T.C.
YEDITEPE UNIVERSITY
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DEPARTMENT OF NUTRITION AND DIETETICS

DETERMINATION OF BETA-LACTAM ANTIBIOTICS IN UNPACKED CHEESES

MASTER THESIS

BURÇİN AYSAN

İSTANBUL, 2020

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This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 29.04.2021 and numbered 2021/04-41.

Prof. Dr. Bayram YILMAZ

Director of Institute of Health Sciences

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 been made in the text.

22.04.2021

BURÇİN AYSAN

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LIST OF SYMBOLS AND ABBREVIATIONS

E.U	European Union
F D A	Food and Drug Administration
g	Grams
HPLC	High-performance Liquid Chromatography Method
IU	International Unit
LAT	Latex Agglutination Test
µ	Micro
Min	Minutes
ml	Mililiter
MRLs	Maximum Residue Limits
ppb	Parts Per Billion
S N F	Solid Non Fat
W H O	World Health Organization

ABSTRACT

Aysan, B. (2020). Determination of Beta-Lactam Antibiotics in Unpacked Cheeses. Yeditepe University, Institute of Health Science, Department of Nutrition and Dietetics, MSc thesis, Istanbul.

For all individuals around the world, milk is a key component of a balanced diet. In addition to milk, many dairy products have been produced and eaten worldwide, such as cream, yogurt, butter, cheese, and kefir. There are several research and studies about the effect of milk and dairy products on people health for this cause. Antimicrobial medications are used for both prophylactic and medicinal uses in industrial milk farming systems. In lactating animals, penicillins, aminoglycosides, tetracyclines, and sulphonamides were commonly used, resulting in the presence of their res idues in milk. Dr ugs are commonly used to promote the health of cattle, to cure diseases and to increase production. One of the big concerns related to the use of antibiotics in an imals is mastitis. Milk containing antibiotics triggers resistance to pathogenic microorganisms. In certain individuals, they cause allergic reactions and therefore cause harmful effects. In this research, 100 cheese samples were analyzed. These samples were analyzed by MiRA as a qualitative analysis; the Beta lactam ELISA test also conducted quantitative checks for antibiotic residue. Finally, positive results was observed by both methods in 6 (6%) of 100 samples. According to these results, the antibiotic residue issue in milk and milk products still persists. On the other hand, the beta lactam levels in the lower limits of the samples have been evaluated as promising when all the studies are reviewed. However, with further examples, these experiments should be improved and milk and dairy products should be systematically evaluated in terms of hygiene requirements. Since it is important to analyze food compounds not just for antibiotic residues, but also contaminants such as mycotoxins, hormones and heavy metals.

Keywords: Cheese, Antibiotic, Beta-Lactam, Elisa, Mira Test

ÖZET

Aysan, B. (2020). Açıkta Satılan Peynirlerdeki Beta-Laktam Antibiyotiklerinin Tayini. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Beslenme ve Diyetetik Anabilim Dalı, Yüksek Lisans Tezi, İstanbul.

Süt insanların tarih boyunca dengeli beslenmenin önemli bir bileşeni olmuştur. Sütün yanı sıra, dünya çapında krema, yoğurt, tereyağı, peynir ve kefir gibi birçok süt ürünü üretilmekte ve tüketilmektedir. Bu nedenle süt ve süt ürünlerinin insan sağlığı üzerindeki etkisine yönelik çok sayıda araştırma ve çalışma bulunmaktadır. Antimikrobiyal ilaçlar endüstriyel süt yetiştiriciliği sistemlerinde hem profilaktik hem de tıbbi kullanımlar için kullanılmaktadır. Emziren hayvanlarda penisilinler, aminoglikositler, tetrasiklinler ve sülfonamidler yaygın olarak kullanılmakta ve bu da sütte kalıntılarının varlığına neden olmaktadır. İlaçlar, hayvan sağlığını geliştirmek, hastalıkları iyileştirmek ve üretimi artırmak için yaygın olarak kullanılmaktadır. Hayvanlarda antibiyotik kullanımıyla ilgili en büyük endişelerden biri mastittir. Antibiyotik içeren süt, patojen mikroorganizmalara karşı direnci tetikler. Bazı kişilerde alerjik reaksiyonlara neden olur ve bu nedenle zararlı etkilere neden olurlar. Bu araştırmada 100 peynir örneği incelenmiştir. Bu numuneler kalitatif bir analiz olarak MiRA testi ile analiz edildi; Beta laktam ELISA test kiti ayrıca antibiyotik kalıntısı için kantitatif analiz için kullanıldı. Sonuç olarak, 100 numunenin 6'sında (%6) her iki yöntemle de pozitif çıkan sonuçlar gözlenmiştir. Bu sonuçlara göre süt ve süt ürünlerinde antibiyotik kalıntısı sorunu devam etmektedir. Öte yandan örneklerin alt sınırlarında bulunan beta laktam düzeyleri, tüm çalışmalar incelendiğinde umut verici olarak değerlendirilmiştir. Ancak başka örneklerle bu deneyler iyileştirilmeli ve süt ve süt ürünleri hijyen gereksinimleri açısından da sistematik olarak değerlendirilmelidir. Gıda bileşiklerini sadece antibiyotik kalıntılar için değil, aynı zamanda mikotoksinler, hormonlar ve ağır metaller gibi kontaminantlar için de analiz etmek önemlidir.

Anahtar Kelimeler: Peynir, Antibiyotik, Beta-Laktam, Elisa, Mira Testi

1.INTRODUCTION AND PURPOSE

Cheese, which is an animal food, is rich in basic nutrients such as protein, fat, minerals and some vitamins. Therefore, it has a very high nutritional value and is also easy to digest. Cheese which can be made from almost all kinds of milk (sheep, goat, cow, buffalo) is a rich source of protein, vitamins and minerals (1). In this context, cheese has an important place among other dairy products in terms of healthy and balanced nutrition. Cheese, which has a very important place in nutrition, has 4000 varieties in the world, and more than 130 in Turkey (2). It is estimated that cheese production in the world is approximately 17 million tons per year and increases by 4%. Today, cheese production has a very robust market, with an increasing volume in international trade (3). Therefore, the food safety is very important for cheese, which is a food with such a high consumption volume.

Clinical studies have shown that there are many chemical contaminants in milk and their harmful effects on human health. Veterinary drugs such as pesticides, antibiotics, hormones, mycotoxins and dioxins are among the most common residues in milk and dairy products. Since milk products are commonly consumed by adults and children worldwide, the presence of these chemical residues in milk is a potential hazard to public health. For this reason, limits on the amount of chemical residues in milk and dairy products have been regulated by many countries (4).

Antimicrobial medicines are used for both prophylactic and medicinal purposes in the current dairy farming environment. In lactating animals, pencillins, aminoglycosides, tetracyclines, and sulphonamides were commonly used, resulting in the presence of their residues in milk. Drugs are commonly used to promote the health of livestock, to cure diseases and to increase production. One of the big concerns contributing to the use of antibiotics in livestock is mastitis (5).

A serious public health concern is the use of antibiotic residues in milk. The requirement to leave statutory deadlines between the application of the medications approved for use and the cutting of the animal in order to protect human health was agreed by law, with the lowest residue levels in foodstuffs.

The purpose of this study is to investigate the levels of antibiotic residues in unpacked sold cheese.

2.LITERATURE REVIEW

2.1.Milk

Milk is very important for the first stage of life in all mammals. It is a porcelain-white liquid food with a distinctive taste and aroma that new born mammals have to consume it which contains all the nutrients necessary for their growth until the day they will be able to feed themselves. The main function of milk is to ensure that offsprings of mammals develop well, protect themselves against external factors and live healthy (6). For milk-fed offspring, milk is not only a nutritious but also a protective food (7). Raw milk is defined as unprocessed and unheated mammary gland secretion excluding colostrum milked from cows, sheep, goats, and buffaloes (8).

According to Turkish Food Codex, in the regulation of special hygiene rules for animal foods, raw milk is defined as "milk that is secreted from the mammary glands of farm animals, not heated above 40 santigrat degree or not treated with any equivalent effect" (9).

Composition of milk varies depending on factors such as age, breed, diet, energy balance, udder health status and the lactation period of the dairy animal. Milk contains important nutrients. Cow milk carries the nutrients required for the growth and development of the calf, as well as sources of fat, protein, amino acids, vitamins and minerals. It includes immunoglobulins, hormones, growth factors, cytokines, nucleotides, peptides, polyamines, enzymes and other bioactive peptides. Fats in milk are emulsified in globules covered with membranes. Proteins are found as micelles in colloidal dispersions. Casein micelles are mainly calcium-containing colloidal protein and salt complexes. Milk contains lactose as carbohydrates, most minerals, some vitamins and many different types of 6 fatty acids. All of these components make milk a rich food item (10). Milk as a rich food source is considered by many people to be a valuable element of the diet. Cow's milk contains many bioactive components that support various physiological processes in the body (11). Many factors such as the characteristics of the milked animal, milking conditions and hygiene, and storage conditions of the milk after milking affect the physical, chemical and microbiological properties of milk (12).

Milk is a valuable food source not only for humans but also for microorganisms. Since this situation makes it difficult to preserve, people have been turning milk into more durable products such as "cheese" for a long time (13).

2.1.1.Cheese

According to the Turkish Food Codex, cheese is obtained by coagulating the raw material using a suitable coagulant and coagulating after the whey is separated from the curd, with different hardness and fat content; in brine or dry salting or boiled, with or without flavor.It is produced in accordance with the technique, consumed with or without matured It is all dairy products that show different characteristics specific to their variety (14).

Cheese demand worldwide is expected to be about 17 million tons per year and to grow by 4 percent. Cheese production today has a very robust market, with an increasing volume in international trade. The cheese industry has a very advanced technology all over the world and its diversity has increased (3). White cheese, cheddar cheese and tulum cheese constitute 85% of cheese production, respectively (15).

Approximetely, 700.000 tons of cheese is produced in Turkey in 2017. It is estimated that the annual cheese consumption per person in Turkey is approximately 17,8 kg (16).

2.1.2.Compound of Cheese and Benefits to Health

Cheese, which contains proteins, fats, minerals, especially calcium and phosphorus minerals and vitamins, especially fat-soluble vitamins A, D, E, K and water-soluble B2, in a concentrated form, is preferred by the all age group in society due to its superior nutritional value and unique taste. The importance of cheese in our daily diet is due to the fact that the digestion rate increases due to the breakdown of proteins during the ripening of the cheese. Also, it helps the digestion of other foods, and finally, the soluble salts, vitamins, serum proteins and other nutritional elements in the milk serum are in the structure of cheese (17). In addition, cheese is a suitable dairy product for patients with lactose intolarance and diabetes because of its low lactose content (1).

Lactose

At the beginning of the cheese ripening process, some of the lactose is washed off with whey and the remaining amount is fermented with lactic acid followed by diacetyl, acetaldehyde, acetic acid, ethanol and CO2 (18). The fact that mature cheese is lactose-free, this is advantageous for most adults. Because approximately 70% of adult people in the world have lactose intolerance, which shows itself with various ailments such as abdominal pain, diarrhea, nausea, bloating, etc. (19). Therefore, individuals with lactose intolerance can easily consume these kind of cheeses that contribute to a healthy diet with a must nutrients for body such as calcium.

Protein

Cheese is a very important source of protein and amino acids. It is well known that cheese meets all essential amino acid requirements (except for methionine and cysteine) which is higher than the recommended amount for children or adults (20). It has a number of defined biological activities including opioid effect, blood pressure lowering, mineral binding, antimicrobial, immunoregulatory, anticarcinogenic, anticaryogenic (against caries), antithrombotic, anti-inflammatory, and cholesterol-lowering effects (21).

Fat

One of the main ingredients of cheese is fat. Fat ratio ranges from 20% to 35% of the dry weight. The amount of fat does not change during ripening and cheese contains, on average, 600 g · kg − 1 saturated fatty acid, 235 g ·kg − 1 monounsaturated fatty acid and 46 g · kg 1 polyunsaturated fatty acid.

Some saturated fatty acids play a very important role in protein modification (acetylation) in cell regulation, organization of genetic regulation and gene expression (22).

Vitamins and Minerals

One of the most important minerals in dairy products, especially cheese, is calcium. In the world, daily calcium intake is met primarily from milk and dairy

products. Cheese is an important source of phosphorus and zinc (including magnesium) as well as calcium. Although a portion of hard cheese meets only 10% of the daily calorie requirement, it provides 15% of the daily recommended vitamin A needs, more than 10% of the vitamin B2 needs, more than 20% of the vitamin B6 needs and almost 40% of the B12 needs (23).

2.1.3.Classification of Cheese

The first process in cheese making is coagulating milk or fermenting milk to obtain coagulated casein. The second stage of production is to purify the casein from water and filter it, and the last stage is salting and maturing to form the desired flavor and texture in cheese (3). Classification of cheese is very difficult due to the large number of varieties and some qualities similar to another. For this reason, many attributes are taken into consideration when classifying.

The most recently published Turkish Food Codex Communiqué includes cheese types, definitions, categories and required standards of 19 articles. The classification of the cheeses in the appendix index, which includes the standard information in the Communiqué, according to the amount of milk fat, is as in the table 1 below (14).

Table 1: Properties considered in the classification of cheese

Origin	Country, Region
Milk Type	Cow, Sheep, Goat, Buffalo
Cheese Type	Coagulation Method
	Maturation Time
	Major type of microorganism involved in maturation
Internal Quality	Texture, color, pores, flavoring agent
External Quality	Shell, smokey, size, shape, weight, packaging material
Chemical composition	% Fat in dry matter; % minimum fat and % maximum moisture

2.1.4.Production Steps of Cheese

According to Kosikowski (1977), the basic principals of cheese production are(24)

(1) Setting milk

(2) Cutting the coagulum

(3) Cooking the cut coagulum

(4) Removing whey from the curd

(5) Allowing curd particles to “knit,”

(6) Salting (this comes at different times in the procedure for different cheeses),

(7) Pressing, and

(8) Ripening of the finişhed cheese.

Explanation of steps and meterials(24):

Milk:

Fresh milk obtained from healthy cows (or other animals that may serve as a source of milk) should be cooled rapidly and then promptly delivered to the cheese factory where it should be converted to cheese as soon as possible. The milk should be free from antibiotic residues, other chemical contaminants, and serious off-flavors.

In addition, excessive growth of psychrotrophic bacteria should not be assisted by raw milk since such growth may cause abnormalities in the development of cheese by affecting the activity of lactic starter cultures and the coagulation of milk by rennet extract. The consistency of cheese resulting from such milk would also be poorer than that of cheese made from milk without psychrotrophic bacteria developing excessively. The difficulties just described occur because proteolytic enzymes that partially degrade casein in milk are produced by many species of psychrotrophic bacteria.

It is usually explained using a centrifuge to extract tiny extraneous particles and somatic cells after milk arrives at the plant. The milk fat content of the clarified milk may be adjusted, depending on the variety of cheese that is to be made. Some cheese is made from raw milk, but it is more common to use heat-treated (heat treatment less than that of pasteurization) or pasteurized milk.

Heat-treated milk is sometimes preferred because the resultant cheese tends to be more flavorful than that made from pasteurized milk. Tests to determine the microbiological quality, composition, and presence of unwanted chemical contaminants in milk are described in Standard Methods for the Examination of Dairy Products.

Starter Culture:

It is common to add one or more species of lactic acid bacteria to pre-warmed milk. The small amount of acid formed by these bacteria early in the process of cheese making (fermentation) promotes the coagulant's eventual clotting of milk. In addition, the starter bacteria become part of the finished cheese where they contribute to the processes that take place during maturation of the cheese.

For eg, one might use Streptococcus cremoris and/or Streptococcus lactis, the so-called mesophilic lactic acid bacteria, to make Cheddar cheese. The form of cheese to be produced decides the microorganisms to apply to milk. Lactobacillus bulgaricus and Streptococcus thermophilus, the so-called thermophilic lactate acid bacteria, will be used to produce Swiss cheese, on the other hand (the concept one prefers for thermophilic bacteria can decide whether certain bacteria are actually thermophilic). There are still more examples, but to highlight the case, these two should suffice.

When cheese is made, microorganisms other than lactic acid bacteria are often added along with them. Examples include Propionibacterium shermanii for Swiss cheese or blue or Camembert cheese molds.

Presently, in preparation for cheese-making, condensed frozen starter cultures can be bought and added directly to milk. Provided that the manufacturer’s instructions are followed, this approach will result in greatest uniformity in culture activity from day to day and also will minimize, if not eliminate, problems caused by bacteriophage infections.

The alternative approach is to transfer the culture from one lot of a suitable medium to another until finally a volume of culture suitable for cheese-making is obtained. When this is done, the problem of bacteriophage infection can be controlled through use of a phosphate-treated medium. Several commercial organizations market such a medium.

Regardless of how the culture is handled before it is added to milk, presence of antibiotics in milk (as a result of administering them to lactating dairy cows) will either retard activity and acid production by the culture or completely stop acid production if the starter culture is inactivated.

Loss of activity by the starter culture will lead to inferior cheese and may cause public health problems. Presence of antibiotics is normally controlled by frequent testing of the raw milk from individual farms according to methods described in Standard Methods for the Examination of Dairy Products.

Coagulant:

In milk, an effective coagulant is applied; normally a short time after the starting culture has been added. The coagulant is an enzyme that in the presence of calcium ions, divides colloidal casein into a carbohydrate-rich peptide fraction and the insoluble paracasein that precipitates.

Rennet extract, derived from the fourth stomach of young calves, has historically been used as a coagulant. The lack of rennet extract has arisen from a worldwide shortage of young calves. As a result, alternatives such as rennin and pepsin blends, rennet extracts from mature cows and fungal coagulants have been produced. Mucor miehei, Mucor pusillus, and Endothia parasitica contain fungi that create coagulants for use in cheese production. Most of the cheese currently manufactured and sold in the United States without excessive ageing is made with a coagulant of fungal origin.

Cutting the Coagulum:

For splitting the coagulated milk into cubes, rectangular frames are used with thin cables, horizontal on some and vertical on others. The surface area of the coagulum, which promotes its whey degradation, is enhanced by such cutting. Coagulum cubes may also be evenly heated during the cooking process. Tiny cubes result in cheese that is low in humidity, whereas big cubes contribute to cheese that is high in humidity.

Cooking the Cut Coagulum:

The cubes of coagulum (curds) suspended in whey are heated to a given temperature at a specific time after the coagulum is cut. This heating is preceded by the swirling of the mixture of curd-whey which allows the curd cubes to contract and thereby convey free whey. Cooking is also used by the lactic starter culture to regulate acid production, to inhibit the growth of some spoilage bacteria, to affect the texture of the curd, and to help control the amount of moisture in the finished cheese.

Draining Whey:

The whey is extracted from the curd after cooking is done. By draining whey from a vat containing the whey-curd mixture, this can be done, using sufficient measures to avoid curd failure. An alternative process known as dipping, involves scooping the whey-curd mixture from the curds.

Any extra lactic acid is produced by the starter bacteria during the period required for the removal of curds from whey. As in the manufacture of Camembert cheese, the curd may be withdrawn from the vat and put into a shape or mold. Alternatively, as in the processing of Cheddar cheese, the curd can stay in the vat so that the knitting of curd particles can take place.

Knitting of Curds:

This step enables lactic acid to be further developed and the curd particles to be modified such that they bind to each other and form a single mass of cheese. This method partly decides the characteristic texture of a given variety of cheese.

Salting of Curds:

Salt is added in one of the forms to curds. As in the processing of Cheddar cheese, dry salt can be poured on loose curds or it may be rubbed onto the surface of freshly made cheese. Alternatively, a nearly dissolved aqueous solution of salt should be dissolved in freshly made cheese. Adding salt adds to the taste, texture and appearance of cheese; controls the production of lactic acid; suppresses the development of microorganisms that are spoiled; and thus decreases the amount of moisture in the finished cheese.

Pressing of Curds:

Before salting (as with cheeses that are soaked in brine) or after, this step also comes. Curds are put into a shape, often referred to as a pipe, and pressure is applied hydraulically by with weights. External pressure should not be added if cheese with an open texture is needed. As in the production of Cheddar cheese, the piling of curds in a vat is a type of pressing, while hydraulic pressure is used later in the production of this variety of cheese.

Pressing gives the cheese its unique appearance and adds to its compactness. During pressing, free whey is conveyed and the knitting of curd particles is done. During or after pressing, use of vacuum chambers can help extract occluded air from cheese and thus give the product a tightly knit body.

Ripening of Cheese:

The finished cheese is placed in a room with a controlled temperature and relative humidity and, depending on the variety of cheese and the degree of ripening desired, is held there for several months to several years.

Ripening causes enzymatically-induced changes to occur in the cheese's protein and fat fractions. The freshly made cheese converts these changes into one with the perfect and distinctive taste, texture, scent, and appearance.

Automation of Cheese-Making:

Making of cheese was and for some cheese-makers continues to be largely a hand operation. However, increases in cost of labor and in amount of cheese a given factory desires to produce prompted development of machines which have automated various portions of the cheese-making process. This also includes preparation of finished cheese for the retail market.

2.2.Types of Cheese Used in This Study

Cheese, an important dairy product with high nutritional value, has many varieties. In Turkey, white cheese, cheddar cheese and goat cheese are the most varieties of cheese produced. Apart from these, it is reported that there are more than 25 local and regional cheese types depending on factors such as the breed of the milking animal, environmental and climatic differences, traditional and technological processes, and even hundreds of unrecognized regional cheese types (25). The majority of the cheese types produced in Turkey are white cheese (60%), respectively kashar cheese (17%), tulum and mihalic cheese (12%) and other local cheese (11%) (13).

2.2.1.White Cheese

Turkish White cheese is seasoned in salt and is aged in brine (~ 12–14 g / 100 g NaCl solution). Cheeses are usually cubic or square in shape and typically measure 7x7x7 or 7x7x10 cm3 and weigh between 350 and 500 g. The cheese is white, tight (without spaces) without crust and has a salty sour taste (26). Cheese matures in 1-3 months. Considering the moisture content (55-65 g / 100g), Turkish white cheese is a soft type cheese. White cheese was mainly produced from sheep or goat milk, but now, cow's milk or combined milk is generally used in cheese production. Since the milking

periods of sheep and goats are short (3-5 months), cow's milk is used in cheese production most of the year (27).

2.2.2.Kashar Cheese

Kashar cheese is the second most consumed cheese in Turkey. It is produced around 49 thousand tons per year (28). It can be produced from both cow and sheep milk, as well as from a mixture of these two and is similar to other types of cheeses such as Caciocavallo, Provolone, Ragusano, Kaskaval and Mozzarella (29). This cheese contains an average of 24.2% protein, 4.2% ash, 41.9% moisture, 25.2% oil, 4.6% salt and its acidity (LA%) is between 0.8-2.3% (30). There is no standard technique for the production of kashar cheese, and since raw milk is used in the production of cheese, preventive measures must be taken to eliminate unwanted bacteria. Stretching applied for 5 minutes at 75 ° C is essential for the elimination of microorganisms. Antimicrobials and metabolic products produced by bacteria in the endogenous flora during the ripening phase of cheese also have a damaging effect on unwanted bacteria (31).

2.3.Unwanted Substances in Dairy Products

Milk, both in its original form and in other dairy products, is a fundamental food in the human diet. It can carry various xenobiotic substances as an excretion of the mammary gland. These chemical contaminants in animal-based foods are causing global public health and economic concerns. These agents can easily be transported into milk and milk products that are evaluated as the most significant sources of human health contamination (32).

2.3.1.Antibiotics

Veterinary drug residues are identified by the Food Safety and Standards Act 2006 as parent compounds or their metabolites or both in any edible portion of any animal product, and include residues of the related impurities of the veterinary drugs in question. The presence of any traces of drugs or antibiotics in milk and milk products is

illegal and also contributes to economic losses for the dairy sector. For several factors, the existence of antibiotic residues in dairy products is an unacceptable scenario. Also the lowest concentrations of antibiotics, for example, have bacterisidic or inhibitory effects on starter culture activity (33).

The antibiotics used in the treatment of cows are now one of the most important issues in the milk industry. There are many kinds of antibiotics used for mastitis, as is known, and some of them are retained by the cells and the majority of the antibiotics (30-80 percent) are extracted with milk from the body. Antibiotic residues may be inserted into the animals or added to the diet of the animals under care and excreted by milk. Evidence suggests that both vaccinations and feeding lead to antibiotic residues in milk. In lactating animals, pencillins, tetracyclines, β-lactams, chloramphenicol, aminoglycosides and sulphonamides were used more commonly resulting in the presence of metabolites in milk. Due to its proven allergenicity, even at low doses, penicillin is one of the specific concerns (34). According to the evidences; both injection or feeding lead to antibiotic residue in milk. Pencillins, tetracyclines, β-lactams, chloramphenicol, aminoglycosides, and sulphonamides were most frequently used in lactating animals, which led to occurrence of their residues in milk. Penicillin is one of the special concern because of its proven allergenicity even in low concentrations (33).

Regular use of antibiotic residues in dairy products may contribute to antibiotic resistance and directly affect human health. Antibacterial residues can pose immunopathological, microbiological, pharmacological and toxicological risks to human health. Possible acute and chronic adverse effects of antibiotic residues have been proposed, such as the transition to human nephropathy of antibiotic resistant bacteria, autoimmunity, carcinogenicity, hepatotoxicity, mutagenicity, toxicity of the bone marrow, reproductive disorders, penicillin allergies (33).

No hazardous or toxic residues, such as antimicrobial products, must be present in good quality milk (34).

2.3.2.Heavy Metals

The toxicity of heavy metals to humans and animals, including in the air we breathe, water, food, and so on is the result of exposure to long-term and decreased

pollution in our climate (35). All metal elements that have a relatively high density and low toxicity are referred to as heavy metals. It contains iron (Fe), lead (Pb), arsenic (As), mercury (Hg), zinc (Zn), chromium (Cr), copper (Cd), and is more than 6 grams per cubic meter in actual volume. Since the late 19th and early 20th centuries, heavy metal pollution has increased widely across the globe due to the growth of industrial activity (36,37). Heavy metals can cause severe health issues, so it is seriously concerned with correctly measuring their residues. Side effects such as renal failure, nervous system disorders, genetic mutations, developmental disorders, forms of cancers, respiratory disorders, weakening of the immune system, cardiovascular diseases and infertility may be caused by heavy metals in the body (38).

It is well known that lead and cadmium are harmful to children, since children are more vulnerable than adults to these heavy metals. Although zinc and copper are important, high doses of them can be toxic (39).

The assessment of residual heavy metal concentrations in milk may be a direct and indirect emission measure. Directly indicating the hygienic status of the milk and implicitly indicating the degree of contamination of the atmosphere in which the milk is processed is a clear result (40). Due to factors such as geographic location, variations between organisms, potential contamination of the equipment during the process and characteristics of the processing practices, the heavy metal content of milk products is variable (41).

2.3.3.Cleaning Materials and Disinfectants

Detergents are used to clean all forms of spaces where milk and goods come into contact, and disinfectants are chemical agents that are used to purify microorganisms from any surface they come into contact with before they are ready for milk processing. After this process, the residues often transfer to milk when the cleaning and disinfectant materials are not efficiently extracted from the surface. The cleaning is carried out not only in processing units, but also during raw milk production, storage, transport and marketing. When the surface residue of the disinfectant comes into contact with the surface, milk is also polluted. The production of lactic acid bacteria is particularly negatively affected by these residues. For this purpose, rinsing with water after cleaning and disinfection will produce a more positive outcome if hot water and steam are observed.

2.3.4.Pesticides

Pesticides are referred to as chemical compounds used in agricultural practices to destroy pests and harmful diseases and to fight diseases, and pesticide residues are referred to as drug residues that are transferred to milk and milk products. It is possible to research them in 6 groups: Insecticide (bee killing), Fungicide (fungicide parasite), Weeds (Hetbicide), Molluscicid (A mollusc of insects), Nematocide (Efficient on parasites), Rodenticide (Rodent Against).

Arsenic, nicotine sulphur and copper drugs were used against toxic species in agriculture until the 1940s. They then used DDT and organic phosphorus compounds. In humans and animals, the anticipated toxic effects of pesticides expected from pesticides should not be observed. The residues have been proven to be dangerous to human health. Pesticides have been determined to maintain their property in the soil for a long time. For instance, within 10 years, DDT continues its operation, 8 years dieldrin, 6.5 years lindan. Urine and feces do not excrete water solubles, whereas oil solubles are contained in fatty tissue. The direct use of pesticides in milk and dairy products does not occur. The pesticides are typically taken with food from dairy animals. It moves through the blood to milk, then to people. Residues remaining in the soil accumulate in plant tubers such as end-beet patatoes and in the leaves, depending on the conditions of use.

In the food crops of grasslands, drinking water, pesticides can exist. Pesticide residues that penetrate the body of the animal through the skin contaminate milk and humans from there. If you drug parasites in the barn and if the animals are taken to the barn immediately, or if you do it moves through the skin of the animal while they are inside. Medicines used to control animal parasites often cause bloodstream transmission. Also causing transmission is the use of contaminant additives in composite dairy items. With respect to pesticide residues, the technological processes applied to milk are not very successful. If it is transferred to the milk, it will certainly move to the product. Strict monitoring of this topic is needed in the case of chronic poisoning and environmental contamination in humans.

The Sub-Committees of the Codex Alimentarius Commission on pesticides and drug residues of the FAO/WHO related committees have identified 85 pesticides in milk and products and have introduced restrictions on their residual levels (42).

2.3.5.Mycotoxins

Mycotoxins are mold metabolites that cause pathological alterations in humans and animals such as mutagenic, carcinogenic, teratogenic, acute and vetoxic results. The most vulnerable group for mycotoxin contamination is milk and dairy products. Mycotoxins, which are the main milk pathogens, are Aflatoxin M1 and aflatoxin B1. In 2 forms, contamination takes place:

Indirect contamination: Containment occurs as dairy cattle eat food. Aflatoxin B1 is synthesized in agricultural products (oilseeds, cereals, dried coconut) by Aspergillusflavus and Aspergillus paraciticus, which are used as feed and feed additives under appropriate conditions of water activity and nutrient temperature. M1 is the 4th hydroxy derivative of B1 in milk. For this reason, in laws and regulations, limits on aflatoxin levels have been imposed.

Direct contamination: Dairy product contamination. In particular, aflatoxins, both of which profit from cheese maturation and are the result of unwanted cheese mold contamination. In mold-ripened cheeses, some strains of Penicillium roqueforti and Penicillium camemberti are used. Mycotoxins such as mycophenolic acid, rocfortine, cyclapiazonicacid are found in these types of cheeses. The other mycotoxins in the cheeses are also ocratoxin A secreted by Penicilliumssp types, penicillic acid and patulin. Other than these, sterigatocystin is also a contaminant found in hard cheeses, synthesized by Aspercillus versicolor. Food can also contain toxins from fusarium fumonisin B1 and B2 (42).

2.4.Analysis Methods Used in Monitorıng Drug Residues

In animal waste, there are various and distinct types of drug residues, and many studies are needed for effective monitoring. The use of screening assays is important for this purpose (43).

A long-standing antibody-antigen response is applied in the identification and characterization of microorganisms. When assessing food pollutants, such as mycotoxins, pesticides or low molecular weight veterinary medicines, immunological approaches are favored. A important mechanism for the rapid detection of all pathogens is the antigen-antibody reaction. Some devices, while others are easy to use are automated at high speed (44). You can classify these tests as follows:

2.4.1.Latex Agglutination Tests

For rapid serological identification or identification and typing of pure bacterial culture isolates, antibody coated and dyed latex or colloidal gold particles are used. Following antigen and antibody conjugation, visible agglutination is produced. The checks for reverse latex agglutination are for soluble antigens and are commonly used to look for toxins (44).

2.4.2.Automatic and Manual ELISA Methods

The use of Enzyme-Linked Immunosorbent Assay (ELISA) systems is the most common use. Due to the use of antibodies produced according to the target molecule, this technology is extremely sensitive. The outcomes of the study are accurate due to its high orderability. A large number of samples can be tested for different drug residues as soon as simple sample preparation procedures are available. The antibody is labeled with an enzyme in this process and the immunological reaction is measured as an outcome of enzymatic activity. The ELISA test is the most widely used sandwich ELISA process, although various types exist, such as direct, indirect and sandwich ELISA. To detect pathogenic microorganisms and toxins, many ELISA tests have been developed Several ELISA kits currently in use have a high standard and automatically improve speed and reliability for their work and minimize human error (44).

2.4.3.Lateral Migration Immunoassay Method

The use of Laterel Flow Technology, which is focused on the antigen-antibody relationship, is another advancement in the immunology field. It's a real rapid test. Bacillus anthracis was designed to quickly detect and recognize different samples of pathogens such as E. Coli 0157, Influenza with Salmonella, Listeria and Avian (44).

2.4.4.Immuno-Magnetic Separation (IMS) Technology

By distinguishing pathogens from the grains, the IMS method saves at least one day of enrichment and pre-enrichment measures. A variety of diagnostic (ELISA) systems have recently been combined with the immuno-magnetic coating system. The incubation time was shortened and the sensitivity increased in this case (44).

2.4.5.Fast Scan Kits

For the detection of antibiotic residues, there are some quick screen kits available. One of them is the MeRA test. Some groups of antimicrobial agents, such as beta-lactams and tetracyclines, are heat sensitive; at the growth temperature of thermophilic bacteria, molecules belonging to these chemical classes are soon inactivated. The MeRA test requires a rapid pre-incubation stage that enables Geobacillus stearothermophilus to expand and multiply. Following this step, interaction is carried out at room temperature between the vegetative form of G.stearothermophilus and heat-sensitive antibiotics, if present in the sample. Finally, a final incubation is performed on the test tubes and a color change is observed (44).

2.4.6.High Performance Liquid Chromatography (HPLC)

It is possible to classify chromatographic methods as separation techniques involving mass transfer between the stationary phase and the mobile phase. One of these techniques, liquid chromatography, although this technique was discovered at the beginning of the 1900s, since the 1960s it has been the focus of extensive research for the development of residue analysis methods. HPLC is a very sensitive process in which it is simple and fast to isolate the liquid phase soluble chemical material mixture into its components. Today, in many regions, HPLC is commonly used. Chemical isolation, purification, detection and determination of concentration are its key uses (44).

2.5. Antibiotic Use in Animals Raised for Milk and Dairy Products

Antibiotics are used in animals raised for milk and dairy production for the purpose of preventing diseases, increasing growth and feed utilization. Antibiotics used in animals can be excreted with feces and urine on the other hand it can pass into milk. Especially the presence of antibiotic residues in the compost product used for fertilizer is a reason for the plants grown in this environment to carry these residues, and also causes the host microflora in the soil to be exposed to antibiotic stress (45).

In a study conducted between 2013 and 2014 in 50 states of the United States and Puerto Rico, antibiotic residues were detected in a total of 3,680,185 raw and

pasteurized milk samples and 703 dairy products (46). Antibiotics are generally used for the treatment of digestive system diseases (36%), acute bronchitis (25%) and foot diseases (16%) in dairy cattle. In this context, a total of more than twenty different antibiotics are used, including beta-lactams, streptomycin, flophenicol and tetracyclines. Among such antibiotics, those containing beta-lactam are widely used especially against mastitis. On the other hand, it is known that 70% of the farms in the USA add oxytetracycline and neomycin to their calf food. Research results show that antibiotics are used for treatment and preventive purposes in dairy cattle. An average of 45 mg of antibiotics are used annually for a kg of meat increase in dairy cattle. This amount is expected to increase by 67% by 2030. It is reported that the increase originates from countries such as Brazil, Russia, India, China and South Africa (47).

2.6. Microbial Criters for Cheese

The main bacteria detected in cheeses are Coliform group bacteria and E. coli 0157: H7, Salmonella species, Listeria monocytogenes, Staphylococcus aureus, Aerobic mesophilic bacteria, yeast and molds. In particular, the characteristics of cheese products contaminated with Enterobacteriaceae strains can vary, as well as causing poisoning and diseases for the consumer (25).

Microbiological values of cheeses are presented under the heading of cheese, melted cheeses and melted cheese products in the related appendix of the Turkish Food Codex Regulation Microbiological Criteria Communiqué (9) (Table 2) in the Milk, Dairy and Milk-Based Products Section (25).

Table 2: Microbial Criters for Cheese

Food	**Microorganisms**	**Sampling Plan**		**Limits**	
		n	**c**	**min**	**max**
Cheese	Ferment and Mold	5	2	10^2	10^3
	Enterobacteriaceae	5	0	$<10^1$	
	S. aureus	5	2	10^1	10^2
	Salmonella spp.	5	0	0/25 g-mL	

	L. monocytogenes	5	0	0/25 g-mL
	E. coli O157:H7	5	0	0/25 g-mL

2.7.Beta-Lactam Group of Antibiotics

Beta-lactam antibiotics are a wide variety of classes of antibiotics with various pharmacokinetic properties, chemical compositions, antibacterial domains. The existence of a beta-lactam ring in all of its structure, the mechanisms of action, and the methods of resistance against them are common features of the members of this community. Included in this group are antibiotics; combinations of penicillins, cephalosporins, monobactams, carbapenems, and betalactam/betalactam inhibitor. Both beta-lactam antibiotics; Blocking penicillin-binding protein (PBP) transpeptidase activity responsible for bacterial cell wall synthesis, inhibiting the synthesis of peptidoglycan. As a result, bacteria will not synthesize and die from the lysis of the cell wall. Antibiotics with beta-lactam are bactericidal (49).

2.7.1.Penicillins

Fleming first accepted in 1928 that an antibacterial agent that caused staphylococcus lysis was secreted by a fungus called Penicillium notatum, and it was called penicillin. It was then purified and clinically used in the 1940's (48).

2.7.2.Cephalosporins

By considering the range of action, cephalosporins are classified into four classes. Roughly speaking, Gram-negative activity rises, decreases and Gram-positive activity increases as the number of generations increases (48).

2.7.3.Monobactatns

Aztroenam is the only example of clinical use of this group of antibiotics. It has good activity with Gram-negative. There is no activity, however, against Gram-positive and anaerobic bacteria. It is an antibiotic with a narrow scope. Therefore in

polymicrobial infections and in the empirical treatment of severe infections of unknown cause, it should not be used alone. The major clinical applications are bacteremia/sepsis, nosocomial pneumonia, nosocomial urinary system infections, osteomyelitis, and septic arthritis caused by susceptible Gram-negative bacteria (48).

2.7.4.Carbapenemler

It is the community with the broadest effect among the currently available antibiotics. In this category, two agents in clinical use were imipenem and later meropenem. No major difference is made between domains. Meropenem Gram-negative, gram-positive activity of imipenem is significantly greater. There is no overlap between the operations of the self-airway (48).

2.7.5.Beta-Lactam/Beta-Lactamase Inhibitör Combinations

The development of beta-lactamase enzymes that inactivate these antibiotics is the most common mechanism of resistance that bacteria use to beta-lactam antibiotics. By enzymatic hydrolysis, these enzymes can neutralize one or more groups of penicillin, cephalosporin, monobactam or carbapenem. There are several common and different types of beta-lactamase enzymes that are synthesized under plasmid or chromosome regulation by various bacteria. More and more, they are a clinically important issue. For this reason, beta-lactamase inhibitors have been created. It is understood, however, that these inhibitors are not effective against all kinds of enzymes (48).

3.MATERIAL AND METHOD

3.1.Material

100 different cheese samples were collected for his study. All of them are bought from the various dairy farms. Accepted samples were transported to the laboratory under steril conditions in cold chain conditions and stored at 2-8 °C until the day of operation.

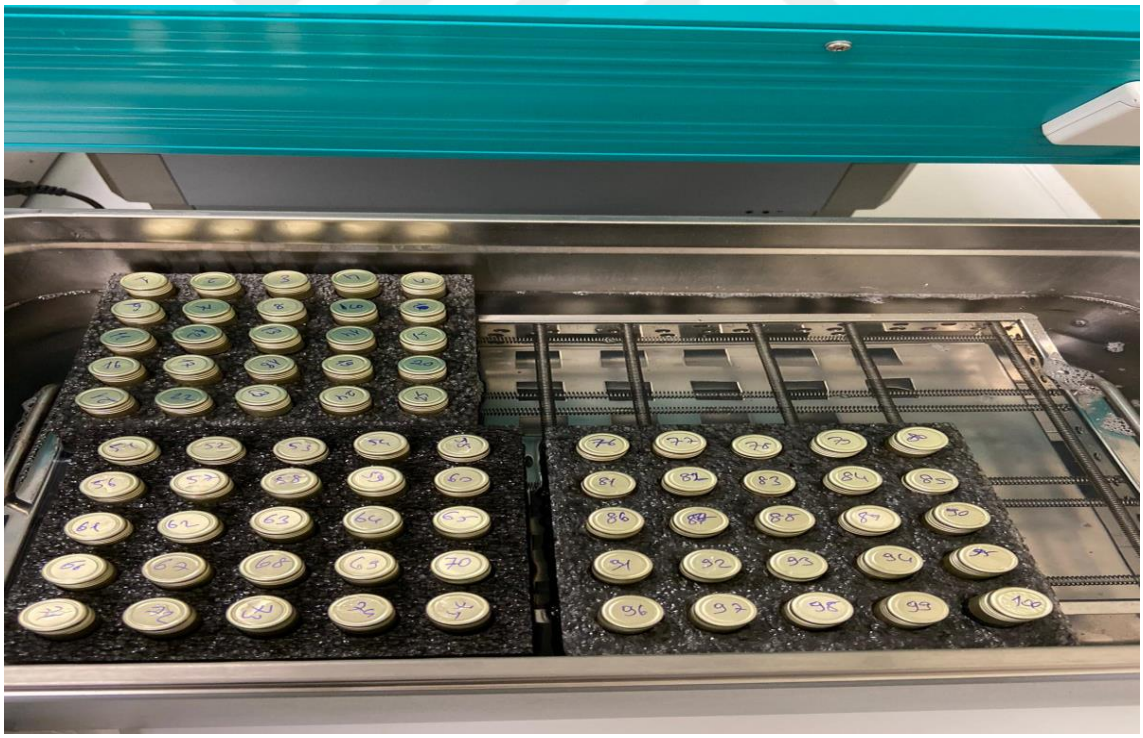
Figure 1: Samples before test

3.2. Method

Firstly, MiRA test qualitatively determined the antibiotic residues in the samples, and then elisa quantitated the beta lactam dose. The antibiotic residues of the beta lactam group are commonly found in milk and dairy products.

Figure 2: Adding 2mg. sample for Mira Test

Figure 3: Mira Test water bath



3.2.1 Antibiotic Residue

The screening test was first conducted with the Mira Test. It is a microbiological procedure for the identification of traces of antimicrobial compounds in milk that contains *Geobacillus stearothermophilus* spores.

Some classes of antimicrobial agents, such as beta-lactams and tetacyclines, are heat responsive. At the growth temperature of thermophilic bacteria, compounds belonging to these chemical groups are inactivated temporarily. The MiRA test requires a rapid stage of pre-incubation that enables G to expand and multiply. Stearothermophilus. Stearothermophilus The relationship between the vegetative form of G follows this step. Stearothermophilus and heat-sensitive antibiotics are administered at room temperature if they are found in the sample. Finally, the test tubes undergo a final incubation phase. This MiRA test incubation stage is a crucial step in achieving extremely low limits of detection. The steps at work are listed below:

1) The Mira test kit is removed from the refrigerator to wait for the room temperature.

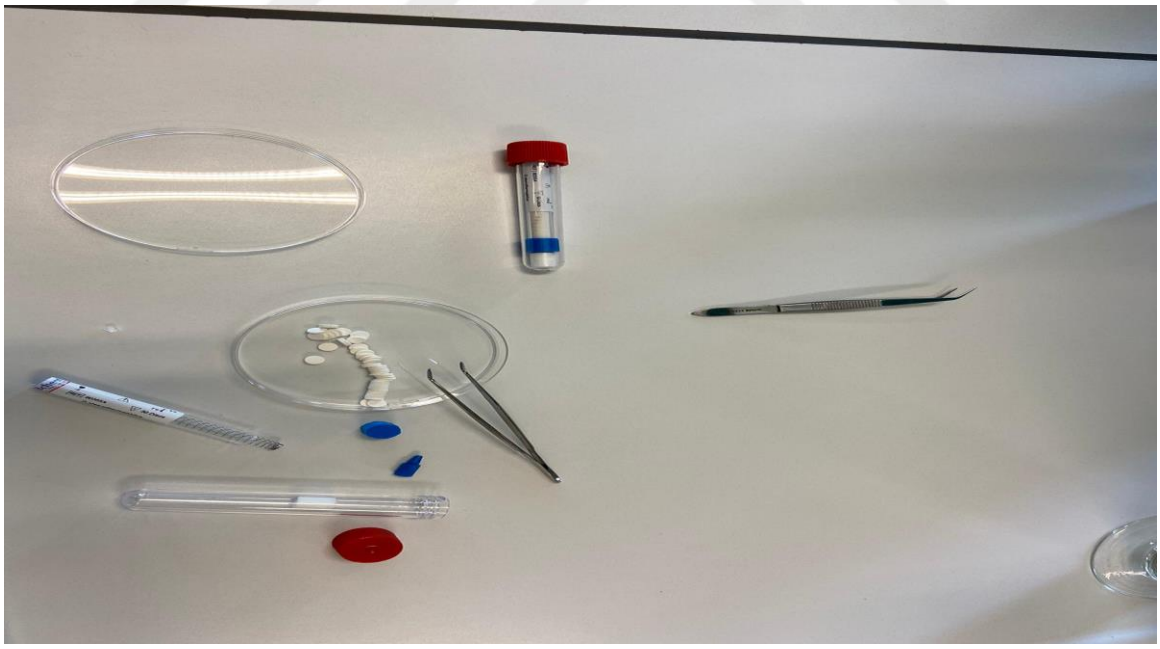
2) The empty tubes were all numbered.

3) 2 mg Examples of the cheese samples to be tested were taken and 6 ml of purified water was added to the 10 ml test tube (cheese/water = 1mg/3 ml).

4) The samples were homogenised for 3-5 seconds by shaking.

5) With each test bottle, a *G. stearothermophilus* disk was added.

Figure 4: Part of Mira Kit



6) The sports disk was pre-incubated at 64 °C with a 20-min solution.

7) 1ml of the homogenized test sample was added to the incubated solution after the incubated solution hit room temperature after incubation.

8) A test sample of 3 to 3 was incubated. 5 hours at 64 ° C in a hot bath or thermoblock.

9) If after observing a color change in the tube, there is no discoloration (Blue-Green Color): the concentration of the antimicrobial agent in the sample is accepted above the limits of detection. (yellow color): No antimicrobial agent or concentration is accepted below the detection limits if there is a color change.

3.2.2.Elisa Test

In addition to the Mira test, the residue levels of the beta lactam group of antibiotics were examined in the cheese samples.

Beta Lactam Elisa Test Working Method

A standart working method for Elisa test is:

Preparation of the samples: 4 gr of cheese 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 for15 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."

(1)

4.RESULTS

4.1.Qualitative Antibiotic Residue Test Results

Antibiotic residues are detected qualitatively using the MiRA test. This test kit will find traces of antibiotics, including tetracycline, and other antibiotic groups, such as macrolides, in addition to the beta lactam group. Table 3 lists these antibiotics as well as the kit's detection limits.

Table 3: Max. Acceptivity Limits of Beta-Lactams

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 the MiRA test results, as we can see in the table 4, positives were found in 6 of the 100 cheese samples. Number 21,22,23,24,25 and 28 samples were found positives which exceeded the acceptamble residual limits.

Table 4: Mira Test Results

Unpacked Cheese Sample No	MiRA TEST RESULT	Unpacked Cheese Sample No	MiRA TEST RESULT
1	NEGATİF	**51**	NEGATİF
2	NEGATİF	**52**	NEGATİF
3	NEGATİF	**53**	NEGATİF
4	NEGATİF	**54**	NEGATİF
5	NEGATİF	**55**	NEGATİF
6	NEGATİF	**56**	NEGATİF
7	NEGATİF	**57**	NEGATİF
8	NEGATİF	**58**	NEGATİF
9	NEGATİF	**59**	NEGATİF
10	NEGATİF	**60**	NEGATİF
11	NEGATİF	**61**	NEGATİF
12	NEGATİF	**62**	NEGATİF
13	NEGATİF	**63**	NEGATİF
14	NEGATİF	**64**	NEGATİF
15	NEGATİF	**65**	NEGATİF
16	NEGATİF	**66**	NEGATİF
17	NEGATİF	**67**	NEGATİF
18	NEGATİF	**68**	NEGATİF
19	NEGATİF	**69**	NEGATİF
20	NEGATİF	**70**	NEGATİF
21	POZİTİF	**71**	NEGATİF
22	POZİTİF	**72**	NEGATİF
23	POZİTİF	**73**	NEGATİF
24	POZİTİF	**74**	NEGATİF
25	POZİTİF	**75**	NEGATİF
26	NEGATİF	**76**	NEGATİF
27	NEGATİF	**77**	NEGATİF
28	POZİTİF	**78**	NEGATİF
29	NEGATİF	**79**	NEGATİF
30	NEGATİF	**80**	NEGATİF
31	NEGATİF	**81**	NEGATİF
32	NEGATİF	**82**	NEGATİF
33	NEGATİF	**83**	NEGATİF
34	NEGATİF	**84**	NEGATİF
35	NEGATİF	**85**	NEGATİF
36	NEGATİF	**86**	NEGATİF
37	NEGATİF	**87**	NEGATİF

38	NEGATİF	**88**	NEGATİF
39	NEGATİF	**89**	NEGATİF
40	NEGATİF	**90**	NEGATİF
41	NEGATİF	**91**	NEGATİF
42	NEGATİF	**92**	NEGATİF
43	NEGATİF	**93**	NEGATİF
44	NEGATİF	**94**	NEGATİF
45	NEGATİF	**95**	NEGATİF
46	NEGATİF	**96**	NEGATİF
47	NEGATİF	**97**	NEGATİF
48	NEGATİF	**98**	NEGATİF
49	NEGATİF	**99**	NEGATİF
50	NEGATİF	**100**	NEGATİF

The samples studied with this test kit are evaluated according to the color change. At the end of the incubation, the blue-green tube was positive; yellow ones are considered as negative.

Figure 4: All samples after Mira Test

Figure 5: Examples of Negative Result

4.2.Quantitative Antibiotic Residue Test Results

The findings of the beta lactam elisa test were reported to be identical to those of the MiRA test. The acceptable beta lactam antibiotics limit is exceeded in samples 21,22,23,24,25, and 28. The maximum consumption of beta lactam antibiotics in milk products is 4 ppb, according to national and international appropriate limits.

Table 5: Elisa Test Results

Unpacked Cheese Sample No	Elisa Test Result	Unpacked Cheese Sample No	Elisa Test Result
1	0,21	**51**	0,21
2	0,21	**52**	0,21
3	0,21	**53**	0,21
4	0,21	**54**	0,21
5	0,21	**55**	0,21
6	0,21	**56**	0,21
7	0,21	**57**	0,21
8	0,21	**58**	0,21
9	0,21	**59**	0,21

10	0,21	**60**	0,21
11	0,21	**61**	0,21
12	0,21	**62**	0,21
13	0,21	**63**	0,21
14	0,21	**64**	0,21
15	0,21	**65**	0,21
16	0,21	**66**	0,21
17	0,21	**67**	0,21
18	0,21	**68**	0,21
19	0,21	**69**	0,21
20	0,21	**70**	0,21
21	4,6	**71**	0,21
22	4,9	**72**	0,21
23	5,4	**73**	0,21
24	7,6	**74**	0,21
25	5,4	**75**	0,21
26	0,21	**76**	0,21
27	0,21	**77**	0,21
28	6,9	**78**	0,21
29	0,21	**79**	0,21
30	0,21	**80**	0,21
31	0,21	**81**	0,21
32	0,21	**82**	0,21
33	0,21	**83**	0,21
34	0,21	**84**	0,21
35	0,21	**85**	0,21
36	0,21	**86**	0,21
37	0,21	**87**	0,21
38	0,21	**88**	0,21
39	0,21	**89**	0,21
40	0,21	**90**	0,21
41	0,21	**91**	0,21
42	0,21	**92**	0,21
43	0,21	**93**	0,21
44	0,21	**94**	0,21
45	0,21	**95**	0,21
46	0,21	**96**	0,21
47	0,21	**97**	0,21
48	0,21	**98**	0,21
49	0,21	**99**	0,21
50	0,21	**100**	0,21

5.DISCUSSION AND CONCLUSION

Since drug residues detected in animal products cause undesirable effects for consumer health such as allergies, cancer, it has become increasingly important to perform residue analysis in these products. Studies that conducted to determine the legal holding time required for the purification of foods from antibiotic residues, discontinuation of drug treatment and the limits of the residue amounts allowed in foods are important for food safety and consumer health (Temamoğulları and Kaya, 2010). Therefore, in this study, the presence of beta lactam antibiotic residues in cheese was investigated and its importance in terms of consumer health was evaluated. In addition, the data related to the method and analysis results used in the study were compared with similar methods and results.

In our study, the presence of beta-lactam antibiotic residue in cheese collected from various dairy farms was investigated by ELISA method. As a result of the analysis, the presence of beta lactam antibiotic residue was detected in 6 samples at varying concentrations and above the maximum permissible residue limits.

Chung et al. (2009) conducted a study in order to determine the presence of residues of sulfonamide and quinolone group drugs in cow and goat milk. According to this study, they found that by using microbiological tests 21 of 269 samples and by HPLC analysis 4 of them contained residues.

In a study conducted by Temiz and Öner (1988), a total of 335 samples of raw milk from two separate dairies, one belonging to the public sector and the other belonging to the private sector in Ankara, were examined in terms of antibiotic residue. It was determined that 6.04% of the milk samples taken from the dairy business belonging to the public sector contain different levels of penicillin. It has been revealed that 14.38% of the milk samples belonging to the private sector contain penicillin and 1.31% contains other substances.

Liman and Karabacak (2003) investigated the residues of tetracycline and streptomycin in 200 raw milk samples collected from the milk collection center in Kayseri by ELISA method between June 2001 and March 2002. Various amounts of tetracycline residues were detected in 19 (9.5%) of 200 tank milk. All of the milk samples were negative for streptomycin and dihydrostreptomycin. The findings of the

study concluded that the use of antibiotics in animals is extremely common, and the milk of the animals treated with the drug is consumed without complying the waiting period, so that the people of our country are faced with many dangers through the foodstuffs containing drug residues, and this may negatively affect the country's economy.

In the study of Temamoğulları and Kaya (2010), they conducted a residue analysis of danofloxacin, ampicillin, enrofloxacin, florphenicol, erythromycin, amoxicillin and cloxacillin in a total of 240 samples consisting of 120 raw and pasteurized milk. For residue analysis, they used bioautographic (DTC / Biootographic) method based on DTC and microbiological disk diffusion technique. According to the results, ampicillin residue was found in 1 pasteurized milk sample. It was emphasized that no antibiotic residue was detected in 239 samples.

In a study conducted in China (Han et al., 2013), 180 UHT milk samples were collected from 25 different cities and various markets and antibiotic residues were investigated. According to this study; sulfamethazine, sulfonamide and quinolone antibiotic levels were found to be high.

According to a study conducted in Croatia (Nina et al., 2011), various antibiotics (chloramphenicol, penicillin, tetracycline, sulfonamide, betalactam, quinolones) were investigated by microbial and immunoassay methods in a total of 1259 unpasteurized milk for 3 years. According to microbial screening; A positive result was obtained in 36 samples, and a positive result was obtained by immunoassay method. In the verifications made by HPLC method, antibiotic residues (12 μg / kg penicillin, 19 μg / kg amoxicillin and 1671 μg / kg tetracycline) were determined above the MRL.

In a study qualitatively investigating the beta-lactam residues in milk samples consumed in Sanliurfa; It has been stated that 64 of 300 milk samples contain beta-lactam antibiotic residues and 32 of them contain other residues with antimicrobial activity (Ardıç and Durmaz, 2006).

Kaya and Filazi (2010) collected 240 milk samples for their study in which they investigated antibiotic residues in raw and pasteurized milk products sold in Ankara and analyzed them in terms of penicillin G, oxytetracycline, gentamicin, streptomycin and neomycin. In one of pasteurized milk samples, 150.4 μg/ L oxytetracycline, 33.5 μg/ L penicillin G in the other one and in one raw milk sample 7688.4 μg/ L neomycin have

been identified. They stated that it was above the maximum allowable residue limits in Turkey and the European Union.

In a study where the performances of 3 different antibiotic test kits based on different principles were evaluated in raw milk samples artificially contaminated with beta-lactam group antibiotics which are penicillin G, ampicillin, amoxicillin and cloxacillin. It has been reported that the detection limits of the methods by HPLC are 8 μg / kg for ampicillin, 8 μg / kg for amoxicillin, 6 μg / kg for penicillin G and 11 μg / kg for cloxacillin. In the study, the detection limits for ampicillin, amoxicillin, and penicillin G obtained with chromatographic methods are above the maximum residue limits for Turkey and Europe (Torlak et al., 2012).

According to the Turkish Food Codex "Regulation on Classification of Pharmacologically Active Substances and Maximum Residue Limits in Animal Origin Foods ", the maximum residue limits for beta-lactam groups of antibiotics of penicilin which are ampicillin, amoxicillin, benzylpenicillin, cloxacillin, dicloxacillin, nafcillin are 4 ppb. When the data obtained in the study were examined, it was determined that 6 samples were above 4 ppb level, and it was determined that they were above the maximum residue limits allowed in dairy products in terms of beta-lactam antibiotics. This situation can be evaluated as the milk which obtained from the animals given antibiotics used for the cheese production before the required withdrawal period is expired.

In addition to being one of the basic foodstuffs, milk and dairy products are also among the dangerous products in terms of veterinary medicines, pesticides, mycotoxins, heavy metals, genetically modified organisms, dioxin and related chemical residues. This contaminants that can be present in milk derive from origins that are manufacturing or agricultural. (20).

In human fat tissue, most of the compounds that illustrate the ability to dissolve in oil are readily absorbed. For many countries, milk and dairy products are an essential source of nutrition and are an invaluable source of food for people of all ages. However, studies suggest that residues of milk and milk products, if not produced under the required conditions, can adversely affect public health and prevent negative external factors from occurring. In this respect, the FAO and WHO, as well as the official agencies of several nations, are seeking to take steps. The Ministry of Food, Agriculture

and Livestock is the designated organization for the disposal of animal products, including milk and dairy products, in our country. For the safety of public health, successful inspections within the framework of the residual surveillance program being carried out by the appropriate ministry are necessary. In this respect, however, veterinarians, animal breeders, suppliers of animal products, drug manufacturers and distributors have significant positions and obligations. (50).

Milk containing antibiotics triggers resistance to pathogenic microorganisms. They induce allergic reactions in certain persons and therefore cause negative effects. (51).

The risk of contamination caused by residues caused by drugs approved for use in foodstuffs is very low relative to the risk of antibiotic resistance. Nevertheless in order to avoid exposure to residues at hazardous levels, the EU has set overall residue thresholds for veterinary drugs in animal feedstuffs.

There are several veterinary medicine laws in the European Union, as well as revisions and changes to these regulations. The use of hormonal, thyrostatic, and β-agonists in animals is limited by simple laws, providing legal grounds for prohibiting growth help. In the case of the old directive on medicinal products which are permitted to be used a list of approved medicinal products and their maximum residue limits has recently been revised. Many of these directives are consistent with the rules of Turkey. Maximum residue levels in animal food products have been identified in the Turkish Food Codex communiqué. (52).

When we look at the Mira test results in this study, it is seen that there are six positive samples. These samples are positively detected with Elisa, too. The same samples were also found to be positive in experiments with beta lactam elisa kit. At this point, the test performance of Mira chitin was determined to be appropriate for beta lactam.

The antibiotic residue issue in milk and dairy products still continues when we check the literature on this subject, the levels of beta lactam in the lower limits of our sample have been assessed as promising. However these findings can be extended with more explanations and systematically reviewed in terms of hygiene standards for milk and milk products. Since different contaminants such as mycotoxins, pesticides, hormones and heavy metals need to be tested for not only antibiotic residues, but also food substances.

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