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HEALTHY FOOD OF TEENAGERS: MONITORING THE CONTENT OF ANTIBIOTICS IN MEAT FROM ORGANIZED CITY MARKETS

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Abstract
Meat is an important source of protein and a popular food product in Ukraine. A survey of teenagers in a secondary school in Bukovina showed that meat makes up almost half of the daily ration of schoolchildren. The most consumed types of meat: pork and chicken (79% of the total meat ration of the respondents), the most popular places for buying meat: organized urban markets (44% of the families of the respondents). Given the widespread use of antibiotics in animal husbandry, the problem of antibiotic content in meat as a food product is very important and relevant. Quality food is one of the components of shaping the health of the future generation. In Ukraine, the analysis of meat regarding the presence of antibiotic residues in laboratories in organized markets is not mandatory. Theoretically, the likelihood of the presence of antibiotic residues in meat and their ingress into the body of a teenager when eating meat is not excluded. To look into this problem, a study was conducted on popular meat varieties from organized markets in Chernivtsi (2017-2018) in terms of the presence of antibacterial residues.
The identification of antibiotic residues in meat was carried out according to standard methods. The following results were obtained: 91% of meat samples contained residues of antibacterial drugs. Of these, 100% pork and 86% chicken (the most consumed meat) contained antibiotic residues. The most commonly detected antibiotics are the aminoglycoside group and the highly toxic streptomycin. 91% of the studied samples in 2017 contained two or more antibiotics, in 2018 – 46%. In 2018, compared with 2017, there was a slight decrease in the number of meat samples containing antibiotics ($F = 4.3$; $p = 0.05$). Studies have shown that more meat samples contain antibiotics. Given the small number of samples, the data obtained are preliminary and require further study.

Key words: *antibiotics; meat; teenagers' food safety; quality reaction; sensitivity threshold.*

1.Introduction

Nutrition is one of the most important factors in human health and its proper physical development. Food, entering the body, performs many important functions.

Healthy lifestyle priorities are food safety and quality issues. More than 90% of human exposure to harmful substances comes from the consumption of contaminated food (1). Due to the fact that the consumption of animal products has

increased significantly, there is a risk of co-occurrence of antibiotics with meat, milk, fish and eggs (2). The developing organism has the greatest risk of exposure to various biologically active substances. Frequent use of such products can cause diseases of the gastrointestinal tract, decreased immunity, allergic reactions and a number of dangerous diseases (3). Eating quality products will help preserve the health of the nation (4).

One of the main foods is animal products. Almost half of the need for proteins, which are the basic structural elements of all tissues of the human body, we get with meat. According to the norms of a balanced diet a person needs an average of 44-45 g of animal protein per day (5). The most protein is found in beef and chicken (up to 20 grams), the lowest protein content in pork – up to 12 grams. In modern animal husbandry for various purposes, antibiotics are widely used (6). The widespread use of antibiotics as medicines in medicine, veterinary medicine, various fields of agriculture, food and canning industry contributed to the creation of a special industry – the antibiotic industry (7). It also caused the emergence of semi-synthetic and synthetic analogues. In animal husbandry, antibiotics are used not only for the treatment and prevention of diseases, but also to stimulate growth, fattening, improve the productive characteristics of animals (8). As a result, the meat supplied to the consumer contains substances that, when they are received continuously, pose a serious risk to human health (9).

The use of antibacterial promotes the formation of antibiotic-resistant bacteria in the body of farm animals that enter the food further and pose a threat to the health of the consumer (10). Passive use of antibiotics can cause intestinal dysbiosis, decreased immunity. A very dangerous and undesirable effect of antibiotics is the sensitization of the human body with subsequent allergic reactions (8). Penicillin, streptomycin and aminoglycosides are considered to be the strongest allergens (4; 11). Streptomycin and tetracycline act on pregnant women as teratogens, causing anomalies in embryonic development. Widely used in veterinary medicine, levomycetin, extremely dangerous for antibiotic sensitive people, can cause toxicosis, aplastic anemia, which turns into leukemia (12).

The problem of residues of antibiotics in food, including their impact (contamination) on human health and the environment, is receiving great attention in almost all countries in Europe, Canada and the United States. The urgency of the problem is determined by three aspects: general biological, medical and socio-economic, which are closely related (10).

The general biological aspect of the problem is that microorganisms resistant to disease are trapped in the digestive canal of diseased humans and animals. Under the influence of antibiotics, sensitive cells die, and resistant, multiplying, become the predominant part of the micro flora. This leads to an increase in the duration of diarrheal and other diseases, the need to increase doses of drugs, which is undesirable both in terms of toxicity and in terms of the possibility of super infections. The problem is acute in pediatrics and in the clinic of immunodeficiency states (13).

For the prevention of the adverse effects of the use of bioactive substances, there are mandatory conditions for keeping animals before slaughter until the antibiotics are completely removed from their body, but these conditions are not always met. According to literature, in Ukraine about 30-40% of all meat contains antibiotics

(14). In Europe, this figure is less than 1% (10).

Meat is one of the most sought after products. For healthy eating, the information on the presence of antibiotics in the meat we consume is very important.

To study this issue, the following tasks and goals were set:

To determine the meat diet of adolescents in Bukovina, students of Chernivtsi Secondary School I-III degrees №4, to study the content of antibiotic residues in meat from the markets of Chernivtsi for 2017-2018.

To substantiate the control of antibiotic content in meat in organized urban markets as a guarantee of a safe and healthy diet.

2. Materials and methods

In order to determine the meat diet of adolescents within one family, we conducted a survey of students of Chernivtsi Secondary School №4 grades 11 to 17 years. Respondents were asked, with the permission of their parents, to voluntarily and honestly answer questions regarding their nutrition. Including:

1. What meat is in your diet?
2. Where do you usually buy meat from?
3. In what markets do you buy meat?
4. Do you know about the physiological protein intake recommended for adolescents?
5. Do you know about the importance of animal protein for the healthy development of a teenager?
6. Do you know about the content of antibiotics in the meat you consume?
7. Do you know about the harm of antibiotics to the human body?

Closed questions (№2, №4-7) were used because they are easy to handle, in addition, the respondent answers the question more willingly and quickly. As well as open-ended questions (№1, №3) were used in order not to influence the respondents by the researcher's opinion, to direct further work and to narrow the circle of purchase of products.

During 2017-2018, 23 meat samples from the Chernivtsi market were surveyed. For qualitative reactions, we used a concentrate extract from the test sample of meat, which was prepared for each sample separately (13). Weighing the prepared minced meat according to state standard 20235.0-74, weighing 5 g, was transferred into a conical flask of 20 ml of twice boiled distilled water; with shaking three times, withstood for 15 min. The resulting extract was filtered. The filtrate was used to perform qualitative reactions. For the study of each sample, 13 standard qualitative reactions were performed (15).

Antibiotics are chemical compounds that have certain chemical functional groups, each of which can be determined by an appropriate qualitative reaction. Due to the fact that amino acids, which are a component of animal protein, may have identical functional groups with antibiotics, they were denatured by ten minutes of boiling the filtrate (14).

It should be noted that in the samples tested, the initial concentration is much lower than the concentration of these substances in the animal body. For each of the qualitative reactions, there is a concept known as a "threshold of sensitivity" that is directly dependent on the concentration of the substance being determined. That is why when studying the filtrate of one sample in the experimental series of qualitative reactions in some cases, we observed the formation of sediment, gas release, turbidity were interpreted as a positive analytical effect of "+" (positive reaction); in others, these signs were absent, which was interpreted as a negative

analytical effect of "-" (negative reaction) (14).

Qualitative analysis of the presence of antibiotics in the samples was carried out taking into account the chemical classification of antibiotics by standard methods.

Tetracyclines

Reaction I. The reaction is carried out with concentrated sulfuric acid. As a result, tetracycline derivatives are formed that have a specific color.

Reaction II. Formation of colored complex salts in an alcohol medium from ferric (III) chloride is brown or reddish brown.

Reaction III. Formation of colored complexes with cuprous salts (II), zinc.

Antibiotics of aromatic series Reaction IV. Hydrolytic cleavage reactions are used in acidic or alkaline media, with subsequent identification of the products formed. Thus, when heated with levomycetin with sodium hydroxide solution, a yellow color first appears, which turns to red-orange (due to the formation of acinitroform), and further heating produces a brick-red precipitate and an ammonia odor.

Reaction V. Levomycetin stearate when heated with hydrochloric acid concentrated hydrolyzes – stearic acid is formed, which floats to the surface in the form of oily droplets that solidify upon cooling.

Reaction VI. In the rapid analysis, the reaction of levomycetin with cuprum (II) sulfate in an alkaline medium is used in the presence of *n*-butanol – the alcohol layer is colored in blue-violet due to the formation of a complex salt.

Penicillins

Reaction VII. Non-pharmacopoeial reactions: reaction of formation of cuprum (II) (green) or ferum (III) (red) of penicillinohydroxamates after hydroxylaminolysis of the β-lactam cycle of penicillins.

Cephalosporins

Reaction VIII. Under the action of a mixture of sulfuric and nitric acids, cephalexin turns yellow, cephalothin turns olive green, then it turns red-brown.

Streptomycin

Reaction IX. When interacted with ferric ions (III) in an acidic environment, maltol forms compounds that have a purple color.

Express methods of identification of streptomycin:

Reaction X. Isolation of ammonia by heating the substance with sodium hydroxide solution.

Reaction XI. Formation of brown color with potassium tetraiodomercurate alkaline (Nessler reagent).

Reaction XII. Formation of red precipitate when heated with copper-tartrate reagent (aldehyde group).

Aminoglycosides

Reaction XIII. Reaction to the aliphatic amino group of aminoglycosides – violet color is formed when heated with ninhydrin.

The results of qualitative reactions to the content of antibacterial drugs in the test samples are shown in tables 1, 2.

3. Results and Discussion.

A survey of schoolchildren (286 respondents) of the Chernivtsi secondary school №4 showed the following results: 48% – most often use pork; 44% buy meat in organized city markets; 62% – recognize the importance of animal protein for the healthy development of adolescents; 93% – do not reliably know about the presence of antibiotics in the purchased meat; 61% of respondents are aware of the dangers of these substances for the human body.

79% of the meat from the diet of Chernivtsi teenagers is pork and chicken.

This result of the survey prompted us to investigate the content of antibiotics primarily in the most common types of meat from organized markets in Chernivtsi.

For the study of antibiotic content in meat samples, 156 qualitative reactions were performed in 2017, 28 of which gave a positive analytical effect, in 18% of specific reactions had a sufficient threshold for sensitivity in antibiotic meat samples tested (Table 1).

It should be emphasized that 100% of the tested samples showed the presence of antibiotics. Most often, the positive reactions were for the following groups: aminoglycosides – 100% (reaction XIII); tetracycline – 50% (reaction II); aromatic antibiotics and streptomycin – 42% respectively (reactions IV; XI).

Table 1

The results of the study of the presence of antibacterial preparations in meat products from the markets of Chernivtsi for 2017

№ meatsample	Kindofmeat	Samplingmarket	Reactionnumber				
			II	IV	XI	XIII	I,III,V,VI,VII,VIII,IX,X,XII
1.	Rabbit	Lower	+	-	-	+	All samples had a negative analytical effect
2.	Chicken	Main	+	+	+	+	
3.	Pork	Main	-	+	+	+	
4.	Chicken	Main	+	+	-	+	
5.	Veal	Central	-	-	-	+	
6.	Pork	Central	-	-	+	+	
7.	Chicken	Central	-	+	-	+	
8.	Chicken	Lower	-	-	-	+	
9.	Veal	Main	+	-	-	+	
10.	Veal	Central	+	+	-	+	
11.	Pork	Central	+	-	+	+	
12.	Pork	Lower	-	-	+	+	

I; II; III; IV; V; VI; VII; VIII; IX; X; XI; XII; XIII – see Materials and Methods Division; "+" – positive reaction; "-" – is a negative reaction.

More than one type of antibiotic was detected in 91% of the samples. Such data are cause for concern, since they indicate the likelihood of concomitant uncontrolled penetration of antibiotic residues into the body of a teenager during meat consumption, which can have adverse consequences. In the theoretical part of our work we have described the negative impact of these substances on the body.

To study the content of antibiotics in meat samples in 2018, we performed 143 qualitative reactions, 21 of which gave a positive analytical effect, that is, 15% of specific reactions had a sufficient threshold for the detection of antibiotics in the tested meat samples (Table 2).

This is a 3% decrease compared to 2017, which may indicate a decrease in the level of antibiotics in consumed meat.

It should be emphasized that in 18% (two samples: №1 chicken; №2 rabbit), the qualitative reactions gave a negative analytical effect, that is, we can assume that the tested meat contains no antibiotics, but 82% (9 samples) – contains antibacterial residues. More than one type of antibiotic was detected in 46% (5 samples), which means that while consuming meat, although in residual quantity, a complex of dangerous groups of antibacterial drugs falls (see Table 2).

Most often in 2018, meat samples tested positive for the following antibiotic groups: streptomycin – 64% (reaction XI); aminoglycosides – 46% (reaction XIII); tetracyclines – 46% (reaction I); aromatic antibiotics – 36% (reaction IV) (see Table 2).

Table 2

The results of the study on the presence of antibacterial preparations in meat products from the markets of Chernivtsi for 2018

№ meatsample	Kindofmeat	Sampling market	Reaction number				
			I	IV	XI	XIII	II,III,V,VI,VII,VIII,IX,X,XII
1.	Chicken	Central	-	-	-	-	All samples had a negative analytical effect
2.	Rabbit	Main	-	-	-	-	
3.	Chicken	Lower	-	-	-	+	
4.	Veal	Central	+	+	+	+	
5.	Pork	Central	+	+	+	-	
6.	Veal	Central	+	-	+	+	
7.	Chicken	Main	-	-	-	+	
8.	Pork	Lower	-	-	+	-	
9.	Pork	Main	+	+	+	-	
10.	Veal	Central	-	-	+	-	
11.	Pork	Central	+	+	+	+	

Notes:I; II; III; IV; V; VI; VII; VIII; IX; X; XI; XII; XIII – see Materials and Methods Division; "+" – positive reaction; "-" – is a negative reaction.

In 2018, the content of two or more antibiotics in the tested samples was 1.8 times lower than in 2017, which may indicate a decrease in the amount of incoming residues of various groups of antibacterial drugs in the consumer's body. The validity of the difference ($F=4.3$; $p<0.05$) was confirmed by the Fisher test. This result is encouraging. However, the number of meat samples containing highly toxic streptomycin has increased.

Interestingly, chicken sample №1, 2018 did not contain antibiotic residues, and sample №2, 2017 revealed 4 different groups (see Tables 1 and 2). This case indicates that the content of harmful substances in the meat does not depend on its type. For safe nutrition, you must focus on the authority of the manufacturer and the protocols of laboratory testing of meat products.

During the experiment, it was found that only 9% of meat (2 of 23 samples: chicken and rabbit) were not contaminated with antibacterial agents. The most common type of meat pork in 100% (8 samples) contained antibiotics, chicken – 86% (6 out of 7). All veal samples contained antibiotics. Half of the rabbit samples were contaminated with antibacterial agents.

Available materials encourage the dissemination of information on the basics of safe and balanced nutrition among adolescents and indicate the need for educational work among the population. Based on an experimental study of the antibiotic content in meat consumed by the population of Chernivtsi, we give some recommendations on how to eliminate the passive use of antibiotics from our menu:

1. Meat must be soaked in salted water for several hours (the minimum procedure should be 15 minutes).
2. Boil meat for 20-30 minutes and drain the broth. Statistics show that boiling for 3 hours reduces the content of antibiotics by 90%. In this case, 70% of the harmful

substances go into the broth, so it must be drained.
3. To minimize the consumption of by-products (kidneys, liver), since they have the highest accumulation of harmful substances (4).
4. Buy meat from certified sellers and private producers who meet the health standards of their products.
5. Require lab meat testing protocols. If consumers pay more attention to the quality of what they buy, then entrepreneurs will also have to improve production technologies and, eventually, we will be able to eliminate antibiotics from the menu.
6. If you do not like cooked meat, you can use the oven. Antibiotics are starting to split already at 130°C.
7. When dividing the meat, be sure to remove the skin. In it often accumulate harmful substances.
8. It is also important to store the meat properly. To do this, select a separate shelf in the freezer, preferably the bottom. Freezing meat reduces antibiotic content by 20-25%.
9. Never buy foods with a very long shelf life: they contain many harmful substances and toxins.
Eating quality products will help keep the younger generation healthy.

4. Conclusion

79% of the entire meat diet of adolescents is pork 48% and chicken 31%;
91% of the test meat is contaminated with antibacterial residues. The most commonly used types of meat, such as pork and chicken, contained antibiotic residues in 100% and 86% of the samples, respectively.
Residues of antibiotics from the group of aminoglycosides and streptomycin were most often found in the studied meat samples.
9% of meat (one sample of chicken and rabbit meat) was not contaminated with antibacterial agents.
The content of harmful substances in meat does not depend on its type. For safe nutrition, it is necessary to focus on the authority of the manufacturer and the protocols for laboratory studies of meat products.
Laboratory monitoring of the content in antibiotics in meat in organized urban markets, as a guarantor of a safe and healthy diet, is advisable.
In order to disseminate information about safe balanced nutrition, educational conversations are necessary.
Given the small number of samples, the data obtained are experimental in nature and require further study.

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