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DETECTING THE PRESENCE OF ANTIMICROBIALS IN RAW MEAT IN THE AKMOLA REGION

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Antimicrobials are important drugs in animal production. However, their prolonged use has led to unexpected threats associated with the emergence and spread of bacterial resistance to antibiotics. Moreover, failure to observe the withdrawal period in animals used for food production, antimicrobial residues may enter the food chain causing direct toxicity, allergies in consumers. During the study, we monitored the content of antibiotics in meat and meat products in the Akmola region using a modern system Evidence Investigator (Randox). The study results revealed that horsemeat, mutton, pork do not contain antibiotics and are safe for consumption. However, the content of antibiotics in beef and poultry meat exceeds maximum concentration limit (MCL) in some cases by more than two-fold, which makes this type of

meat unsafe for consumption. At the same time, the applied analysis system showed a high level of sensitivity and low labor intensity. The results obtained indicate a low level of control of meat and meat products, as well as the abuse of antibiotics in the cultivation of animals and poultry.

Key words: *antibiotics, meat products, sausage products, Evidence Investigator (Randox).*

АҚМОЛА ОБЛЫСЫ БОЙЫНША МИКРОБҚА ҚАРСЫ ДӘРІЛЕРДІҢ ШИКІ ЕТ ҚҰРАМЫНА ТИГІЗЕТІН ӘСЕРІН АНЫҚТАУ

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Микробқа қарсы препараттар мал шаруашылығында маңызды дәрілер болып табылады. Дегенмен, оларды ұзақ мерзімді пайдалану салдарынан антибиотиктерге бактериялық төзімділіктің пайда болуы және олардың таралуымен қоса көтпеген кері әсері болуы мүмкін. Препараттарды қолдану мерзімінен алып тастау шараларын қадағаламау салдарынан микробқа қарсы препараттар қалдықтары азық-түлік өнімдерін өндіру тізбегіне еніп, тұтынушыларда тікелей уыттылық пен аллергия тудырады. Біздің зерттеуіміз бойынша Ақмола облысындағы ет және ет өнімдерінің құрамындағы антибиотиктердің мөлшерін қазіргі заманауи дәлелді зерттеуші (Randox) жүйесін пайдалана отырып бақыладық. Нәтижесінде жылқы, қой, шошқа етінің құрамында антибиотиктер жоқ және тұтынуға қауіпсіз екені анықталды. Алайда, сиыр және құс етіндегі антибиотиктердің мөлшері кейбір жағдайларда ШРК-нен 2 есеге асады, аталмыш көрсеткіш бойынша еттің бұл түрін тұтынуға қауіпті екенін көрсетеді. Осы ретте қолданбалы талдау жүйесінің сезімталдығы жоғары және төмен еңбек сыйымдылығын көрсететіні анықталды. Алынған нәтижелер бойынша ет және ет өнімдеріне бақылау деңгейінің төмен екенін және жануарлар мен құстарды өсіруде антибиотиктерді шектен тыс пайдаланылғаны анықталды.

Түйінді сөздер: *антибиотиктер, ет өнімдері, шұжықтар, дәлелді зерттеуші (Randox).*

ВЫЯВЛЕНИЕ ПРИСУТСТВИЯ АНТИМИКРОБНЫХ ПРЕПАРАТОВ В СЫРОМ МЯСЕ В АКМОЛИНСКОЙ ОБЛАСТИ

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Противомикробные препараты являются важными препаратами в животноводстве. Однако их длительное использование привело к неожиданным угрозам, связанным с возникновением и распространением устойчивости бактерий к антибиотикам. Более того, при несоблюдении периода отмены у животных, используемых для производства продуктов питания, остатки противомикробных препаратов могут попасть в пищевую цепочку, вызывая прямую токсичность, аллергию у потребителей. В нашем исследовании проведен мониторинг содержания антибиотиков в мясе и мясной продукции в Акмолинской области с привлечением современной системы Evidence Investigator (Randox). По результатам исследования выявлено, что мясо конины, баранины, свинины не содержат антибиотиков и являются безопасными для употребления. Однако содержание антибиотиков в говядине и мясе птицы превышает ПДК в некоторых случаях больше, чем в 2 раза, что делает употребление данного вида мяса небезопасным для употребления. При этом примененная система анализа показала высокий уровень чувствительности и низкую трудоемкость. Полученные результаты свидетельствуют о низком уровне контроля мяса и мясной продукции, а также о злоупотреблении антибиотиками при выращивании животных и птицы.

Ключевые слова: *антибиотики, мясная продукция, колбасные изделия, Evidence Investigator (Randox).*

Introduction. Antibiotics are widely used in veterinary medicine and continue to be the most cost-effective measure to protect livestock/birds against disease and infection [1, p. 113]. As a result of misuse and abuse of antimicrobials, antimicrobial residues can persist in food, causing adverse health effects to the consumer [2, p. 16, 3, p. 28, 4, p. 1025].

In Kazakhstan, there are no statistics on the level of antibiotic use in livestock and poultry farming. Large agrofirms keep records of antibiotic use, but there is no guarantee that they are used as intended and in the required doses. At the same time, private farms, often lacking sufficient knowledge, use drugs uncontrollably, which leads to negative consequences.

To protect public health from the side effects of antibiotics, maximum permissible levels (MPL) of antibiotics in food products have been established [5, p. 759]. Microbiological assays, instrumental methods and immunologic reactions are available as tools to monitor antibiotic content. Microbiological assays are characterized by low sensitivity and specificity [6, p. 126], while instrumental methods such as HPLC, liquid chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry are expensive, time-consuming and require sophisticated sample preparation and trained personnel [7, p. 299. 8, p. 943].

Evidence Investigator™ (Randox, UK) is the most advanced system to date for the detection of different classes of antibiotics in food. It is a semi-automated system with biochip kits. The ULTRA I (AM I ULTRA) and ULTRA II (AMII) microarray kits are capable of detecting several compounds belonging to different antibiotic families. The performance of this innovative system has been evaluated for the detection of antibiotic residues in novel matrices, in muscle tissue of different types of animal products [9, p. 243].

The advantages of this system are lower material costs and overall lower cost of analysis compared to LC-MS/MS, better sensitivity compared to microbiological methods, wide detection spectrum. Thus, this system is of potential interest for the detection of antibiotic residues in meat and meat products. Various kits are available for use in the Evidence Investigator™ system. Microarray I (AM I) and II (AM II) kits identify virtually all classes of antibiotics used in the livestock and poultry industries. Thus AM I performs quantitative analysis on sulfadimethoxin (SDM), sulfadiazine, sulfadoxine, sulfamethizole, sulfachloropyridazine, sulfamethoxypyridazine, sulfamerazine, sulfisoxazole, sulfathiazole, sulfamethazine (SM), sulfacquinoxalin, sulfapyridine, sulfamethoxazole, sulfamonomethoxine (SMM), trimethoprim and dapsone (DAPS) simultaneously, when as AM II defines quinolone (Qs), ceftiofur (CTF), thiamphenicol (TAP), streptomycin (STR), tylosin (TIL) and tetracyclines (TET) [10, p. 93].

Purpose: The purpose of our study was to apply modern technology in the study of raw meat of various animals and birds (beef, horsemeat, mutton, pork, chicken) purchased in retail outlets of the Akmola region to identify the level of antimicrobial content in meat and meat products.

Tasks: 1. Detect different classes of antibiotics in food products using a semi-automated system with biochip arrays.

2. Detect multiple compounds belonging to different families of antibiotics.

3. Evaluate the result and analyze the methods used and their effectiveness.

Materials and methods. Sampling of muscle tissue – meat (beef, horsemeat, pork, mutton and other types of agricultural and commercial animals), by-products was carried out at meat processing plants, slaughterhouses, refrigerated bases (chambers) of organizations storing meat products, warehouses and slaughterhouses of different agricultural formations in the Akmola region and imported products from Russia, Belarus (Figure 1).

Figure 1 – Individual samples of meat raw materials in retail outlets of the Akmola region

The total number of samples studied: 134 samples of cattle meat, 100 samples of small cattle meat, 50 samples of horsemeat, 50 samples of poultry, 20 samples of pork, 60 samples of meat products, including wurst, sausages, wieners, etc., at the same time – 11 samples from sausage products (boiled, semi-smoked) produced in the Russian Federation and Belarus.

The following criteria were used for selecting the animal carcasses or animal products intended for sampling:

- type of animal;
- information about the supplier;
- period of slaughter.

Sampling of domestic and imported meat products included the following criteria:

- type of finished product,
- name,
- information about the manufacturer,
- shelf life.

When collecting meat and by-product samples for laboratory testing, efforts were made to avoid taking large quantities of samples from animal carcasses and meat products provided by a single supplier.

When sampling meat from a batch, the sample included no more than 10% of carcasses (half carcasses) of cattle, 5% of carcasses of sheep, pigs and 2% of frozen or chilled blocks of meat and by-products, but not less than three carcasses or blocks. A point sample of muscle tissue was taken weighing at least 100 g which contained layers of fatty tissue, large connective-tissue elements.

The selected samples, immediately after their collection, were packed separately in individual sealed packaging – polyethylene bags, providing protection from contamination, damage and leakage. The serial registration number of the sample was used as an identification number and recorded in the sample logbook. The sample registration log was numbered, bound and sealed with the seal of the Faculty of Veterinary Science and Technology of Animal Husbandry, S.Seifullin Kazakh Agro Technical Research University. Immediately after collection, labeling and packaging of samples were cooled and (or) frozen.

Collected samples of meat and meat products were delivered to the laboratory in refrigerators or insulated containers at a temperature of -1-18°C not later than 36 hours from the collection date.

The analysis was carried out in accordance with GOST 34285-2017 (Interstate standard food products, food raw materials. Method of detection of chemotherapeutic drugs for veterinary use by enzyme immunoassay with chemiluminescent detection using biochip technology) according to the following procedure:

1. Sample preparation. Muscle tissue is homogenized, 1 g is placed in a polypropylene tube, 9 ml of reaction buffer solution is added, vortexed and then centrifuged. 200 microliths volume is taken from the top layer and placed in a microcentrifuge tube, the reaction buffer solution is added, and vortexed. For some kits, sample preparation includes adding chemical reagents to the muscle tissue, incubation in a water bath and evaporation with nitrogen currents.

Sample preparation of the feed includes homogenization, vortexing with methanol, centrifugation and sampling of the resulting supernatant 1. Feed extraction buffer is added to the precipitate, vortexed, centrifuged and the resulting supernatant is taken to the supernatant 1, dabbed with a working buffer solution and vortexed.

2. Analyzing. Take the test system out of the refrigerator 30 minutes before the analysis.

Prepare solutions of working concentration in accordance with the instructions for the test system. The required amount of samples and solutions is individual for each type of test system. Before analyzing the samples, it is necessary to calibrate them according to the instructions for the test system.

Add analytical buffer and analyzed sample to each well of the biochip (for milk samples also milk buffer is added) and incubate in the thermal shaker for 30 minutes; add conjugate and incubate in the thermal shaker for 60 minutes; wash the biochips and add signaling reagent to each biochip in turn, incubate in turn and obtain images on the analyzer. Obtain the results of the study in the software.

Measurement using the Evidence Investigator™ system (Randox, UK)

Calibration was performed using calibrators for AMI and AMII kits, which cover the measurement range of all analytes (Figure 2).

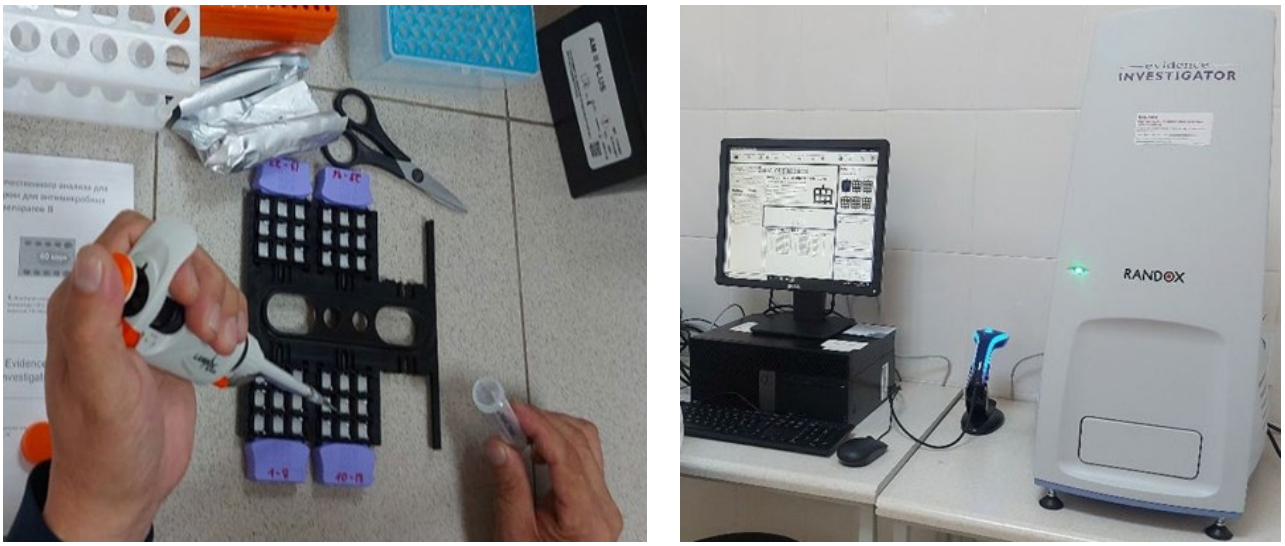

Figure 2 – Biochip loading and analysis on Evidence Investigator™ (Randox, UK)

MicroArray I (AM I) kit

The Anti Microbial Array I (AM I) kit quantitatively tests Sulfamonomethoxin, Dapsone. The Anti Microbial Array I ULTRA kit is based on a competitive chemiluminescent immunoassay. An increase in the concentration of sulfonamides in the sample leads to a decrease in the binding of horseradish peroxidase (HRP)-labeled sulfonamides, resulting in a decrease in chemiluminescence intensity.

MicroArray II (AM II) kit. The Anti Microbial Array II (AM II) kit quantitatively tests quinolones, ceftiofur, thiamphenicol/florfenicol, streptomycin/dihydrostreptomycin (DHS), tylosin/thylmycosin and tetracyclines simultaneously. Each kit includes 6 media, 9 calibration points, buffers required for recovery, and other reagents (conjugate, chemiluminescent solution). Each carrier consisted of 9 microarrays (1 cm by 1 cm) [11, p. 220].

Statistical analysis. Analyses were performed in twofold biological repetition. The mean and standard deviation were calculated in Microsoft Excel 2010 program. The reliability of the data was determined by calculation of Student's standard deviation criterion (p).

Results. Collected samples in the amount of 414 samples after delivery to the laboratory were analyzed for the content of antibiotic drugs in them. Before the start of work, the adjustment and a series of calibrations of the apparatus “Randox” were carried out in terms of the analysis of meat and meat products according to the manufacturer's instructions. Then all samples were analyzed for the level of 6 most used antibiotics. According to the results, antibiotics were found in beef (20 to 23.5% of each type of antibiotics) and poultry meat (5 to 24.6% of each type of antibiotics). The results of the analysis are presented in (Table 1).

Table 1 – Results of sample analysis for antibiotics in meat and meat products

Name of samples	Number of samples, pcs.	Types of antibiotics									
		SMM	DAPS	STR	TIL	TET	TAP	CTF	SDM	SM	Qs
		Number of positive samples									
Horse meat	40	-	-	-	3	2	2	1	-	-	-
Beef	118	17	22	21	33	32	21	12	-	-	-
Lamb	11	-	-	-	-	-	-	-	-	-	-
Pork	10	-	-	-	-	-	-	-	-	-	-
Poultry meat	48	5	14	3	12	9	2	1	2	1	3
Meat products	13	-	-	5		-	-	-	-	-	-

Of the total number of samples of meat and meat products of domestic production, namely 118 samples of meat from cattle, 11 samples of small cattle, 40 samples of horse meat, 48 samples of poultry (liver, drumstick, breast) 10 samples of pork, 13 samples of meat products, including sausages (5 samples of sausages from domestic producers, as well as imported products from Russia 4 samples, Belarus 4 samples), 10 types of antibiotics were detected. Thus, 7 types of antibiotics were detected in beef, of which SMM (14,4%) and CEF (102%), TIL (27,9%) and TET (27,1%) and TAP and STR (17,8%) and DAPS (18.6%). In 96.6% of cases, more than one antibiotic was detected in one sample. TAP, TIL, STR and TET were most often found simultaneously in a sample (27.1%). As in beef, a wide range of antibiotics was detected in poultry meat, with a total of 10 species detected. An additional three types of antibiotics SDM, SM, Qs were detected in 4.2, 2.1, 6.3% of samples, respectively. DAPS (29.1%), TIL (25%), TET (18.8%) and SMM (10.4%) were the most common. Other antibiotics were detected in 2.1-6.3% of samples. In horse meat, 3 types of antibiotics TIL, TET, TAP were detected in 7.5, 5, 5, 5% of samples. Antibiotics were mainly detected in the liver (12.5%) and shank (16%). In horse meat, the presence of 4 types of antibiotics (TIL, TET, TAP, STF) was detected in 2.5-7.5% of samples. However, it is not possible to find out whether the antibiotic was applied during the rearing of the animal or was used after slaughter to increase the shelf life of the product [12, p.102]. Researched antibiotics were detected in poultry meat. DAPS content in poultry meat was detected in 24.6% of samples. At the same time, this antibiotic is banned in the EU for use in livestock used for food production [13, p. 251]. Side effects such as dose-dependent hemolysis (which can lead to hemolytic anemia) and methemoglobinemia, abnormalities in white blood cell formation, including aplastic anemia, are rare but are responsible for the majority of deaths due to DAPS therapy [14, p. 288]. In 10% of the samples, the presence of TAP was noted, which is generally used in poultry for the treatment of respiratory diseases, as TAP degrades to acetylcysteinate, which provides a mucolytic effect [15, p. 492]. All samples of horsemeat, lamb and pork meat did not contain the tested antibiotics. However, SRT was detected in meat products, which is one of the main allergens in the same range as penicillin, oleandomycin and TIL [16, p. 6].

The MPL of each antibiotic are strictly regulated according to ISO applicable in Kazakhstan. For each antibiotic, both minimum and maximum values detected in the tested samples were determined. The results are presented in (Table 2).

Table 2 – Residual amounts of antibiotics in meat and meat products

Name of samples	№ of samples, pcs	Types of antibiotics									
		SMM	DAPS	STR	TIL	TET	TAP	CTF	SDM	SM	Qs
		MPL, mg/kg									
		0,01	0,0046	0,014	0,0009	0,0048	0,0013	0.0046	0.0065	0.002	0.005
		Min- max (aver.)	Min- max (aver.)	Min- max (aver.)	Min-max (aver.)	Min- max (aver.)	Min- max (aver.)	Min- max (aver.)	Min- max (aver.)	Min-max (aver.)	Min- max (aver.)
Horse meat	40	-	-	-	0,0012-0,0029 (0,0023)	0,00864-0,00979 (0,00922)	0,00279-0,00307 (0,00293)	0,00509	-	-	-
Beef	18	-	0,0053-0,0056 (0,00543)	0,0141-0,0143 (0,01415)	0,00092-0,002 (0,0011)	0,00484-0,0051 (0,00497)	0,00134-0,0014 (0,00132)	0.00463-0.00792 (0.00582)	-	-	-
Lamb	1	-	-	-	-	-	-	-	-	-	-
Pork	0	-	-	-	-	-	-	-	-	-	-
Poultry meat	48	-	0,005-0,025 (0,0136)	0,015-0,027 (0,0191)	0,001-0,0025 (0,0016)	0,0049-0,0055 (0,0052)	0,0014-0,0015 (0,00141)	0.0051	0.00653-0.00742 (0.006975)	0.0023	0.00539-0.09197 (0.06101)
Meat products	13	-	-	0,015-0,016 (0,0154)	-	-	-	-	-	-	-

As a result of the analysis, antibiotics above the MPL were detected in beef, poultry and meat products from a domestic producer. In beef samples the maximum value of TIL was detected – 0.002 mg/kg, which is almost 2.2 times higher than the norm. Also, a significant increase in MPL was detected in DAPS by 1.2 times. The antibiotic levels of STR, TET and TAP were insignificantly higher than normal. In meat products, the detected amount of STR was slightly above the norm. However, most of the antibiotic content was in poultry meat. DAPS at maximum content exceeded 5.4 times the MPL, STR – 1.3 times, TIL – 27.8 times, TET – 1.1 times. TAP content was insignificantly higher than the norm (0.0014 mg/kg). STF maximum was at 0.00792 mg/kg, which is 1.7 times the MPL. In poultry meat, the maximum detection level of DAPS was 0.025 mg/kg, which was 5.4 times the MPL. The CTR value was 1.9 times higher than normal. The levels of other antibiotics were insignificantly higher than the norm. In horse meat, TIL was detected at 2.6 times higher than normal, TET at 2.1 times higher, TAP at 2.3 times higher, with CTF slightly higher than normal. Foreign literature more often presents data on antibiotic content indicators in pork and poultry meat, which are most used in Western countries, the USA and China [17, p.233], poultry meat is more frequently tested for oxytetracycline, SRT and TIL content [18, p. 468].

Thus, the presented results indicate the presence of excessive amounts of antibiotics in beef and poultry meat, exceeding MPL up to 5,7 times. Almost all 10 antibiotics were detected in poultry and beef meat. The Evidence Investigator™ system (Randox, UK) allows a large number of samples to be analyzed in a short period of time and is highly sensitive. The use and implementation of such automated systems can expand the scope of antibiotic testing of meat and meat products and reduce the risk to animals and humans.

Discussion. Antibiotics are secondary metabolites of certain bacterial and fungal species that have deleterious properties against other bacterial species. These compounds play a key role in agriculture, veterinary and clinical settings. They are also widely used in food-producing animals for therapeutic, prophylactic and metaphylactic purposes [19, p.417]. Meat is a highly nutritious food due to its high-quality proteins containing all essential amino acids, as well as various minerals, namely iron, zinc, selenium and magnesium. It is also a major source of five B vitamins, which are important cofactors for energetic metabolic pathways [20, p.264]. The human population is growing rapidly, which increases human consumption of food, especially animal products. Therefore, the demand for animal protein is increasing significantly worldwide. To meet this demand, intensive animal and poultry farming is gaining popularity and becoming an important area in the food industry [21, p.301].

The presence of antibiotic residues in combination with multidrug residues in some meat samples is of concern because it may pose serious threats to human and animal health, such as toxicity and resistance development [22, p.643].

As a result of our research, 10 types of antibiotics were detected, including SMM, STR, TIL, TAP, TET, SM, SDM, CTF, DAPS, Qs. Meanwhile, all 10 types of antibiotics were detected in poultry meat, mainly in shank and liver. In beef, 7 antibiotic species were detected, mostly more than one per sample. Four types of antibiotic were detected in horse meat, the number of samples was not significant from 1 to 3, for each type of antibiotic.

Various methods have been developed for the determination of antimicrobial residues in food of animal origin, including screening and chromatographic methods. To date, the Randox system is the most optimal in terms of speed and quality of results obtained. As for the spectrum of the AM II kit, it is ideally suited for the screening of antibiotic residues in meat products.

The most frequently tested types of meat are pork and poultry. Meanwhile, lamb and horsemeat are less frequently investigated. In our study, no antibiotics were detected in either horsemeat or mutton. However, in a study by Khalid Ibrahim Sallam et al of lamb meat in Kuwait for the presence of amoxicillin, oxytetracycline, tetracycline and tylosin showed that the levels of almost all antibiotics do not exceed the MAC, while in some samples several types of antibiotics were detected at the same time, and the lamb is safe for consumption [23, p. 1209 2].

The study of Ali Aydin et al of horse meat from Kazakhstan and Kyrgyzstan, where this type of meat is most often consumed, the presence of antibiotic-resistant Staphylococcus pasteuri species to penicillin, cefoxitin and oxacillin was noted, which has a potential threat to human health in case of poorly cooked meat.

In our research, no antibiotics were found in pork, but according to foreign researchers, pork is the main source of antibiotics in food.

According to Monger X. et al. TRC is the main antibiotic used in pork and which is most frequently detected in pork meat and meat products.

Presence levels exceeding MPL values in meat were found in beef and poultry. Beef samples showed maximum value of TIL was detected – 2.2 times higher than the norm, DAPS by 1.2 times. The antibiotic levels of STR, TET and TAP were insignificantly higher than normal. In meat products, the detected amount of STR was slightly above the norm. In poultry meat maximum content of DAPS was found by 5.4 times higher than MPL, STR – 1.3 times, TIL – 27.8 times, TET – 1.1 times.

It is important to note that the presence of antibiotics cannot be traced during animal rearing and handling of finished products.

Thus, meat and meat products in the Akmola region have antibiotic levels both within the norm and above the MAC. However, mutton, pork and horsemeat are safe for consumption, which corresponds to all ISO norms. Conducting this kind of research is mandatory to improve the quality of products on the local market.

Conclusion. The obtained results indicate that meat and meat products in Akmola region is not dangerous to human health. It is important to improve and expand the detection of antibiotics in meat and meat products, to strengthen control in this area. In this case, it is important to implement the most simple automated and non-labor-intensive methods, with a high level of sensitivity, which is Evidence Investigator™ (Randox, UK). According to our work, this system has proven itself for screening meat and produce samples for antibiotics. Increasing the level of control over the content of antibiotics in meat and meat products will improve the quality of meat, reduce the level of antibiotic resistance of bacteria, and reducing the number of applications and the transition to organic animal husbandry will increase the volume of organic products in the local market.

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