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COMPARISION OF TWO DIFFERENT METHODS FOR THE DETERMINATION OF RESIDUES OF TETRACYCLINE ANTIBIOTICS IN BEEF AND CHICKEN MEAT

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ABSTRACT

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NİL KOÇ

Yeditepe University

Faculty of Health Science

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In this study, it is aimed to determine the presence of antibiotics in chickens and calves, which are important for the nutrition due to the protein, fat, minerals and vitamins in the composition of the meat products. By evaluating the factors such as the amount of antibiotic residues, usage areas of antibiotics and frequency, it was tried to contribute to the work done in terms of food safety and community health.

Even if there is a small amount of meat and meat products in their diet, the antibiotics may accumulate in the body, resulting in resistance to antibiotics in some bacterial strains, which may lead to a reduction in the effectiveness of antibiotics in human treatment.

For the study, 20 poultry meat, 20 pcs of calf meat, which were sold outdoors in various sterile conditions, were collected from various butcheries in Samsun province and delivered to the laboratory in the cold chain. It was stored at -20 ºC until the day of operation. 12 of the beef samples taken from the breast were collected from a slaughter house on different days, 5 of them were butcher, and 3 of them were collected from the meat department of the big markets.

Firstly, the antibiotic residues in the samples were qualitatively detected with the MeRA Test. MeRA test; Is a microbiological test containing Geobacillus stearothermophilus spores for the detection of antimicrobial substance residues. Tetracycline levels were quantitatively analysed by elisa (R-Biopharm) for the samples detected positively as a result of this test.

Of the 20 red meat samples studied by the MeRA test, 10 were used; Of the 20 chickens, 19 were positive. That is, of the 40 meat samples studied in total, 29 were found above the acceptable limits. Positive samples were then examined for tetracycline levels by elisa. According to this test, tetracycline was found above the acceptable limits of 4 red meats and 17 chicken meat.

Our study is a regional study, with more samples and samples in different regions should be expanded. However, it can be said that it is necessary to ask about the safety of the products that are sold outdoors.

Keywords: Antibiotics,antibiotic residues, elisa test, MeRA test

ÖZET

DANA VE TAVUK ETİNDE TETRASİKLİN ANTİBİYOTİĞİ KALINTILARININ TAYİNİNDE KULLANILAN İKİ FARKLI YÖNTEMİN KARŞILAŞTIRILMASI

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Bu çalışmada, et ürünlerinin tümünde olduğu gibi bileşiminde bulunan protein, yağ, mineral ve vitaminler nedeniyle beslenmede önem taşıyan tavuk ve dana etindeki antibiyotik varlığının farklı yöntemlerle tespit edilmesi amaçlanmıştır. Antibiyotik kalıntıların miktarı, antibiyotiğin kullanım alanları ve sıklığı gibi faktörler değerlendirilerek de gıda güvenliği ve toplum sağlığı yönünden yapılan çalışmalara katkı sağlanmaya çalışılmıştır.

Et ve et ürünlerinin beslenmedeki yeri göz önünde bulundurulduğunda az miktarda da olsa, devamlı antibiyotik alınması sonucu, bu antibiyotikler vücutta birikmekte, bazı bakteri suşlarında bu antibiyotiğe karşı direnç meydana gelmekte, bu durum antibiyotiklerin insan tedavisindeki etkinliğinin azalmasına neden olabilmektedir.

Çalışma için, Samsun ilinde çeşitli kasaplardan steril şartlarda açıkta satılan 20 adet tavuk eti, 20 adet dana etinden 50 şer gram toplandı ve soğuk zincirde laboratuvara ulaştırıldı. Çalışma gününe kadar -20 ºC'de saklandı. Göğüs bölümünden alınan dana eti örneklerinin 12 tanesi farklı günlerde bir mezbahadan, 5 tanesi kasaptan, 3 tanesi ise büyük marketlerin et reyonundan toplanmıştır.

Öncelikle MeRA Test ile örneklerdeki antibiyotik kalıntıları kalitatif olarak saptandı. MeRA test; içerisinde antimikrobiyal madde kalıntılarının tespiti için Geobacillus stearothermophilus sporları içeren mikrobiyolojik testtir. Bu test sonucunda pozitif olarak saptanan örnekler için tetrasiklin düzeyleri kantitatif olarak elisa (R-Biopharm) ile çalışılmıştır.

MeRA test ile çalışılan 20 kırmızı et örneğinden 10 tanesi; 20 tavuk örneğinden ise 19 tanesi pozitif olarak saptanmıştır. Yani toplamda çalışılan 40 et örneğinden 29 tanesi kabul edilebilir limitlerin üstünde saptanmıştır. Pozitif çıkan örneklerin daha sonra elisa yöntemi ile tetrasiklin düzeylerine bakılmıştır. Bu teste göre 4 adet kırmızı ette, 17 adet tavuk etinde kabul edilebilir limitlerin üstünde tetrasikline rastlanmıştır.

Çalışmamız bölgesel bir çalışma olup, daha fazla örneklem ile daha farklı bölgelerdeki örneklemler genişletilmelidir. Ancak bu çalışma ile açıkta satılan ürünlerin güvenliğinin sorulması gerekli olduğu söylenebilir.

Anahtar Kelimeler: Antibiyotikler, antibiyotik kalıntı, elisa test, MeRA test

I. GENERAL INFORMATION and INTRODUCTION

To treat and prevent diseases, to faster the growth, to increase productivity and the utilisation of forage, control of parasitic disease and to support nutrition, lots of medicine, vitamin, minerals and hormone are used. Due to the different reasons, nearly all of the animals are exposed to medicine in their course of live (1).

80 % of the antibiotics sold in the USA are used in chicken and meat farming. Most of them are used to develop the growth of healthy animals or to prevent the diseases in unhealthy situations. Antibiotics are being used since 1940s, from that time on there has been a dramatic decrease in deaths and diseases caused by infections. According to US origin Interagency Task Force on Antimicrobial Resistance (ITFAR), over usage of antimicrobial medicines results in counter-resistance to medicine and usage of antibiotics widespread and for long time is a threat to the public health. Using medicine in animals is now an indispensable practice. Consumers Union has declared that usage of antibiotics in growing and edible animals has ill effects on public health. Potential existence of bacteria resistant to antibiotics in meat products and also migration of these bacteria to environment resulting in illness for humans make risks human beings.

Lots of health organizations like "American Medical Association, American Public, Health Association, Infectious Disease Society of America and World Health of Organization" calls for a decrease in the usage of antibiotics in animals for food production (2).

Antibiotics are widely used in farm animals to increase their productivity and growth and to treat infective illnesses. B-lactam, tetracycline, chloramphenicol, macrolide, spectinomycine, lincosamide, sulfonamide, nitrofuran, nitroimidazols, trimethoprim, polymyxin, quinolone and macrocyclic are the most used medicines for the mentioned objectives. However, as a result of inappropriate and illegal usage these medicines, there arises residues in meat, milk, egg, honey and the other edible tissues of the animals (3).

Existence of antibiotic residues may result in allergic reactions in human beings; even it may result in serious situation such as increase in antibiotic resistance in pathogenic bacteria that can cause dangerous health problems. In addition to this, residues can cause decrease in the quality of fermented foods. Because of all these

serious and dangerous problems, it is important for consumers to identify the residues in food products(3).

Today, lots of advanced analytical methods capable of quantitative measuring are used for the identification of antibiotic residues in different food products. Elisa, Charm II, GC, HPLC and LC-MS/MS are among the used methods. To provide an effective food security, unconscious usage of antibiotic should be avoided and potential antibiotic residues in foods should be frequently watched by a legal authority (3).

More than 20 years ago scientists have concluded that there is a connection between the decrease in the medicines used for human beings in medicine and the antibiotics used in animals.In 1988, the Institute of Medicine, until that time although frequently, has identified that Salmonella clones migrates to human beings through food products from farm animals, which are treated with antibiotics used lesser concentrations than the necessary curative amounts. Accordingly, human beings are exposed to infections caused by Salmonella bacteria (2).

Ten years later, the National ResearchCouncil states that there can be a connection between the usage of antibiotics in edible animals and growth of resistant microorganisms in those animals and dissemination of animal-borne pathogens.

In 2003, World Health Organization, Food and Agricultural Organization (FAO) ve World Animal Health Organization (OIE) states that there emerges ill effects in human health because of the resistant organism rising through antimicrobials that human beings do not use. Among those ill effects, rise in infections' severity, emergence of infections never occurred before, rise in the frequency of failed treatments and even deaths can be listed.

In 2010, Food and Drug Administration (FDA) and the Centre for Disease Control (CDC), proves that there is a connection between the routine usage of antibiotics for meat production and the decrease in the antibiotics efficiency for human beings. Dr. Thomas R. Frieden and managers of CDC has recorded that there is a strong scientific proof about the relation between antibiotic resistance in human beings and the usage of antibiotics in edible animals.

At the end of 2012, FDA states that rise in the amount of germ resistant bacteria as result of wrong and over usage of antimicrobial medicines is much more than the germ-sensitive bacteria. This rise brings about the increase in the number of people infected by those resistant bacteria.

In the same time period, FDA prohibited the usage of cephalosporin antimicrobial medicines in the food production obtained from animals. Exposition of animals that used for food production to antimicrobials indirectly threatens public health via people eating those foods containing germ resistant bacteria.

Despite all, despite the development of antibiotic resistant bacteria in the farm, industry of farm animals continues arguing that usage of in widespread necessary situations has not a significant impact on human health (2).

Finally, towards the end of 1970s, usage of penicillin and tetracycline as forage support has been prohibited. Today Turkey allows usage of avoparcin, bacitracin, phlavophospholipol, monencin, sypiramicin, virginiamycine (4).

One of the serious harms of penicillin type antibiotic residues in the animal source food is that it may cause derma reaction and even anaphylactic shock that can result in deaths. Tolerance levels regarding the medicine residues in the food show the amount in raw tissue and organs. It is stated that antibiotic residues in animal foods become harmless by transformation them to ineffective metabolites through cooking, roasting, frying or keeping in cold. However whatever the reason unconscious usage of antibiotics is risky for both human and animal health and also for economics (4).

I.1 Antibiotics

Antibiotics are substances that can inhibit the development of microorganisms or that can kill them. They are largely used for the prevention and treatment of infectious diseases. They are used in a curative way to secure the health and wealth of human beings and animals. Some of the antibiotics emerge through microorganism but today most of them artificially produced (5).

Within the context of national residue plan, antibacterial medicines including quinolone and sulphonamide group medicine are analysed in fisheries, milk and honey (6).

Chemotherapy is term first used by German researcher Paul Ehrlich at the end of 19^{th} century. It is used to identify the treatment way that prevents the development of protozoa, virus, bacteria, external and internal parasites or kill them without giving harm to the host. There are lots of factors that may cause the illness in the body such as Helminth, protozoa, mycetes, virus and insects; therefore, medicine used is also several. Pasteur in 1871, for the first time, mentioned about antibiosis that means the prevention of the factors resulting in illness or killing of these factors through the synthesis of

various microorganism within the culture medium. The term antibiotics is used for substances formed by microorganism such as myctetes, bacteria, actinomycites or sytnthetically prepared and the substances that prevents the development of bacteria or kill them even with very low densities. Like other medicines they may cause undesirable effects for the patient. Drug allergy, increase in the frequency and the intensity of undesirable effects as result of drug interaction, breakdown of the normal microflora balance, rise of resistance in the bacteria, immunosuppression or deterioration of immune system, tissue damage after injection and medicine residue in edible tissue and organs are among those undesirable effects (6).

Antibiotics can be classified according to chemical structures and operating mechanisms. Mainly, they are molecules having different functions within the same molecule. For this reason, in different Ph levels, antibiotics can be neutral, cationic or anionic. Fluoroquinolones, tetracycline, aminoglycosides, β-lactams, macrolides and amphenicol are some of those antibiotics (5).

I.1.1 Beta-lactam antibiotics

Beta-lactam antibiotics is a large group including lots of antibiotics having different chemical structures, different antibacterial effect area and pharmacokinetics features. Common features of the members of this group are the beta-lactam ring all have and the ways of resistance against them. Antibiotics in this group are penicillin, cephalosporin, monobactams, carbapenems and beta lactam beta lactamase inhibitor combinations(7).

All beta-lactam antibiotics operates via preventing peptidoglycan synthesis by blocking the trans peptidase activity of penicillin binding protein (PBP) responsible from the synthesis in bacteria. At the end, bacteria lacking epicyte synthesis is exposed to lysis and dies. Beta-lactam antibiotics have bactericidal effect (7).

Antibiotics have a crucial role in the treatment of bacterial infections. Spectacular researches on antibiotics and their intense usage are followed by the discovery of penicillin (5).

I.1.2 Penicillines

Penicillin is the first discovered and one of the most significant antibiotics. Its efficacy spectrum is narrow but as a result of the researches on penicillin main molecule; penicillin derivatives having wider efficacy spectrum such as ampicillin,

amoxicillin, aslocillin, carfenecillin and lots of semisynthetic penicillin derivatives have been discovered and began to be used in treatments (8).

A. Fleming introduced to the initial information about penicillin in 1928. He observed that bacteria circling the penicillin mould contaminant to staphylococcus colonies, do not multiply and called them antibiotics. He gets suspicious about the fact that the mould releases and forms a substance that prevents the reproduction of the staphylococcus and names this as penicillin. In 1956, Di Vigneaud has synthesized penicillin and a year later group of Batchclor, Doyle, Meyler and Robinson has developed methods regarding synthesis of the nucleus of penicillin that is 6-amino penicillacanic acid. Penicillin is majorly obtained from Penicillium notatum and Penicillium mycetes. Today, more than 40 penicillin derivative have been prepared. Some of them are obtained from original culture medium, some are obtained as biosynthetic from inclusion of pro-substances and some are obtained as semisynthetic from binding of 6-APA groups (8).

It is reported that, for the effectivity against bacteria the integrity of 6-APA should be maintained, penicilloic acid are formed by the opening of β-lactam ring by β-lactamases, this does not have effect on bacteria however combined with the proteins in the body they gain an antigenic character and this becomes harmful. For this reason, penicilloic acid is seen responsible for the penicillin allergy. The effectivity of natural penicillin is evaluated by University of Oxford and International Department (8).

Penicillines after absorbed are dispersed within the extracellular liquid. Since they are ionised and highly dissolved in water, they hardly exceed the biological webs. 65% of the dispersed penicillines are bound to albumins and 10% goes into erythrocyte.

They are not easily smashed in the body and 90% removed from kidneys without any transformation; their density in the milk is 13% and 30% of the plasma. The removal of ampicillin and cloxacillin is higher (24-30%). This negatively effects the milk production and also plays a significant role in allergic situation in the penicilin-sensitive people (8).

I.1.3 Tetracycline

First member of these medicine chlortetracycline has been obtained by Duggar in 1948, a year later oxytetracycline has been obtained from Streptomyces rimosus cultures by Finlav.With the withdrawal of 1 molecule from chlortetracycline, in 1952 semisynthetic tetracycline has been prepared. Tetracycline are amphoteric substances,

that is they make salt with acid and base. It has been reported that they are dissolved very slightly at pH 7 and that circulating medicines oxytetracycline are bound to plasma proteins at a level of 20-40% and, tetracycline at a level of 45-65% and chlortetracycline at a level of 50-70%. This group of antibiotics are composed of four hydrocarbon derivatives. They are defined a sub-group of polyketides having the skeleton ofOctahydrotetracene-2-carboxamide and known as derivative of polycyclic naphthalene carboxamide, oxytetracycline, chlortetracycline, doxycycline and tetracycline (5).

It is notified that metabolites mostly found in urine, tissue and stool are the major material of tetracycline and they are partially exposed to biotransformation in the body. It is reported that tetracycline is found 5-10 times dense in the gall compared to plasma (8).

Tetracyclines are mostly removed via urine or secondarily via gall. It is detected that some of the tetracycline coming to the guts with this way are absorbed back and partakes to entero-hepatic circulation and it proceeds into in milk at notable densities (8).

It is reported that all tetracycline permeates the placenta and joins foetal circulation and by this means it considerably permeates the milk and egg by prostatorrhea joint fluid and eye fluid. It is also reported that giving tetracycline for long time during pregnancy may result in maldevelopment and deformation and pigmentation in fetus, bones and primary tooth (8).

It is reported that tetracyclines causes gastrointestinal disorders at different levels, it is experienced as diarrhoea, anorexia, gripe, vomiting, nausea by monogastric animals and those effects of tetracyclines can be prevented by taking tetracycline with antacids that does not contain calcium, magnesium and aluminium or with foods other than milk products (8).

Tetracyclines, which are broad spectrum antibiotics, owing to their high antimicrobial efficiency are used in medicine and veterinary for the treatment of various diseases.They are used for the treatment of most of the bacterial illnesses such respiratory system diseases and for different types of infectious illnesses.

I.1.4 Aminoglycosides

Aminoglycoside antibiotics has a significant place in the treatment of infections developing especially in hospitals and cliniques. Except for spectinomycin all

aminoglycosides have amino diabetes in their structure. Main structure of spectinomycin is aminocyclitol but it is still involved in this group. Those antibiotics classified in two groups according to streptidine or 2-deoksistreptamin they contain. In the group containing streptid, streptomycin is the only clinically important compound. The group containing 2-deoksistreptamin has two sub-groups: neomycin, paromamycin, lividomycinand butirosin that 2-deoksistreptamin are bound at 4^{th} and 5^{th} positions or kanamycin, amikacin, tobramycin, gentamycin, sisomycin and netilmycin that 2-deoksistreptamin are bound at 4^{th} and 6^{th} positions (9).

Aminoglycosides are antibiotics that are also effective against penicillin resistant germs. However, since because of their relatively higher toxic effect compared to other antibiotics their usage is limited. Regarding this one should pay attention to the dose and length of treatment.

Insufficiency of penicillin and sefalosporines in the treatment of infections caused by various germs, made researches to search for new antibiotics.

Different from accidentally discovered penicillin, streptomycin is found as a result of conscious and purposeful researches. When it becomes apparent that like other antibiotics usage of streptomycin results in emergence of resistant germs, new antibiotics called aminoglycoside produced from semisynthetic way from streptomycin. They have some features for antibacterial effect and possible side effects.

Aminoglycosides are used as treaters for ophthalmitis, urinary tract infection and for endocarditis (5).

I.1.5 Fluoroquinolones

Typically, they have florin atom bound to centre ring that is found in 6 positions. Eurofloxacin, ciprofloxacin and norfloxacin are among fluoroquinolones. Fluoroquinolones form a class of antibacterial that are active against important synthetic, gram positive and gram negative antibacterial. Besides, these bacterial have some activities against mycobacteria, mycoplasmas and rickettsia. This group of antibiotics can easily enter into the inner cell and for this reason they are frequently used in the treatment of inner cell pathogens. Fluoroquinolone group antibiotics like Ciprofloxacin are used in the treatment of infectious diseases of human beings (5).

Table I-1: Maximum Residue Limits of Veterinary Residues (MRL) (µg/kg) (16)

ANTIBIOTIC	MRL	ANTIBIOTIC	MRL
Benzyl penicillin	4	Sulphonamides	100
Ampicillin	4	Trimethoprim	50
Amoxycillin	4	Spiramycin	200
Oxacillin	30	Tylosine	50
Cloxacillin	30	Erythromycin	40
Dicloxacillin	30	Quinolones	75
Tetracycline	100	Polymyxine	50
Oxytetracycline	100	Ceftiofur	100
Chlortetracycline	100	Cefquinome	20
Streptomycin	200	Nitrofurans	0
Dihydrostreptomycin	200	Nitromidazoles	0
Gentamycin	200	Other chemotherapeutics	0
Neomycin	100		

I.2 Antimicrobial Resistance

Antimicrobial resistance originally means that antimicrobial medicine resistant microorganisms develop resistance. Resistant organisms (bacteria, mycoses, virus and some parasites) have the ability to resist against the attacks coming from antimicrobial medicine. Thereby, standard treatments become ineffective and the risk of spread of infections increase. Development of resistant strain, which is a natural event, occurs when microorganism are exposed to antimicrobial medicine and some resistant characters of different bacteria replace. Wrong and Over Usage of Antimicrobial Medicine speeds up this event (5).

Most of the medicine-sensitive microorganisms arises from the genetic transformations that are obtained through selection processes following the lifelong transfer and mutation of genetic materials of microorganism. There arises mutational resistance as a result of natural mutation formed on microbial chromosomes controlling the sensitivity of given antimicrobials. Besides, there may emerge resistance as a result of genetic material transfer among bacteria.

Resistance depends on different mechanisms and more than one mechanism can work for the same antimicrobial. Microorganisms that are resistant to particular antimicrobials are resistant to other antimicrobials that have an effect mechanism.Some

relations known as cross resistance relation (Neomycin-kanamycin) between agents having close chemical relations are also found in unrelated chemicals (Erythromycin-lincomycin). Microorganisms may resist against lots of antimicrobials without any relations (5).

Food borne germs can resist to antibiotics used in human disease's treatment as a result of usage of antibiotics in animals. When an animal is treated with antibiotics, there is selective pressure over all bacteria exposed to the medicine. Bacteria having the ability to resist antimicrobials have the advantaged of growing faster compared to antimicrobial-sensitive ones, sensitive bacteria are killed by antimicrobials. In addition to this, bacteria become resistant through resistant gens transferred to sensitive bacteria from antibiotics resistant bacteria.

Animal borne food plays a role as a resource of food borne diseases in humans. For this reason, they are thought as the source of resistant genetic material and resistant food borne pathogens. Resistant bacteria reporuces rapidly and makes other bacteria types in animal's resistant (5).

Resistant bacteria migrates to human via animal stool, air, soil and water contamination, consumption of meat and food containing resistant bacteria and direct contact with farm animals and animals in abattoir (5).

I.2.1 Antibiotic Residue and Antibiotic Resistance in The Farm

Lots of researches shows that routine usage of antibiotics in the farms result in the development of antibiotic resistant bacteria in the plant. As a result of FDA practises of fluoroquinolones group antibiotics including ciprofloxin, which is used in farm animals since 1995, there appears the most dramatic proves. In 1999, almost 20% of chicken breast sample contains ciprofloxacin resistant bacteria *Campylobacte* that causes disease. After its long-lasting usage in the field, in 2005 FDA prohibited the usage of medicine and almost containing 30% *Campylobacter coli*ciprofloxacin resistant chicken breast's resistant has declined to %13.5 by 2010.

For this reason, when we feed animals with antibiotics, bacteria around and inside the animal are exposed to medicine and lots of them die. But medicine does not always kill some of them. They survive and multiply. Industry does not pay attention to these realities but discuss the situation of the farms.

In the farm, there are some antibiotics resistant bacteria but they do not effect humans. Those bacteria have two main ways. There antibiotic resistant bacteria may

leave the farm of infect humans. One of these ways is the direct way from meat and farm animal and the other is the indirect way from the environment (2).

I.2.2 Antibiotic Resistance Bacteria from Farm to Our Kitchen

Most of the antibiotic resistant bacteria in the farm are certainly carried to kitchens from farms via uncooked meat and farm animals. In 2006 and 2010 in consumer reports, it becomes evident that there is antibiotics resistant pathogens in the production of retail farm animals. In both years, more than 2/3 of the chicken samples were contaminated by *Salmonella* and *Campyolobacter* and more than 60% of those bacteria became resistant to one or more than one antibiotics.

People from the industry argues that this is not a perturbational situation because people know that farm animals should be completely cooked. Actually, these are donebut packages can drop off to refrigerator or can spoil the cutting board. There is no exact information about how and to what extent this situation results in occurrence of a disease that is difficult to recruit.

Centre for Disease intervenes when antibiotics resistant bacteria frequently blows up. In 2011, crashed turkeys has resulted in one death and 136 disease arising from Salmonella, which means four different antibiotics (gentamicin, tetracycline, streptomycin and ampicillin), resistant bacteria (2).

In 2011, in another institution, Hannaford Food Store Chain in North East America, 7 hospitalizations and 19 infections was observed. It was understood that those were linked to antibiotic resistant Salmonella strains that is found in crashed meats containing amoxicillin/clavulanic acid, ampicillin, ceftriaxone, cefoxitin, kanamycin, streptomycin and sulfisoxzole (2).

I.2.3 Antibiotic Resistance Bacteria Reaching Our Environment From The Farm

Antibiotic resistant bacteria can disperse by environmental transmission and threatens public health. This situation especially arises through various ways like farm river or workers. Farm borne antibiotic resistant bacteria can exchange genetic material after leaving the farm and can give the resistant feature they have to other bacteria, even to different type and kind bacteria that never host antibiotics. This situation can even take place in wild animals, human digestive system and in lakes (2).

Workers deal with resistant bacteria coming from especially animals and different place. In Delmarva peninsula, a research of workers in the poultry farm confirms that there are 32 times more gentamycin resistant *Escherichia coli* compared

the other community members and 5 times multiple medicine resistant *E.coli.* Another research finds methicillin resistant Staphylococcus aureus among 64% of workers and 70% of pigs in a plant in the Middle West. Research also finds out that there isn't any bacteria in a plant that is warned against methicillin resistant Staphylococcus strains.

In a genetic research, methicillin resistant Staphylococcus aureus found in pigs are seen as methicillin resistant Staphylococcus aureus against pigs in humans. Those sensitive bacteria passing to pigs, after gaining resistance against methicillin and tetracycline passes back to humans and this is known as the methicillin resistant Staphylococcus aureus related to stock breeding. Those bacteria are quite widespread in Netherlands.

In addition to this resistant bacterium can be transferred via confined animal feeding operation, or CAFO, fertilization for productivity, animals carried by trucks, wind coming from pig factories or flies stacked to fertilizer. Recent days, in a research on the North Platte River including antibiotic resistant genes (coded for sulphonamide resistant); it has been detected that downstream sediments are larger and there are these genes 100.000 times more when compared to upstream sediments in the feed yards having 10.000 cattles. In a research on the same antibiotic resistant genes, it has been observed that when old sediments are compared to sewage treatment plant disposing 10 million gallon sewage, there are just 1000 times of these genes.

In lots of environment, bacteria can change antibiotics resistant gene codes with adjacent bacteria easily. Antibiotic resistant genes especially such as plasmids, transposons and integrons, are placed frequently on mobile genetic substances that can be easily transferred between the same or different kinds bacteria enabling the contagion of the resistance against multiple medicine by the multiple kinds of bacteria.

According to one industry, 40% of antibiotics are used in farms and medicines called ionophore, which are not used in animals, therefore even if antibiotics come resistant to these medicines, it does not pose a problem. A research on Monensin antibiotics, which is an ionophore used most in meat production, carried by Cornell University and United States Department of Agriculture (USDA) shows that usage of monensin in cattle's nutrition and selection of monensin resistant bacteria has 32 times increased the resistance against bacitracin, that is used in human medicine. This research argues that no one can argue that ionophores cannot be selected as cross resistant against any antibiotics used in human medicine. This research requires further

research and therefore it deems more suitable to consider ionophore as a part of antibiotics used in animal breeding (2).

I.3 Factors resulting in Residues in Animal Resource Food

Antibiotic residues are mostly discovered in animal source foods; streptomycin, penicillin, oxytetracycline and neomycin are among the antibiotics that have the biggest residue problem. Most of the foods that antibiotic residues discovered are mature beef, meat and pork. These are some of the reasons residues in animal source foods:

- Mistakes of animal keepers and breeders
- Incompliance to legal dwell time and uneven treatment records
- Residues to the medicines used via injections
- Incompliance to legal dwell time more than less dosage and wrong medicine (1).

If we talk about usage of medicines in animals, we should talk about residues in foods such as meat, milk, egg and honey. Here are the reasons of medicine residues in those foods:

- Overdose and excessive usage of medicine
- Not considering the formulation of the medicine and the way of medication. Slaughtering the medicated animals without considering these or consuming the meat, milk, egg and honey of these kind of animals.
- Using unconfirmed and unlicensed medicine in animals
- Not complying with the instructions of the doctor or with the prospectus during medication
- Mismedication or using ready-made drug or method or formulation
- Usage of licenced human medicine in animals (like anticancer drugs, cardiac glycosides, insulin)
- Existence of conditions or diseases in the medicated animal slowing the removal of medicine (renal failure)
- Breeding the animal with medicated forage too much and for long time (1).

I.3.1 Losing track of Residue Washout Period of the Body

Factors such as dwell time before slaughtering, non-use time of egg and milk and non-fishing time effects the washout period of body from the antibiotic residues. Transfer of those residues to people consuming animal foods and as a result diseases are

inevitable. For this reason, slaughtering of the animal should be executed according to the washout time of the medicine from the medicated animal's body.

I.3.1.1 Dwell Time Before Slaughtering in Meat Products

Dwell time indicates that medicated animals should not be slaughtered until the level of medicine or chemical residues, which have undesirable or toxic potential, in edible tissues and organs decrease to a secure level for the consumers.

Dwell time also means the time period between the completion of treatment and butchering of the animals. After this period, it is accepted that the level of chemical residues and antibiotics in the edible part of the animals does not have adverse effects for human health.

Dwell time before slaughtering is valid when the appropriate medicine for the animal is used in the recommended dose, in appropriate dosage range and with appropriate medication method for the targeted animal.

Dwell time before slaughtering is determined with the consequence of experimental studies on the healthy animals. This period can change between a couple of days and couple of weeks according to factors such as type of animal, medication method, circulation of medicine in the body, formulation and form of formulation (like water-soluble and dispersible powder, solution for injection with short-long effect), producing company and sort of medicine and accordingly for a drug substance there can be more than one dwell period.

The same situation is relevant for milk and egg. Since the medicine is used in ill animals, its movement in the body can change and cannot comply with the dwell time before slaughtering of the healthy animal. When there is such a situation, a doctor should evaluate. If there isn't any record about the medicine, the dwell time before slaughtering is defined as 28 days temporarily (6).

Table I-2: Dwell period of some antibiotics before slaughtering (1)

Type of Medicine	Type of Animal	Medication Method	Dwell Time (Day) Meat
Diminazen	Gevişenler	Enjeksiyon	28
Sulfaquinoxaline	Kanatlı	Oral	10
Sulfadimidin	Gevişenler	Enjeksiyon	10
	Gevişenler	Oral	12
	Kanatlı	Oral	14
Amprolyum	Kanatlı	Oral	3
Imidocarb	Gevişenler	Enjeksiyon	28
Lasalosid	Kanatlı	Oral	5
Buparvaquon	Gevişenler	Enjeksiyon	42
Tetrasiklinler	Gevişenler	Enjeksiyon	15
	Gevişenler	Uterus içi	7
	Kanatlı	Oral	7

I.3.1.2 Non-use time of egg and milk

After the end of medication in dairy animals and laying hens, since the milk and egg of the animal already has the medicine and metabolite, for a certain time period those kind of foods should not be consumed until the residue level decreases to a harmless level for the consumer health.

For instance, organic alkaline medicine easily mixes into milk and its washout takes long time; therefore, if there isn't any record about the medicine, temporarily during the treatment period and the seven days following the treatment period, eggs and milk should not be consumed as a rule (6).

I.3.1.3 Non-fishing Period

Physiology of fishes change according the temperature of the water. Since their breeding, metabolism and pharmacokinetics of the medicine is closely related to the temperature of the water, it is difficult to determine the non-fishing period after the medication. For this reason, dwell time regarding the medicine is determined with reference to a general principle. According to this, 500 degrees day unit is taken into consideration, this number is divided to temperature and non-fishing time or dwell time is defined for that substance (6).

I.3.2 Pharmaceutic Form

Formulation of a medicine is very important in respect to the danger of causing residue. Especially the medicine for injection ready for usage, tablets, growth factors and intramammary preparates are much more important than others. Among preparates

for injection long retard and slow-release formulation (long retard forms like penicillin, amoxicillin, oxytetracycline) are particularly important (6).

I.3.3 Way of medication

The way of medication follow the line defined for Pharmaceutic Forms. Injection, tablets taken orally, medicating by mixing the forage, intramammary medication are more important than others in regard to causing residues.

Especially in the case of medicine usage for long term, even if the level of medicine that can be found in plasma, tissues and organs is under the legal level, there is the risk of finding residue for long term (6).

I.3.4 Type of Medicine

There are antibiotics such as streptomycin, penicillin, oxytetracycline, gentamicin, neomycin, sulphonamides, ivermectin among the medicines causing residue. The reason for the medicines like penicillin and oxytetracycline is the usage of retard salts or medicines. In medicines like Streptomycin, neomycin, gentamicin, this situation occurs as a result of their attachment to the kidney with specific interest (6).

I.3.5 Unlabelled Medicine Usage

This situation arises from the usage of unconfirmed medicine or usage of its formulation in edible animals or sing the confirmed medicine or formulation in an inappropriate way for the animals (like increasing dosage, changing the way of medication, shortening the dosage range).

As a general principle, any medicine should not be used in edible animals or food of animal origin exclusive of its prospectus; but in some situations one has to use unlabelled medicine. Some of the primary reasons of this situation can be the lack of licenced medicine for the existing disease or inefficiency of the licenced medicine for the recommended dose. For this reason, situation may require the usage of the medicine at higher doses to reach the necessary effectiveness (6).

I.3.6 Usage of Human Medicine

When there isn’t licenced medicine, some of the licenced medicine for human (such as cardiac glycosides, anodyne, anaesthetics, insulin and antidotes) are used by necessity in veterinary. Since there isn’t any regulation regarding the residues in these medicine, when they are used in edible animals, there is the risk of contamination. These medicines should not be used without necessity and when they are used doctor should watch the animal for the danger of residue (6).

I.4 Problems Medicine Residues Can Cause

I.4.1 Drug Allergy

Most of the drugs by initiation of the immune system can result in various types of allergic reaction. Foods containing the residues of these type of medicine results in the same effects. For instance, penicillin with very low amount of allergic reactions can cause deaths.

Chloramphenicol by allergic reaction can suppress bone marrow that may result in deaths. For this reason, in most of the countries including our country, usage of it in edible animals is forbidden (6).

I.4.2 Pharmacological Impact

Medicine residues in foods are usually lower the levels that can cause Pharmacological Impact. Some substances enormously effective and when animals are slaughtered not considering the dwell time and when people consume these foods there can be some undesirable consequences. In 1990 in France, 22 people faced with such a situation after eating liver of a calf that had clenbuterol (6).

I.4.3 Carcinogenic Impact

Usage of substances, that are proved to be carcinogenic by the researches on experimental animals, in edible animals is forbidden. Long term usage of these kind of substances results in carcinogenic risk for people. Chloramphenicol, nitrofurans, imidazole compounds (like metronidazole, ronidazole); some sulfonamids, some aristolachia types, colcicihine, some anodyne (like ksilazin) and some pesticide are listed among the substances having carcinogenic impact (6).

I.4.4 Food Industry

Antibiotics can leave the body with milk. Antibiotic residues in milk seriously affect the processing of foods like cheese and milk. Some antibiotics can mix into the milk couple of times of the plasma. These antibiotic can exist in the milk one the one hand at a level of effecting consumer health negatively and on the other hand suppressing the bacterial culture in the milk.

There cannot be formed nitrosomyoglobin because of the fact that medicine residues in the meat prevent the enzyme activity like nitrate reductase that enables the reduction of nitrate to nitrite. This situation prevents the formation of natural colour of the sausage, meats of animals that had antibiotics is not appropriate to make sausages or similar products (6).

I.4.5 Formation of Resistant Strain

Antibiotics, which can be found as residue or at very low density in the foods like meat and milk, are expected to result in the occurrence of resistant strains in bacteria in such environment but there isn't enough information about this. Foods that are obtained from animals, which were exposed to antibacterial substance, result in severe and frequent food poisonings in consumers. It is seen as a result of resistant bacteria strains from the medicated animals (6).

I.4.6 Transformation of Gender Characteristics

It is accepted that with the usage of anabolic effective substances residues found in the foods may cause changes in the gender characteristics. In animals, except for wrong implementation or mandatory slaughtering, medicine residues in the animals are under the level that can cause changes in the gender characteristics. When you eat 500 gr. Meat from the animal slaughtered in appropriate ways, the residue you will have Ostradiol 17β; this amount is 1/15000 of the released in men and one per million in women. The amount of progesterone is 1/500 (300 gr) of the released in preadolescents. Amount of testosterone is one of couple of thousands of the released in the body (6).

I.4.7 Transformation of Digestive Track Bacterial Consorm

There is 1×1011/g bacteria inside the gut. These bacteria help the digestion and besides they prevent the introduction and reproduction of disease causing bacteria. Antibiotic residues in the foods can ruin the ecological balance of the bacteria community in human gut. In residue form, daily 1.5/mg/60 kg c.a. antibiotics are allowed at most. This amount is based upon that a human being consumes 1.5 kg food daily and 1mg antibiotics in 1kg food will not have nu microbiological harm. While determining the daily dose and tolerance levels of antibiotics, this fact should be taken into consideration (6).

I.4.8 Impact of Antibiotics Residues on Human Health and Disease

I.4.8.1 Drug Allergy

Almost any antibiotics, beta-lactams first can cause the situation known as Type 1 allergy. This can appear as a skin rash mediated by IgE type anticor and even as an anaphylactic shock or death. Penicillin and sulphonamides are among the first medicines causing serious allergic reaction. During the usage of antibiotics there can be occur permanent organic disorder (deafness) in some tissues and organs (6).

I.4.8.2 Neurotic Disorders

During the usage of aminoglycosides almost 1% of the patients, there is observed disorders in organ of hearing and organ of balance. Aminoglycosides, lincosamides, polimiksin, cholistin and capreomycin neuromuscular can cause the stimulus transmission disorder at the junctions; penicillin, nalidixic acid, cycloserine, ethionamid and quinolones can cause clonus; isoniazid, cycloserine and ethambutol can cause neuritis; cholistin, chloramphenicol, streptomycin, sulphonamides, sulphonamide -trimethoprim medleys and nalidixic acid can cause paraesthesia; isoniazid and ethambutol can cause visual disorders. The frequency of undesirable effects is 1% or less (6).

I.4.8.3 Gastrointestinal Disorders

Tetracycline, sulphonamides, chloramphenicol, cephalosporins, ampicillin, isoniazid and quinolone by startling digestive tract mucosa can result in nausea, vomit and diarrhoea. Besides broad spectrum medicine can cause superinfection. In addition, synthesis of K and B vitamins can decrease as a result of disruption of gut flora caused by antibiotics.

I.4.8.4 Renal Disorder

Aminoglycosides being in the first place, polimiksins, bacitracin and amphotericin B and to a certain extent sulphonamides may cause damages in the renals (6).

I.4.8.5 Liver İnjury

Medicine such as erythromycin estolat, novobiocin, isoniazid, chlortetracycline, triacetyloleandomycin and chloramphenicol may cause liver injury (6).

I.4.8.6 Suppression of the Bone Marrow

Long term usage of Chloramphenicol can cause liver injury that can result in extreme decreases in blood shape cells (6).

I.4.8.7 Endotoxic Shock

Especially in heavy gram-negative illnesses, when antibiotics are used higher doses for treatment, endotoxins released from crashed bacteria results a reaction and this is followed by stenosis of arterioles and extension of venuls; this situation is known as endotoxic shock. During the treatment of typhoid with chloramphenicol typhoid, syphilis with penicillin, pestis with streptomycin and tuberculosis with isoniazid, there can occur these type of situations (6).

I.4.8.8 Resistance Against Antibiotics

There emerges resistance among the bacterial consorm exposed to antibiotics and this is one of the most attractive issues for doctors and veterinaries. Bacterial resistance generally means the non-response of bacteria to the medicine. Clinically, medicine resistance means the living and reproduction of strains and types of bacteria known as sensitive in the density provided by the healing doses within the plasma. Response of these kind of bacteria requires the existence of density ten times of higher than the normal densities in the plasma (6).

I.5 Usage of Antibiotics in Chicken Production

In raising of winged animal, usage of antibiotics among feed supplements for the acceleration of growth has a significant role. Anabolic impact of antibiotics is first realized via the increase in the live weight by means of their usage in chicken rations. Even if the effectiveness of antibiotics and similar substances as growth factors cannot be explained, there are some hypothesis on that.

- By inhibiting the production of toxic metabolites preventing the absorption of nutrients
- Preventing the development of pathogen microorganisms in the gastrointestinaş system,
- Preventing or decreasing the subclinical infections

As a result of occurrence of resistant bacteria due to over and inappropriate usage of antibiotics, European Union to a large extent has forbidden the usage of antibiotics for growth in winged animals breeding in 1998-1999. In recent years, researchers are seeking for substances for growth to replace antibiotics and probiotics, organic acids and enzymes are begun to be used as alternatives (10).

I.5.1 Risk of Antibiotic Residue in Chicken

In regard to residue, antibiotics are more important compared to other pharmacological medicine. Over dose medication and slaughtering without considering the legal dwell time after medication are among the reasons for this. As a result of this, non-metabolizing of antibiotics or non-washout from the body, there can be antibiotics residue in animal foods obtained from animal tissue and organs. Tetracycline group antibiotics (tetracycline, oxytetracycline, chlortetracycline) are used widely in winged animals breeding since they are broad spectrum and their toxic effect is low. For this reason determination of Tetracycline group antibiotics in chicken is significant (10).

In our country, a research carried by Acet and friends it is observed that after oxytetracycline (20 mg/kg) and tetracycline (50 mg/kg) is orally given to broiler chickens, oxytetracycline residues are observed at level of 0.32-2.56 ug/g just in kidneys and tetracycline residues are observed at all tissues except for the plasma.

A research carried on 300 samples, composed of 175 chicken and 175 chicken liver, which has sought for chloramphenicol, erythromycin, monensin and tylosin residues, reports that in 2.3% of chickens there is chloramphenicol, in 2.3% there is erythromycin, in 1.1.% there is tylosin; in chicken livers there is 0.57% chloramphenicol, 1.4% erythromycin and 1.7% tylosin residues and notified that these values are above the tolerance limits. There is no monensin in any of the samples (22).

In a research done by The Ministry of Agriculture and Rural Affairs, in 200 chicken and chicken liver samples obtained from different cities in Turkey, with HPLC technique tetracycline group antibiotics (tetracycline, oxytetracycline and chlortetracycline) are watched. As a result, in chicken 8.1% oxytetracycline, 7% tetracycline and 5.5 % chlortetracycline; in chicken liver 74% oxytetracycline, % 47 tetracycline and 5.5 % chlortetracycline is determined. Residue levels are under the tolerance level of the samples that have positive residue results.

In some researches done abroad on broiler chickens and laying hens, for seven days 480 mg/kg tetracycline is given orally to broiler chickens and 840 mg/kg oxytetracycline is given to laying hens orally and in this research done with HPLC method there observed 76% residue in briskets and egg.

When samples of chicken muscle samples are analysed regarding the existence of amoxicillin with LC (Liquid Chromatography) method, there is observed 81.7- 82.9 % residue at an average of 20 ug/g level.

In Japan, sulfadimethoxine and sulfadimethoxine hydroxyl metabolites are analysed on chicken and chicken liver by HPLC method. At a level of 0.1 and 0.5 ppm, 0.5% and 4.8% residue has been identified in this research. Again with the same method in a research done China, 74.7-86.5% sulfamethazine and sulfamethoxazole residues has been discovered in chickens.

With quartile plaque test in Malesia, in 17 of 47 samples examined in terms antibiotic residues there has been identified 36.17% residue and notified that in the samples beta-lactam and tetracycline residues are more widespread (10).

I.6 Usage of Antibiotics in Meat Production

Antibiotics knowns as antimicrobials are medicine to fight against bacterial infections. Antibiotics, especially done for meat and are used to support the animal health and secure meat production (5).

I.6.1 Reasons for Giving Antibiotics to Calf

Antibiotics given to sick animals to make them feel better, to heal and to reduce their pains and stress due to their illnesses. They are also used to prevent the infection or disease of the animals that are open to risk of illnesses. After all, antibiotics are not effective on animal diseases caused by virus or parasite or other bacteria near to bacteria. Although its reason is not known, some antibiotics result in the acceleration of calf growth and support their nutrition. These medicines are used in lower concentrations than the ones used for treatments and used in the feed of the calf. Decisions regarding the usage of some substances is made according to the individual calf producer's usage of them. Not all but antibiotics to be used together is selected with this way (11).

I.6.2 How to Give Antibiotics to Calf?

Antibiotics is given into sub surface skin or to intramuscular via mixing their nourishment or their water or by injection. Injectable antibiotics are used when the risk of illness is high or when calf is already sick. When animals have to be waited for the usage of injectable antibiotics, antibiotics can be given if the injections used in human and animals is secure. This situation can result in stress in the animal and this made calf producer look for further researches. For this reason, usage in the animals should only be in times of necessity. Most of the products are required to be prescribed by a veterinary that already saw the animals. Calf breeders are in contact with veterinaries to prevent the diseases and to find the best way of treatments. Based on these advises, calf breeders can give antibiotics to their calf in their farms and plantations. Necessary approval for the usage of these products and directions are controlled by the FDA to secure the appropriate usage of animal health products and to maintain the reliability of the meat. This includes the minimum contraction periods that prevents the antibiotic residues in the meat we eat (11).

I.6.3 Specific Examples of Usage of Injectable Antibiotics in the meat

Compared to giving antibiotics as forage injecting to animals results in existence of antibiotics in the body in higher concentrations at a short time. If a baby calf faces

pneumonia or diarrhoea antibiotics may be required for treatment. Calf during ablactation if they have pneumonia should be treated with antibiotics. During their life cows, calves and bulls can face with bacterial infections such as contagious and infected scars that require antibiotic treatment. In these situations, penicillin, tetracycline, ceftiofur, florfenicol, tilmicosin, enrofloxacin and tulathromycin are among the widespread used antibiotics. These treatments are necessary not only for the treatment but also to reduce the pains caused by the disease (11).

I.6.4 Specific Examples of Usage of Antibiotics in Stock Farming

Not all cattle that we obtain meat is fed by antibiotic added food. Cattle feeders, due to different reasons, choose the animal to which they will medicate by nutrition (21).

Sometimes, when babies are removed from the mother or weaned, there can be used more antibiotics. Together with the weather change and the stress of the removal babies can be more sensitive to pneumonitis. To prevent pneumonitis or to help the healing of the disease antibiotic should be added to their nutrition. If this disease occurs within 2-3 weeks after the stress, antibiotics are used short-term but higher concentrations. Widely used antibiotics in this situation are tetracycline and some sulphonamides. Additionally, antibiotics used in the nutrition when babies are growing fast and they are close to their final weight. Big portion of the diet of baby calf is composed of cereals and there may appear infection cells in their livers. In some situations, these pyin masses result in diseases. This situation in the lviers is not healthy for the foods. To prevent this, in the last phase of the feeding period of calf there is used antibiotics like tylosin. Although tylosin belongs to the human antibiotics family, they are not used in humans. Ultimately FDA decides the medicines to be used in animal feeding. No one, even the licenced veterinaries cannot allow the usage of medicines in higher concentration above the levels mentioned in the labels or cannot allow the usage of unconfirmed medicine (11).

I.6.5 Injecting antibiotics to animals: with feed path or other ways?

If calves get sick they would not eat too much and they cannot have antibiotics with this way. In such cases, animals are treated with injection of other methods individually.

If calves still able to eat, usage of medicine in their food or in their drinking water would enable the cowman to medicate lots of animals at the same time (11).

I.6.6 Can We Give The Same Antibiotics Used in Human to Animals?

Regarding types of the antibiotics used in calf feeding, Merk Manual Health Professionals argue that among the common prescribed antibiotics, which lists human antibiotics, among 74 2 of them neomycin and tetracycline are appropriate to use.

Tetracycline or its equivalent oxytetracycline and chlortetracycline is used to treat or prevent pneumonitis in the baby calves. These antibiotics are also used for the rapid growth of baby calves and development of their feed efficiency. Neomycin is used as a supplementary in the formulation of some fundamental antibiotics to support growth but rarely used in calf feeding.

In this list, human antibiotics different from each other, procaine penicillin, benzathine penicillin, ampicillin, amoxicillin and spectinomycin, are used for the way of injection in the calves and they are not allowed to be mixed to their feeding. Other antibiotics that are not in the list are also used and some of them are medicine groups including important human antibiotics. For instance, an injectable antibiotic enrofloxacin that are used in calves are not used in humans but they are in close relation with medicine ciprofloxacin that is important for humans.

In the existing calf breeding systems, is it possible for animals to survive without antibiotics?

It is not compulsory. For instance, some consumers prefer to buy meat of animals that never given antibiotics. Some farm owners and feedlots prefer to breed animals that are going to be classified in the markets as naturally fed meat.

In lots of herd, at least some of the animals require antibiotic treatment to support their nutrition at a particular level and to prevent the diseases. Meat producers for responsible farmers and feeding units and even for natural markets give antibiotic to animals when they get sick and these animals are distinguished from the ones that are selected for natural markets. Most of the calf breeder prefer some methods such as making close herd (not buying new animals to the herd) and vaccine program preventing diseases to avoid the need for antibiotics and to keep the animals healthy (11).

I.6.7 How Do Animals Treated With Antibiotics Effect The Meat?

Lots of the antibiotics given to the animals are absorbed by bloodstream at the end but the antibiotics given orally move within he gut and does nor pas to bloodstream. Antibiotic given to an animal is used by the metabolism and changed by the physical

chemistry to be more effective against bacteria, to circulate in the body easier and to be easily removed from the body. A metabolism is different for any different antibiotics and ways of medication but at the end it is removed from the body.

People should follow the remission period after the treatment when they give antibiotics to edible animals. Remission period is the waiting time after the treatment. This remission period gives animals some time to decrease the medicine concentration in their body. This period is different for every antibiotics and it is defined by Food and Drug Administration and it is a short time period.

US Department of Agriculture's Food Safety Inspection Service (FSIS tests the existence of antibiotics in animal bodies used in meat production. They do not test each animal but randomly animals with the body samples are chosen from farms that experienced problem earlier. People selling the animals containing antibiotics over the limits are punished by penalt fine and prison sentence. Number of violations is low and they are published on the FSIS web site (11).

I.7 Antibiotic Regulations in Chicken and Meat

World Health Organization (WHO) and Food and Agricultural Organization (FAO), Food and Drug Administration (FDA), relevant units of the EU and Ministry of Food, Agriculture and Livestock, Ministry of Health work in unity and compliance regarding the topics of securing consumer health and preventing the economic lost caused by drug residues (10).

I.7.1 Antibiotic Regulations in Chicken

European Union

EU parliament and commission’s regulation, numbered 854/2004 and dated 29 April 2004, lays down conditions of establishing specific rules for organization that makes formal control on animal source foods consumed by people.

In European Union, identification of maximum residue levels of veterinary medicine used in animal source foods like meat, fish, egg and honey is regulated by the council regulation for the proceeding numbered 2377/90. Some substances are linked to determine the maximum residue levels of veterinary medicines. It is thought that these added substances are not enough to determine the maximum residue level and these are used in the determination of temporary MRL and they are not allowed to be used in food production.

The 96/23 numbered regulation of the European Commission settles measurements to watch some substances and residues. For this reason, veterinary medicines for livestock and animal source foods are separated into two groups. According to EU regulation numbered 1831/2003 since 2006 antibiotics cannot be used as additives for forage (5).

Veterinary Department Centre is responsible to the violative residues notified by American Ministry of Agriculture Food Safety and Infection Service. Drug residue compliance team regularly provides support and social aid to prevent the illegal residues by watching the inspectional findings send to Veterinary Department Centre from FDA Regional Management. This finding is analysed to see its conformity with food, drug and cosmetics implementations and regulations. U.S. Food and Drug Administration (USFDA) defines specific margin of error for animal medicine (5).

In 1977 USFDA suggests the prohibition of tetracycline and penicillin that is not yet used as additives in breeding farm animals. USFDA since 12 September 2005, prohibits the usage and distribution of Enrofloxacin that is used to treat bacterial infection interacting with fowl.

In 2012 and 2013 Veterinary Department publishes two policy documents known as Guidance for Industry (GFI). First of these documents, GFI≠209, is used for the correct usage of antimicrobial medicines that is medically significant for food producing animals. The second one, GFI≠213, is used for the medication of food and drinking water of the animals, which produce foot, or for the new animal drugs and the products containing new animal drugs (5).

Canada

Veterinary Drugs Directorate (VDD), is responsible for the security of the foods obtained for animals that are medicated for food production. To do this VDD does some supplementary observations like watching the maximum residues limits in the tissues or in the animal source foods, before the sales and establishment of the standards for veterinary medicine.

In human health branch of VDD, examines the health risk with the demand of Canadian Food Inspection Agency (CFIA) and evaluates the information on new medicine if there is a potential risk for the human health regarding the veterinary medicines used in the animals used in food production. This branch develops the warning reports for veterinary medicine labels, defines compulsory withdrawal period

and maximum residue limit of the veterinary medicines in animal source foods. Together with this, this branch is in close relation with CFIA and develops principles regarding the binding human health to veterinary medicines including the situation of antimicrobial resistance (5).

India

Food Safety Standards Authority of India (FSSAI), which is established under the Food Safety and Standards Act (FSS Act), in 2006 lays down the condition of standard based science for the regulation of s production, storage, distribution, sale and export and for the definition of foods to provide the consumption of beneficial and secure foods. FSS Act, has repealed some central acts to establish a single reference point regarding the all situations of food security and standards in 1973, in 1954 repealed the regulation of meat products and in 2006 to prevent the fraught in food.

In 2011 FSSAI establishes tolerance limits in antibiotics for sea food including shrimp and scampi or any other type of this fish and other fishes and in other pharmacologically active substances under the title of food security and standards (contaminants, toxins and residues) regulations.

There is no tolerance limits established by FSSAI for antibiotics and other pharmacologically active substances in the poultry (5).

I.7.2 Antibiotic Regulations in Meat

In calf breeding, calf breeders are tested with forceful processes under the control of FDA, before the usage of antibiotics.

Together with this, The Centre for Veterinary Medicine (CVM), which is a branch of FDA, is responsible for the purveyance of the secure, effective and high quality medicine to the animals. There are different views regarding the antibiotic usage in animals, human health is thought to be the key compound here. A withdrawal period is determined as a part of the implementation process to specify the number of days that is compulsory period between the last antibiotic treatment and animal's entrance to the food chain. This time provides the depuration of antibiotic from the animal's body totally. A person legally giving antibiotics to farm animals should watch the withdrawal period. In addition, FDA and USFDA has a coordinated watch program to follow the antibiotic residues (12). VDD should be implemented to all veterinary medicine before the sales of meat. A medicine is used to reveal the risk for human health and to watch the regulation of animal security, efficiency, treatment and production.

Antibiotic residue in meat is very rare in Canada. CFIA shows 100% conformity with the residue test program.

Table I-3: Maximum Residue Levels in Tissue Chemicals (mg/kg)

Chemicals	Meat	Chicken
Gentamicin	0.1	-
Neomycin	0.25	0.25
Novobiocin	1.0	1.0
Monensin	0.05	0.05
Bcitracin	0.5	0.5
Viginiamycin	-	0.1
Salinomycin	N.D	-
Streptomycin	-	N.D
Spiramycin	0.025	0.025
Amoxicillin	0.01	-
Ampicillin	0.01	-
Erythromycin	N.D	0.125
Oxytetracycline	0.1	0.1
Oleandomycin	-	0.15
Chloramphenicol	N.D	N.D
Chlortetracycline	0.1	1.0
Tetracycline	0.25	0.25
Tylosin	0.2	0.2
Penicillin	0.05	N.D
Hygromycin B	-	N.D
Nicarbazin	-	4.0
Nitrobin, Panazon	0.1	0.1
Decoquinate	2.0	2.0
Sulfadimethoxine	0.1	0.1
Sulfamerazine	0.1	0.1
Sulfamethazine	0.1	0.1
Sulfamonomethoxine	0.1	0.1
Sulfaquinoxaline	0.1	0.1
Albendazol	0.1	-
Amprolium	0.5	0.5
Ethopabate	-	0.5
Olaquindox	0.05	-
Oxolinic acid	0.05	-
Ormethoprim	-	0.1
Isometamidium	0.1	-
Zoalene	-	3.0
Thiabendazole	0.1	0.1
Thiamphenicol	0.5	0.5
Cabadox	-	-
Clopidol	0.2	5.0
Furazolidone	-	-
Flubendazole	-	0.2

N.D.:Not Detected

I.7.3 Legal Regulations and Current Situation in Turkey

There isn't enough control on veterinary medicines, antibiotics at first, in Turkey. Veterinary medicines are misused and abused by animal owners, animal keeper or breeder. Veterinary medicines, because of their impact on human and animal health and also environment, should be prescribed by veterinaries and obtained from pharmacies.

Due to the lack of necessary controls on the production, distribution and consumption of the veterinary medicine, each year there occurs critical amount of economic loss. Together with this food contamination and harmful environment caused by the veterinary medicines threatens public health.

Veterinary medicines accredited by the Ministry of Food, Agriculture and Livestock are used unconsciously.

There are 1800 veterinary medicines accredited by the Ministry of Food, Agriculture and Livestock and average of 500 among them used widespread in the field.

Lots of the medicine used by the Veterinary can be obtained easily and used arbitrarily by the animal breeders. Breeders do not have information about the concepts such as legal dwell time and medication.

State intervention is not enough on the usage, production and distribution of the medicines.

For the reasons such as lack of infrastructure and equipment, confusions among ministries about the authority, a permanent and effective market control cannot be done.

There are economic losses every year due to the unconscious consumption of medicines.

In our country, there isn't reliable data on the extent of the pollution since there isn't infrastructure enabling to watch the residues in the food.

Researches indicates that there can be extensive amount of residues in our food.

All developed countries with their national authorities carry out researches to reduce the veterinary medicine residues and to secure public health.

Acceptable daily limits of residues in the foods are determined by the Commission of Codex Alimentarius. This commission is responsible for the food standard program of FAO and WHO. Food Alimentarius Commission, which is founded in 1962, aims to secure consumer health and ease the international trade of food.

Commissions establishes the examples for the relevant standards regarding processed, semi- processed or raw food.

There is the conditions about methods of analysis and sampling, form of labelling and presenting, polluters, additives, pesticide residues and also the conditions

about the microbiological norms of the substances and hygienic and nutritive feature of them in the Codex Alimentarius (1).

In harmony with the EU legislation, maximum residue limits of a medicine are regulated by Turkish Food Codex and the regulation has been promulgated in 28.04.2002 with the number 247739. According to the regulation promulgated in 19.01.2005 with the number 25705, entitled "Instructions about the Actions to be taken for the Watch of Particular Animal Products and Livestock and the Residues of them".

The number of samples and the substances to be sought according to type of animal and sort of food are prepared in conformity with EU Directive numbered 96/23/EC. The Ministry authorizes Ankara and İzmir Province Control Laboratories and Etlik, Bornova and Pendik Veterinary Control and Research Institute to watch the residues (10).

I.7.4 Institutions that Support the Limitation of Antimicrobials in Animal Products

- **American Medical Association, 2001**

On the usage of antimicrobials and their resistance AMA argues that the usage of microbial, which are pesticide or other antimicrobials used to support growth in humans and in agriculture, should not exceed the levels required for treatment (2).

- **American Public Health Association, 1999, 2004**

APHA works on the possible problems regarding the emergence of antimicrobial agents and resistant bacteria (2).

They require the observations of FDA that works on the regulations on antibiotics used out of medicine and the limitation of antibiotic used in animal food.

In 2004, according to a resolution to protect the production policy a wholesale is began to applied and this encourages the usage of antibiotics apart from their curative features in the production of milk, meat and fish products (2).

- **Infectious Diseases Society of America, 2009**

It supports the phasing out of antimicrobial medicine usage to prevent routine diseases in animals, to support growth and forage effectivity in edible foods (2).

- **World Health Organization, 2001**

To prevent antimicrobial resistance, according to the advises of the WHO, states should phase out or terminate the usage of antimicrobials especially if they are used in human treatments (2).

I.8 Method of Analysis in Observing Medical Residues

There are many and variety of medicine residues in animal source foods and this requires lots of analysis for an effective observation. For this reason, usage of scan analysis is compulsory (14).

For so long, anticor-antigen reaction is used for the determination and characterization of microorganisms. Immunologic methods are preferred to define the food contaminants like Mycotoxin having low molecular weight, pesticide or veterinary medicine.

Anticor-antigen reaction is powerful system for the rapid determination of all pathogens. Some systems are automatized at high levels while some have a simple usage. These tests can be classified as such (15):

1) Latex Agglutination Test

Anticor covered and dyed latex or colloidal gold particles are used in rapid serologic identification or in the recognition and typing of pure bacterial culture isolate. As a result of Anticor-antigen reaction there apparently occurs agglutination. Reverse latex agglutination test is for resoluble antigens and mostly used to seek toxins (15).

2) Automatic and Manuel Elisa Scan (Enzyme-Linked Immunosorbent Assay) Methods

Most widespread implementation is elisa (Enzyme-Linked- Immunosorbent-Assay) systems. This technology is very sensitive because it is used in the anticor developed according to the targeted molecule. Analysis of different drug residues of lots of samples is possible in a short time because it includes easy sample preparation procedures and also results of analysis are reliable because it has high specify (14).

In this method antigen or anticor is marked by an enzyme and immunological reaction is measured as a result of enzymatical activity. There are types of elisa test such as direct, indirect and sandwich elisa but most used one is sandwich elisa method.

To identify pathogen microorganism and toxins lots of elisa test has been developed. Most of the elisa kit used today has high standards and since they operate autaomatically they increase the speed and efficiency and reduces human errors (15).

3) Latera Migration İmmunoassay (Immunochromatography) Method

Another development in the field of immunology is the usage of "Lateral Flow Technology" based on antigen-anticor relation. It is a rapid test. It is developed for the rapid recognition and identification of various samples from pathogens like Bacillus anthracis, E.coli 0157, Salmonella, Listeria and Avian influenza (15).

4) Immuno-Magnetic Separation (IMS) Technology

IMS system provides at least one day saving from the steps of pre-enrichment and enrichment in the identification of pathogens from the food. Recently, lots of recognition system (elisa) are combined with the system of Immuno-Magnetic coating. By this means, sensitivity has been increased and the period of incubation has been shortened (15).

5) Rapid Scan Kits

There are some rapid scan kits for the recognition of antibiotic residues. MeRA is one of them. Some antimicrobial agent groups like beta-lactams and tetracyclines are temperature-sensitive; molecules belonging this group of chemicals become inactive in a short time in the growth temperature of thermophilic bacteria.

MeRA test involves a step of rapid pre-incubation that provided the growth and reproduction of *Geobacillusstearothermophilus.*

Following this step, interaction of *G. stearothermophilus* vegetative form in the room temperature and temperature sensitive antibiotics, if there is in the sample, is realized. Finally, test tubes are exposed to a final incubation and colour change is watched.

I.8.1 Methods Used for the Detection of Different Antibiotic Residues

It is increasingly important to analyse the animal source food since they cause undesirable effect on human health. Legal dwell time, which is a necessary for the withdrawal of antibiotic residues of animal source foods, cessation of medicine treatment and the analysis to define the permitted residue limits in the food has made contributions to the researches regarding public health and food security (3).

In residue analysis, there used different methods. In the search of antibiotic residues in the food, high performance liquid chromatography (HPLC) and microbial tests for chloramphenicol sulfamonomethoksin, sulfadimethoxine, sulfamethazine, sulfamerasin, sulfakuinoksalin, enrofloxacin and ciprofloxacin and especially for tetracycline, macrolide, penicillin, aminoglycoside are among the methods used. Some

researches show that LC-LIV technique is also used in the recognition of antibiotics and other recognition methods based on capillary electrophoresis (CE) are also defined in the literature (3).

For the analysis of Nitrofuran AOZ in some food products, several methods such as HPLC-LIV and LC-MS/MS are used.

Yibar and friends in 2011 made the chloramphenicol analysis in chicken with the elisa and LC-MS/MS techniques.

Charm II test can be used in the scan of sulphonamides, tetracyclines, β-lactam, macrolides, amphenicol, streptomycin and aminoglycosides. With tetrasensor method, tetracycline residues in honey are recognized rapidly.

In 2009 Wang and friends used elisa analysis to follow the neomycin, from the aminoglycoside group, residues in kidney, fish, egg, meat, chicken and pork and used HPLC for the verification. In 2008, Chang and friends made use of the elisa test to search the residues caused by the illegal usage of nitrofuran AOZ (3).

II. MATERIAL and METHOD

II.1 Material

50 gr. For each 20 chicken and 20 meats are collected in sterile conditions from various butchers in Samsun and transmitted to laboratory in cold chain. It has been kept under -20 ºC till the working day. 12 of the meat samples from the breast are collected from slaughter house and 5 of them from butcher and 3 of them from the meat counter of groceries. 15 chicken breast samples are collected from a plant ad 5 of them form the butcher.

II.2 Method

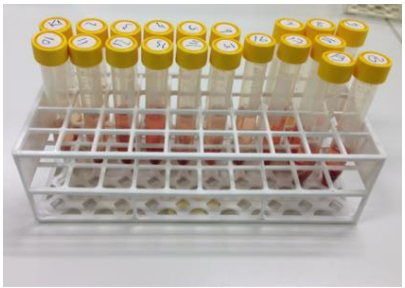
First of all, with MeRa test antibiotic residues in the samples are detected qualitatively. Then, tetracycline levels of all samples are determined quantitively with elisa method.

II.2.1 MeRA Test

MeRa test is a microbiological test including the Geobacillus stearothermophilus spores for the recognition of antimicrobial residues. Like beta-lactams and tetracyclines some antimicrobial agent groups are temperature-sensitive; molecules belonging this group of chemicals become inactive in a short time in the growth temperature of thermophilic bacteria. MeRA test involves a step of rapid pre-incubation that provided the growth and reproduction of *Geobacillusstearothermophilus.* Following this step, interaction of *G. stearothermophilus* vegetative form in the room temperature and temperature sensitive antibiotics, if there is in the sample, is realized. Finally, test tubes are exposed to a final incubation, this incubation step of the MeRa test is a critical step enabling to reach very low recognition limits (23).

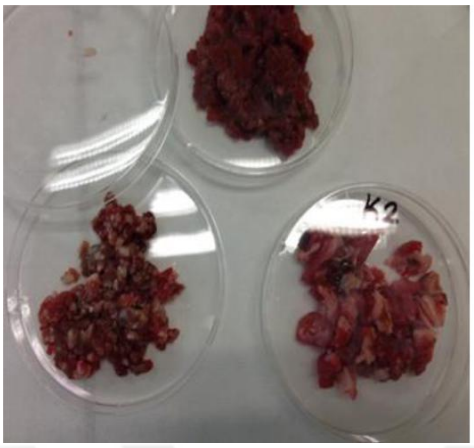
Performing the MeRA Test

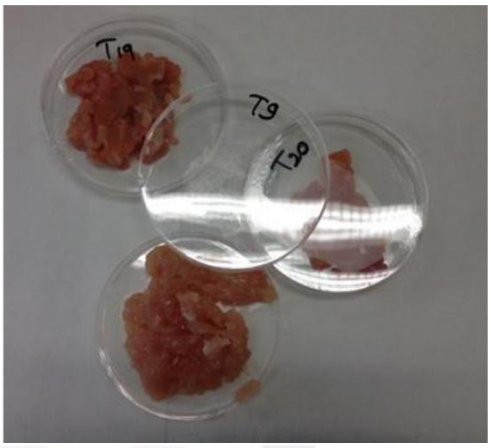
1. After crashing the 50 gr. Meat and chicken samples with blender; minced samples of 2 gr. Per and 6 ml distillate water is put into the test tube. (meat: water should be 1:3)



Picture II-1: Preparation of the Samples

2. In vitro, test sample is homogenized at 13000 rpm for a few seconds
3. Homogenizes sample is centrifuged at 4000 rpm for 15 min.
4. 1 spore disc is added into the medium solution





Picture II-2: MeatPicture II-3: Chicken

5. Pre-incubation of spore disc with solution is realized at 64^{o}C for 20 min.
6. When the incubated solution reaches room temperature after incubation, 1 ml homogenized supernatant (test sample) is put into the incubated solution and solution is left in room temperature for 20 min. for the affection of antimicrobial agent (if there is) in the test sample
7. Test sample is incubated in 64^{o}C water bath or thermo block for 3 -3.5 hours.

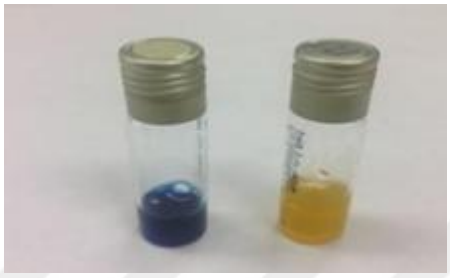
Picture II-4: Incubation in water bath

8. After observing the colour change in the tube, if there isn't colour change (Blue-Green Coloured): Antimicrobial agent residue in the sample is accepted as above the recognition limits. If there is colour change (Yellow coloured): Antimicrobial agent residue in the sample is accepted as below the recognition limits or non-existent (23).

II.2.2 Elisa Test

Elisa technology is very sensitive and has high specifity due to the usage of anticor specifically developed for the targeted molecule. Because of the specifity reliability of the analysis result is high and also analysis of different drug residues of lots of samples in a very short time period since they involve relatively easy sample preparation.

A typical Elisa kit is composed of 96 wells, coated by anticor developed against targeted veterinary medicine (tetracycline) (12 separable the 8 well strain)



Picture II-5: Elisa; Positive Control and Negative Control

Analysis is made within these wells, by adding certain amount of sample extract and adding a range of reactive and after then by washing up. At the end of the analysis, with the help of the spectrophotometer amount is determine. Some liquid samples (like urine and plasma) being diluted by buffer solution are directly analysed; solid samples like meat and fish should be mixed with extraction solvent before Elisa and requires filtration.

Tetracycline Elisa Test Performing Method

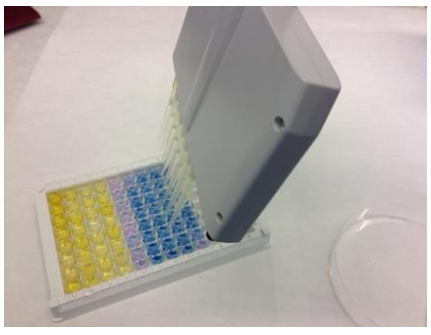
Preparation of Samples: Firstly, meat samples are homogenized in a bowl.4 gr meat sample are put into 50 ml falcon tubes and 20 of 50 mm succinic acid has been added on. Falcon tubes are shaken in shaker incubator for 15 minutes in room temperature. After, it has been centrifuged for 15 min. at 4000 g. After centrifuge, supernatant has been diluted 1/10 (100 µl supernatant, 900 µl PBS- Phosphate buffered saline). 50 µl of this mixture has been used in the experiment.

Preparation of the Standards: Concentrates standards has been diluted. For each standard (50 µl), 450 µl sample buffer has been diluted with 1. For the freshness, standards have been prepared in the work day.

Performing Elisa Test: Solutions and plate within the kit are kept in room temperature and the steps below followed and then the research has been completed (Picture 6).

Picture II-6: Elisa Test Kit

1. Test wells at a number of sample and standard are replaced to plate.
2. Standards and samples are pipetted into the 50 µl wells, respectively.
3. 50 µl anti-tetracycline antibody are pipetted into each well. Then it incubated for 1 hour under room temperature.
4. In automatic elisa washer, it has been washed 3 times for each wash to come 250 µl wash buffer.
5. To each well, 100 µl conjugate has been added with the help of multi-channel pipette and shaken and incubated in room temperature for 15 min (Picture 7).
6. In automatic elisa washer, it has been washed 3 times for each wash to come 250 µl wash buffer.
7. To each well, 100 µl substrate/chromogen has been added and shaken and incubated in room temperature for 15 min.
8. 100 µl stop solution has been added and by using 450 nm' filter it has been read in elisareader.
9. Standard curve graphic has been drawn by Rida soft program (Graphic 1). Tetracyline values are counted in terms of ppb using the formula of Absorbents sample/zero standard “absorbents x 100”



Picture II-7: Performing elisa test

III. DISCUSSION and RESULTS

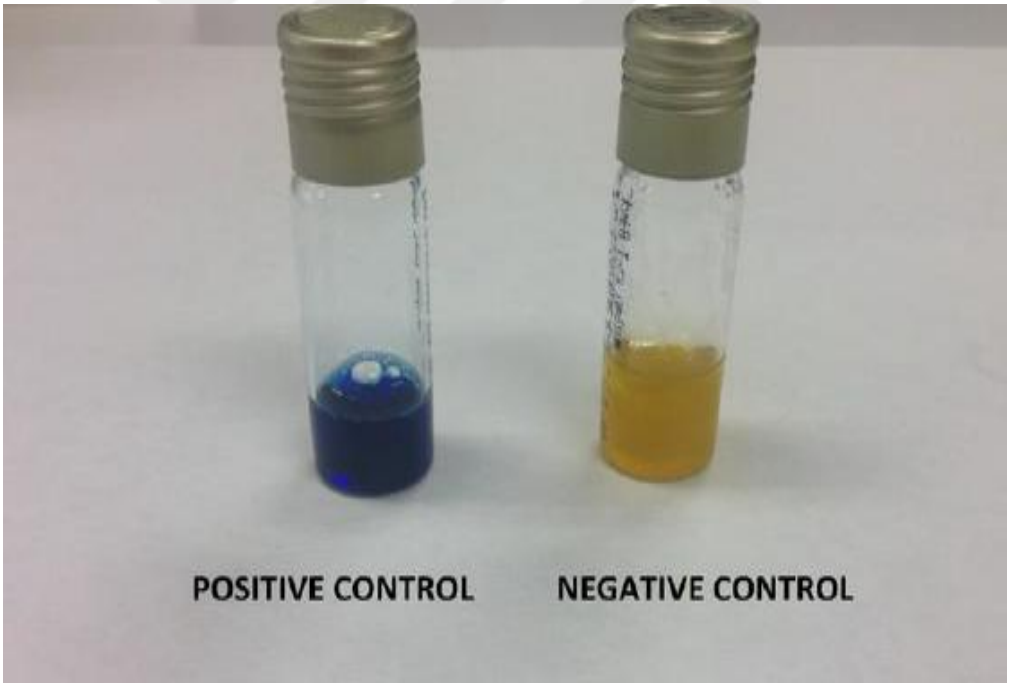
III.1 Results

As seen from Table 4, 10 out of 20 meat samples (Picture III-2) and 19 out of 20 chicken samples (Picture III-3) analysed with MeRa tests are detected as positive.

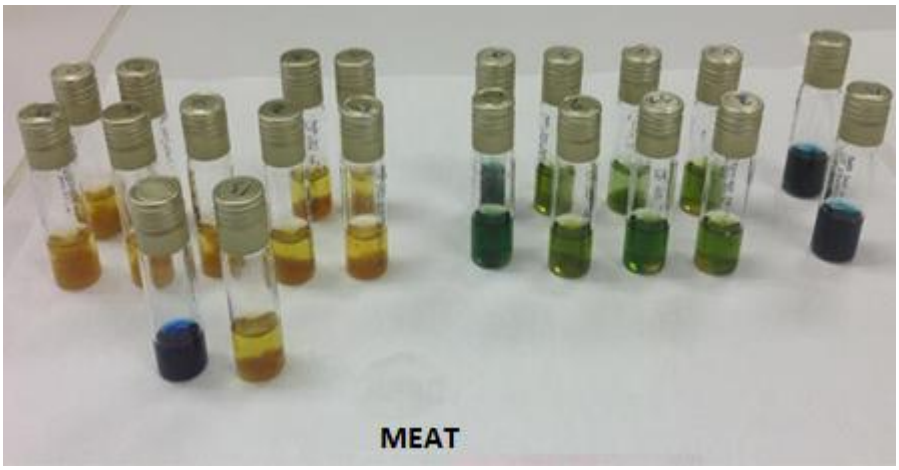
As seen in Picture 8, blue-green colour has been observed in meat samples since the antimicrobial agent concentration is above the limit of detection and they are detected as positive. When there isn't any agent in the meat samples or if they are below the limit of detection, yellow colour is observed and they are detected as negative. That is to say, 29 out of 40 meat samples at the total are detected above the acceptable limits.

After with elisa method tetracycline levels of the positive resulting samples have been watched. According the results indicated in Table 5, in 4 meat and 17 chicken, there is tetracycline above the acceptable limits. When we compare the chicken and meat samples, percentage of presence of tetracycline is higher in chicken samples than the meat.

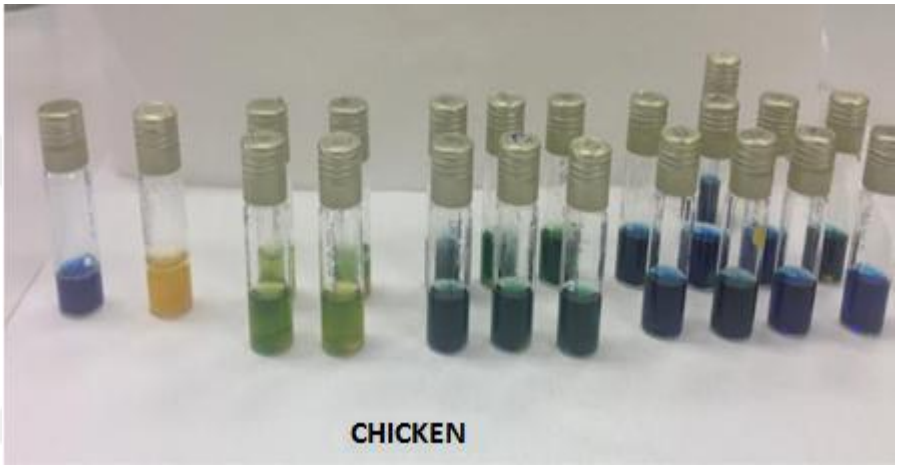
III.1.1 MeRA Test Results



Picture III-1: Positive-Negative Control



Picture III-2: Positive-Negative Control Result of Meat



Picture III-3: Positive-Negative Control Result of Chicken

Table III-1: MeRA Test Results

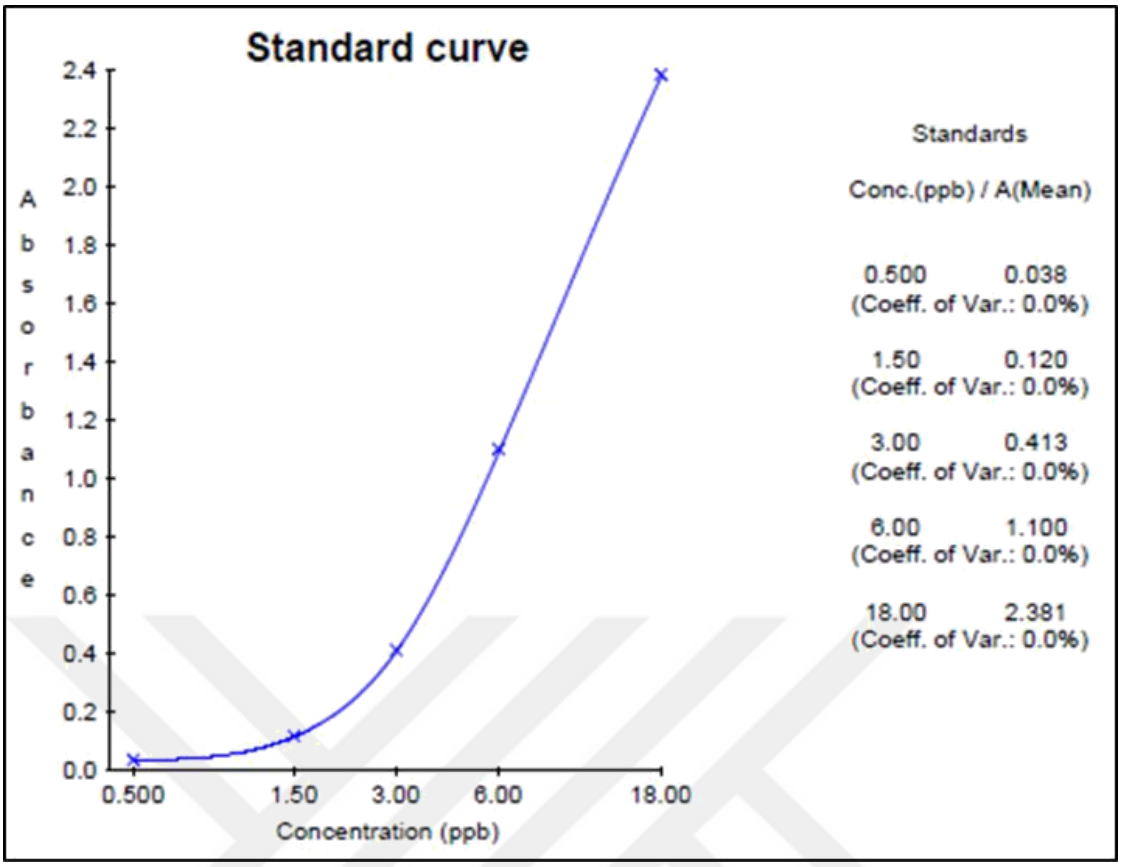
Meat Sample No	Result	Chicken Sample No	Result
1	Positive	1	Positive
2	Positive	2	Positive
3	Positive	3	Positive
4	Positive	4	Positive
5	Negative	5	Positive
6	Positive	6	Positive
7	Negative	7	Positive
8	Negative	8	Positive
9	Negative	9	Positive
10	Negative	10	Positive
11	Positive	11	Positive
12	Negative	12	Negative
13	Negative	13	Positive
14	Positive	14	Positive
15	Positive	15	Positive
16	Positive	16	Positive
17	Negative	17	Positive
18	Negative	18	Positive
19	Positive	19	Positive
20	Negative	20	Positive

III.1.2 Elisa Test Results

Table III-2: Tetracycline Elisa Test Results

Meat Sample No	Numerical Value	Result	Chicken Sample No	Numerical Value	Result
1	116	Positive	1	> 180	Positive
2	5,47	Negative	2	> 180	Positive
3	> 180	Positive	3	> 180	Positive
4	5,47	Negative	4	> 180	Positive
5	1,3	Negative	5	> 180	Positive
6	5,47	Negative	6	> 180	Positive
7	1,2	Negative	7	> 180	Positive
8	1,2	Negative	8	> 180	Positive
9	1,2	Negative	9	> 180	Positive
10	1,2	Negative	10	80,9	Negative
11	5,47	Negative	11	77,6	Negative
12	1,7	Negative	12	1,7	Negative
13	1,6	Negative	13	> 180	Positive
14	> 180	Positive	14	> 180	Positive
15	> 180	Positive	15	> 180	Positive
16	5,47	Negative	16	> 180	Positive
17	1,2	Negative	17	> 180	Positive
18	1,2	Negative	18	> 180	Positive
19	5,47	Negative	19	> 180	Positive
20	1,2	Negative	20	> 180	Positive

Samples worked on elisa plates are read by elisa reader to examine the values and standard curves and to check the accuracy of the test (Graphic III-1).



Graphic III-1:Standard Curve Graphic of Tetracycline Elisa Test

Standard values taken from the elisa reader and examined according to expected values. Later, test results attained as ≥ 1.7-180 ppb at chicken and ≥ 1.2-180 ppb at meat (Table III-2). For EU, FDA and Turkish Food Codex results should be maximum 100 ppb.

III.2 Discussion

Medicines and other chemical substances are used to treat the diseases, to prevent and control and to prevent the speed up of its development. Following this, unchanged metabolites, which are hoarded and stored in the tissues and organs having nutritional value and products obtained from them (like meat, milk, egg, and honey), and degradation products, free or bound substance are defined as residues (1).

Usage of veterinary medicine is a matter of debate but their usage in modern breeding techniques has become inevitable. One of the biggest concerns in this matter is the fact that as a result of widespread usage of veterinary medicine microorganisms becomes resistant to antibiotics. Also, concerned of the its effects on human health. In such situation concerns about the decreasing impact of human medicines for human

resulted in the reviewing the usage of antibiotics for nonhuman and in 2005 usage of enrofloxacin in chickens has been prohibited in USA.

WHO demands the prohibition of usage of a serious of antibiotics in animals that have critical importance in human treatment. Because of the concerns of antibiotic resistance, in EU usage of antibiotics to support growth has been prohibited. When compared to the danger of antibiotic resistance, risk of toxicity caused by the residues of the medicines allowed to be used in food products is slightly low. Despite this, to prevent human's exposure to residues at harmful levels, EU identified Maximum Residue Limits (MRL) for the veterinary medicines in animal source food products. Regulation of EU Commission numbered 37/2010 and dated 22.12.2009 on Active Pharmacological substances, lists the limits maximum residue limits (MRL) for the veterinary medicines in animal source food products (14).

The biggest share of world total cattle and veal production belongs to USA with 20.6% and EU with 14%. Share of our country is only 1.6 -1.8%, chicken production in our country since 1990 has been more than both sheep and cattle production.

Recent years it is seen that production of winged approximates to the sum of sheep and cattle production. In the total meat production by 48% chicken and turkey comes first and it is followed by cattle by 25% and sheep by 23%. Despite the increase in both read and white meat in worldwide, in EU there is decrease in the red meat production obtained from cattle and buffalo and sheep and goat.

Usage of antibiotics in breeding can bring about serious dangers via animal source food regarding human health. Medicines taken accumulate in various tissues and organ, kidney and liver being the first. There is observed development of resistant bacteria, change in gut flora, fertility disorders, toxic symptoms, allergic reactions in people that consumed the food containing drug residue.

In the turkey legs, breast and meat cubes obtained from the groceries in Ankara, there has been found tetracycline at a level of 50.9% isolated Salmonella spp., penicillin at a level of %83.3 Listeria monocytogenes and resistances against the trimethoprim at level of %100 Clostridium perfringens and it is detected that isolates have multi antibiotic resistance.

As a result of widespread usage of Fluoroquinolone and tetracycline group antibiotic in winged, an increasing resistance of thermophilic Campylobacter spp against antibiotics has been observed. Correspondingly, meat of the winged is notified

as an important source for the transfer of enteropathogenic bacteria types that have antibiotic resistance. Widespread usage of avoparcin as a growth factor in winged breeding, there appear cross resistance against vancomycin in enterococcus and it is notified that this resistance is transferred to S.aureus.

Increase of multiple resistance features of pathogen microorganisms gains antibiotics and usage of similar antibiotic groups in human and animal treatment makes us think that there can be an important decrease in the number of antibiotics that can be used for human treatment. Effectivity of antibiotic causing the occurrence of resistant bacteria is decreasing, correspondingly usage of medicine is increasing (10).

In a research carried by Lee and friends, in 2007 in which they scan 13 antibiotics including the types of tetracycline, macrolide, penicillin, aminoglycoside and chloramphenicol by microbial tests; they detect that 34 of the scanned 459 samples are possibly positive.

Oruç and friends, in their research in 2006 done by elisa, detected streptomycin between the range of 25.2 µg/ kg and 31.4 µg/ kg in 4 of 60 cattle meat collected in 2005 and 2006 and in one of them sulfamethazine residue at a level of 12 µg/ kg.

Again in a research done in 2011 with elisaanalysis, there is detected chloramphenicol residues in 15 of 180 chicken samples. Later, as result of the LC-MS/MS verification analysis of all elisa positive samples and 60 negative samples, they detect 2 of the positive samples and 1 of the negative samples contain the mentioned antibiotic (3).

There is very little research on chicken and veal in our country.

In Akar's research in 1994 in Ankara on 175 chicken, in 5.7% of them chloramphenicol, erythromycin and tylosin antibiotic residues has been detected. Again in Ankara, in 2011 made by Yüksek on 50 chickens, there wasn't oxytetracycline, chloramphenicol and zinc bacitracin residues in muscle tissues of the chickens.

In a research of Kaya and friend in 1992, on 85 calves, on the 18% of the samples they do no observe antibiotic residues. In 2006 in Ankara in a research carried on 240 veal and sheep meat, no quinolone antibiotic is observed (19).

In this research, we analysed the tetracycline group antibiotic on the 20 chicken and meat obtained from different animals and by this we tried to contribute to the researches regarding food security and public health. We have found, according to elisatest results, ≥ 1.2-180 ppb tetracycline in meat samples (Table 5) and ≥ 1.7-180 ppb

tetracycline in chicken samples (Table 5). When two are compared, percentage of tetracycline presence in chicken is higher than the percentage of the meat.

Consequently, in the production of elisa test kits usage of anticor having cross reaction against the other substances and also test is very sensitive and has high specificity due to the usage of anticor specifically developed for the targeted molecule. Because of the specificity, reliability of the analysis result is high and also analysis of different drug residues of lots of samples in a very short time period since they involve relatively easy sample preparation (14).

In our research, amount of tetracycline in the samples obtained from meat and chicken is examined with elisamethod.

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EĞİTİM

Lisans Öncesi	2003-2007	Huriye Süer Anadolu Lisesi-Samsun
Lisans	2008-2013	Yeditepe Üniversitesi Sağlık Bilimleri Fakültesi Beslenme ve Diyetetik Bölümü (İngilizce) (Burslu)
Yüksek Lisans	2013-2016	Yeditepe Üniversitesi Sağlık Bilimleri Fakültesi Yüksek Lisans Programı Beslenme ve Diyetetik Bölümü (İngilizce)

DENEYİMLER

2012 staj	Samsun Mehmet Aydın Eğitim ve Araştırma Hastanesi
	İstanbul Özel Gaziosmanpaşa Hastanesi
	İstanbul Süreyyapaşa Göğüs Hastalıkları Hastanesi
	İstanbul Bahçelievler Medicalpark Hastanesi
	İstanbul Kartal Koşuyolu Yüksek İhtisas Eğitim ve Araştırma Hast.
2013 staj	İstanbul Üniversitesi Çapa Tıp Fakültesi
	Üsküdar Devlet Hastanesi
	Ümraniye Devlet Hastanesi
	Dr. Siyami Ersek Göğüs Kalp Ve Damar Cerrahisi Eğt. ve Arşt. Hast.
2013 Haziran-	Samsun Özel Mediva Hastanesi
2016 Haziran	Samsun Özel Gatohospital Hastanesi*
	Özel Fresenius Medical Care Çarşamba Diyaliz Merkezi**
	Özel Fresenius Medical Care Samsun 19 Mayıs Diyaliz Merkezi**
2016 Eylül	HDL Beslenme Danışmanlığı

Ticari unvan değişikliği sebebiyle çalışılan kurum ismi değişmiştir.

****İçinde bulunulan ayın belirli günleri gidilerek hizmet verilmiştir.***

EĞİTİM ve SEMİNERLER

- İstanbul Sağlık ve Beslenme Bienali
- Kendini Tanımak ve Kişisel Farkındalık Semineri
- Career Management Resume Writing & İnterview Tecniquies
- Yeditepe Üniversitesi Kariyer Günleri
- Güncel Bilgiler Işığında Diyabet Ve Beslenme Tedavisi Mezuniyet Sonrası Eğitim Kursu
- Hastalılarda Diyet Tedavisinin Klinik Uygulamalara Yansıması Sempozyumu
- Diyet Programlarına Besin Destekleri Yaklaşımı ve Yaşam Koçluğu
- 3.Ulusal Sağlıklı Yaşam Sempozyumu
- 3.Ulusal Sağlıklı Yaşam Sempozyumu Kapsamında Kardiyoloji Diyetisyenliği Kursu

YABANCI DİL

Okuma – Anlama	4/5
Yazma	3/5
Konuşma	3/5