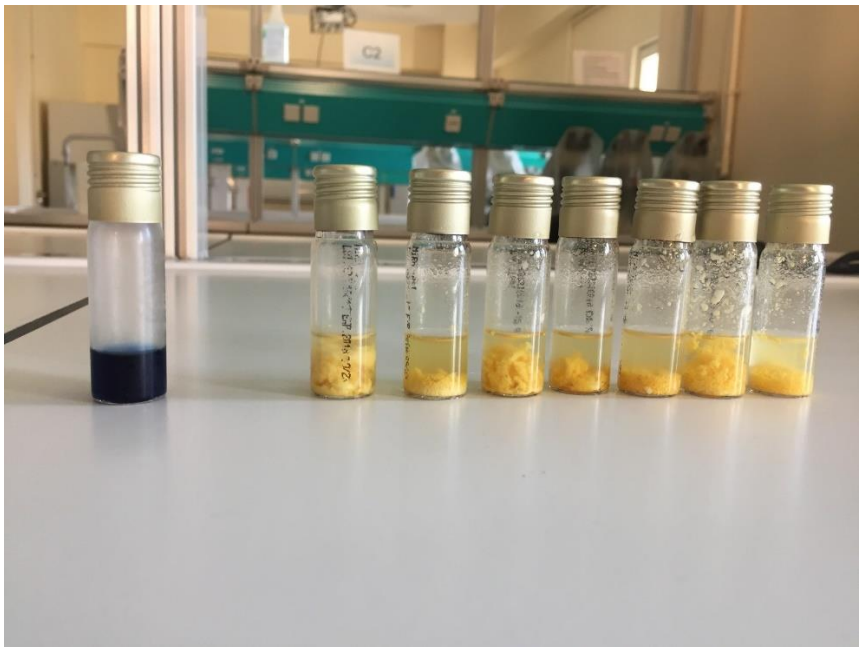


Figure.6: Control group and the negative samples

IV.2. Quantitative Antibiotic Residue Test Results (Beta Lactam Elisa Test Results)

Beta lactam elisa test results showed that the same results as the MiRA test results were obtained. All samples were below the acceptable limits. According to national and international acceptable limits, the maximum amount of beta lactam antibiotics in milk products is 4 ppb.

Table.8: Beta Lactam Elisa Test Results

Packed Yoghurt Sample No	Numerical Value	Result	Unacked Yoghurt Sample No	Numerical Value	Result
1	0,21	Negative	**1**	0,21	Negative
2	0,21	Negative	**2**	0,21	Negative
3	0,21	Negative	**3**	0,21	Negative
4	0,21	Negative	**4**	0,21	Negative
5	0,21	Negative	**5**	0,21	Negative
6	0,21	Negative	**6**	0,21	Negative
7	0,21	Negative	**7**	0,21	Negative
8	0,21	Negative	**8**	0,21	Negative
9	0,21	Negative	**9**	0,21	Negative
10	0,21	Negative	**10**	0,21	Negative
11	0,21	Negative	**11**	0,21	Negative
12	0,21	Negative	**12**	0,21	Negative
13	0,21	Negative	**13**	0,21	Negative
14	0,21	Negative	**14**	0,21	Negative
15	3,2	Negative	**15**	0,21	Negative
16	0,21	Negative	**16**	0,21	Negative
17	0,21	Negative	**17**	0,21	Negative
18	0,21	Negative	**18**	0,21	Negative
19	0,21	Negative	**19**	0,21	Negative
20	0,21	Negative	**20**	0,21	Negative
21	0,21	Negative	**21**	0,21	Negative
22	0,21	Negative	**22**	0,21	Negative

23	0,21	Negative	**23**	0,21	Negative
24	0,21	Negative	**24**	0,21	Negative
25	0,21	Negative	**25**	0,21	Negative
26	0,21	Negative	**26**	0,21	Negative
27	0,21	Negative	**27**	0,21	Negative
28	0,21	Negative	**28**	0,21	Negative
29	0,21	Negative	**29**	0,21	Negative
30	0,21	Negative	**30**	0,21	Negative
31	0,21	Negative	**31**	0,21	Negative
32	0,21	Negative	**32**	0,21	Negative
33	0,21	Negative	**33**	0,21	Negative
34	0,21	Negative	**34**	0,21	Negative
35	0,21	Negative	**35**	0,21	Negative
36	0,21	Negative	**36**	0,21	Negative
37	0,21	Negative	**37**	0,21	Negative
38	0,21	Negative	**38**	0,21	Negative
39	0,21	Negative	**39**	0,21	Negative
40	0,21	Negative	**40**	0,21	Negative

V. DISCUSSION

All veterinery drugs including antibiotics are the most essencial and efficient elements for the production of food. Now, drugs are commonly used for treatment of the animals that used for food production.

Sulphonamides, nitrofurans, beta lactams etc. can contaminate the milk and milk products and leads to occurance of antibiotic residues. Because of the usage of high dosage of these antibioticsin dairy cattle, human health might be affected directly. Milk products that has antibiotic residues may lead to allergic reactions or antibiotic resistance in sensitive people (42).

Compared with the antibiotic resistance, the risk of toxicity caused by the residues and the drugs permitted for use in foodstuffs is very low. Neverthless, The EU has set the Maximum Residue Limits (MRLs) of veterinary drugs in animal feedstuffs to prevent exposure to residue at harmful levels (4).

These antibiotic residues also effect the quality and fermentation process of yoghurt as well. Technological operations during milk processing don't bring to inactivation of antibiotic residues in milk. Due to increased resistance to antibiotics, bacteria of yoghurt cultures do not stand for a barrier any more for antibiotics in milk, as the raw material for production of fermented milk products. The maximal allowed amounts of beta-lactam residues in milk is 0,067 IU/ml according to EU. In order to prevent the problem during milk processing and production, it is necessery to obey the deadlines of waiting period of used antibiotics in the system for raw products and materials (10).

In European Union, there is a rule to control using antibiotics for veterinary called Council Directive 96/23/EC. One of the most importatnt society that cares about residues in food is World Health Organizaton (WHO). Many organizatıon related with food are trying to make provision for residues and give some information to veterinary physicians, medicine providors and public. In our country, laws are also introduced by Food and Agriculture Ministry. (43,44). FDA has a survey about milk drug residue sampling and gave the safe levels of residues.

Lee at al. (2007), made a rewiev about 13 antibiotic types that used in livestock and they studied on 459 sample. At the end of the research they found 34 potential positive sample.

Chung at al. (2009), made a research on sulfonamid and quinolon types. They had 269 cow and goat milk samples and at the end of the microbial tests 21 of them, at the end of the HPCL analyze 4 of them were positive (43).

In Turkey, Aksu et al.4 studied the tetracycline and streptomycin residues in milk samples by ELISA. In this study, streptomycin was found in 98 of 126 samples (77.8%). Only one milk sample was higher than the maximum tolerance limit (200 ppb) accepted by Turkish Food Codex. Tetracycline was determined in 161 of 167 samples (96.4%) and all tetracycline residues were found to be below legal limit (100 ppb) in Turkish Food Codex (11).

In 2006, Khaskheli et al. Showed that 36.5 of all raw cow milk samples were contaminated by beta lactam antibiotic residues in Pakistan (47).

Liman and Karabacak made a research at Kayseri, Kocasinan and they collected 200 raw milk samples from the milk collecting centers. They worked on the milks with ELISA method to investigate tetracycline and streptomycin residues. Tetracycline residues are detected in 19 of 200 milk samples (%9.5). All milks were negative for streptomycin. At the end of the study, they found that antibiotic usage is very common and farmers do not obey the rules about milking protocole just after the antibiotic usage (48).

In our research, according to our Mira test results, all 80 sample, both packed and unpacked, were negative.

When we studied on the same samples in Elisa Test, all samples were below the acceptable level for antibiotic residue levels.

Milk samples contaminated with cloxacillin, benzylpenicillin, nafcillin, oxacillin, ampicillin or dicloxacillin above and at maximum residue limit concentrations were used to produce yoghurt according to the typical industrial process. Expectedly, increased concentrations of penicillin residues influence coagulation negatively. In addition to this, the concentration of the penicillin might reduce with different parameters while the production process. To decrase the penicillin residues in milk following factors are important: The temperature during heat treatment (90°C, 15min)

of the milk just before the yoghurt cultures were added, the fermentation temperature and time, the binding of the penicillins to milk proteins, the effect of the starter cultures (45).

According to another study; boiling slowly reduce the antibiotic residues in raw milk (46). So our yoghurt samples were negative in both tests but it might be related with the fermentation or heating process of milk. While milk is processing to yoghurt, antibotic levels might effected by the external factors.

In this research we found that in Turkey, the yoghurts that we studied were safe in point of antibiotic residues.

VI. SUGGESTION AND CONCLUSION

The chemicals that can solve in oil can easily stored in the fat tissues in human body. Milk and dairy products are one of the main source of the nutrition, that's why they are really important and necessery for the public health. But the researches show that if the effects of the residues or contaminats can not prevent, dairy product will be harmfull for the public health. In our country; Ministry of Food, Agriculture and Livestock is the autorized organization about the food safety. Precautions that are supplied by the ministry is essencial to protect the public health. In addition to ministry; veterions, farmers, drugmakers and doctors also have the responsibilities.

In our study there is no antibiotic residues in the samples but the studies about food safety can improve with more research and more sample. There are many different studies about milk but there are limited studies about dary products such as yoghurt, cheese, kefir etc. To make more research and laboratory studies will make the dairy products more reliable.

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