



Review Article

Identification of Residual Traces of Antibiotics in Food

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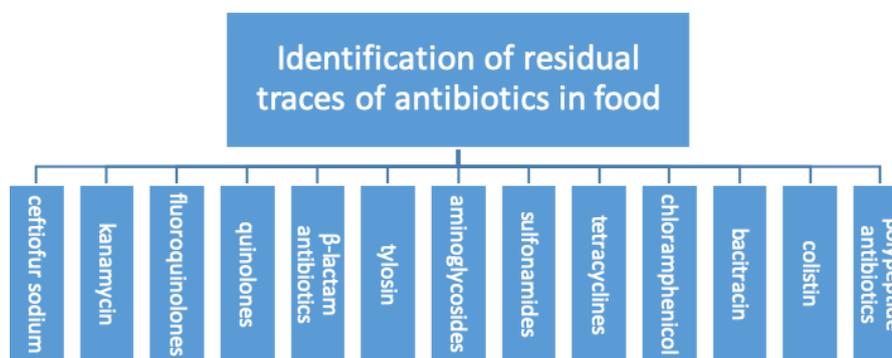
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ABSTRACT

Due to the widespread use of veterinary drugs and antibiotics in animal husbandry, one of the most critical problems in ensuring food safety is the presence of residual antibiotic traces in animal products. Concentrations of such traces in food products pose a danger to both humans and the environment as a whole. Irrational use of antibiotics in agriculture stimulates resistant bacteria that can cause infectious diseases in humans and animals that cannot be treated with modern drugs. Due to the potential risk to human health in many countries, maximum permissible limits for the content of residual traces of antibiotics are regulated. This review aimed at investigating the recent publications in the field of identification of residual antibiotic traces in food. Various types of high-performance liquid chromatography (HPLC) and tandem mass spectrometry (MS/MS) combined with solid-phase extraction were mainly used for the quantitative determination of analytes. Methods of enzyme immunoassay, capillary electrophoresis, and infrared spectroscopy with Fourier transform, biosensor, and fluorescence analysis methods are also presented. It is shown that the development of new susceptible, accurate, simple, and cost-effective methods for the determination traces of antibiotics in food remains an urgent task and is highly demanded in the modern world.

GRAPHICAL ABSTRACT



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Introduction

The widespread use of antibiotics has gradually led to modern veterinary and animal husbandry being utterly dependent on the availability of antibiotics. This addiction has led to heavy and sometimes reckless use of antibiotics [1,2]. It has been established that when antibiotics are added to the feed, there is a decrease in the mortality of young animals, an acceleration of their growth and development processes, and a decrease in the volume of feed consumption by 5-10% [3]. However, it should be borne in mind that entering an animal body, antibiotics can circulate in it for a long time, and then their residues end up in products of animal origin (milk, meat, eggs, etc.). Moreover, traces of antibiotics can be found in crops and vegetables due to fecal fertilizers [4,5].

Antibiotic residues in food or the environment can have many serious health consequences. They can cause intestinal dysbiosis, metabolic disorders, allergic reactions, including anaphylactic shock, and produce such effects as mutagenic, toxic, teratogenic, carcinogenic, etc. [6]. The ingestion of antibiotic traces with food into the human body stimulates the growth of the microorganisms' resistance to them. Antibiotic resistance has become one of the leading international problems of modern health care. More than 25,000 people die annually in European countries due to infections caused by antibiotic-resistant bacteria [7]. The emergence of pathogens provoking infectious diseases that are resistant to antibiotics makes the course of the disease worse and increases the cost of their treatment; such diseases are difficult or even impossible to cure [8,9].

As a result of the current situation, animal products should be monitored for antibiotic residues in products to ensure that the maximum residue limit is observed. Today, many antimicrobial drugs are known, which are intensively used for feeding animals on industrial farms and agricultural holdings [10].

The primary purpose of this study was to review publications describing the latest advances in identifying residual traces of antibiotics in food.

Polypeptide Antibiotics

Polypeptide antibiotics are a group of broad-spectrum antimicrobials against many gram-negative and gram-positive bacteria. Antibiotics of this group have a high molecular weight and have a typical structural structure, consisting of a heptapeptide ring with a polypeptide side chain. The article [11] describes the method of ultra-HPLC and MS/MS to identify polypeptide antibiotics' residues in food of animal origin. Extraction was carried out with a mixture of acetonitrile, water and 25% ammonia solution at a volume ratio of 80/10/10, the stages of evaporation, reduction and filtration were carried out. Mass spectral measurements were carried out in the mode of selective monitoring of multiple reactions using a triple quadrupole mass spectrometer, and chromatographic separation was carried out on C18 column in the mode of gradient elution. By using this method, the concentration of polypeptide antibiotics can be determined in the range from 10 to 1000 µg/kg.

Colistin

Colistin inhibits bacteria by destroying the outer and inner membranes of the bacterial cell. The interaction of colistin and the bacterial cell membrane is carried out by electrostatic interactions between the positively charged diamminobutyric acid in colistin and the phosphate groups' anions of the cell membrane. The antibiotic actively replaces the cationic Mg⁺² and Ca⁺² and the phosphate group anions of the bacterial cell membrane. This mechanism leads to the destabilization of the lipopolysaccharide molecule, which contributes to the weakening of the bacterial cell membrane molecule. Consequently, the cell is going to be destroyed by antibiotics. It is toxic to the kidneys, neurotoxicity has also been noted, and is the last reserve drug in treating infectious diseases caused by gram-negative bacteria with a high degree of resistance to most drugs [12].

In [13], a method for colistin determination by the LC-MS / MS method was proposed. The extraction process was carried out using solid-phase extraction in the presence of methanol oxidized with 0.1% formic acid. The analysis was performed using an Atlantis dC18 column (2.1×100 mm, 3 µm) at room temperature and a

mobile phase of 0.2% formic acid in acetonitrile and water (50:50 v:v) supplied at a flow rate of 0.2 mL per a minute.

Bacitracin

Bacitracin is an antibiotic of the polypeptide group, synthesized by strains of the *Bacillus subtilis* bacterium. Bacitracin acts on gram-positive microorganisms (beta-hemolytic streptococci, staphylococci) and some gram-negative pathogens. The mechanism of action is to inhibit the bacterial cell wall synthesis.

The study [14] presents a portable enzyme-linked immunosorbent assay method with the lateral flow to identify traces of colistin and bacitracin in milk. The method modification replaced the gold nanoparticles used in the traditional lateral flow immunoassay with fluorescent microspheres for labeling monoclonal antibodies. Based on the principle of competitive binding with labeled monoclonal antibodies between analytes in samples and fixed antigens on the membrane, the detection limits for bacitracin and colistin were 100 and 50 ng/ml, respectively.

H. Kumar et al. described a method to determine colistin in eggs and poultry meat. Colistin b was extracted with a solution of acidified methanol and water at a ratio 1:1, then it was centrifuged and filtered through a membrane filter. The analysis was performed using ultra-HPLC and MS/MS. The limit of the colistin quantitative determination by the developed method was 10 µg/kg in poultry and 5 µg/kg in eggs. This method is more cost-effective as compared to analogs due to the reduction in the cost of sample preparation [15].

Chloramphenicol

Chloramphenicol is a broad-spectrum antibiotic applied to treat infectious diseases in humans and animals. However, when consumed excessively, a number of serious side effects appear. Consumed excessively, a number of serious side effects appear. It can have a negative effect on the cardiovascular system, bone marrow, be embryotoxic, and cause diseases such as leukemia, aplastic anemia, and Gray's syndrome. Therefore, many countries, including the USA, Canada, and China, have banned

chloramphenicol in animals used in food production [16].

Simultaneous determination of eight veterinary drugs and three metabolite residues of four categories (chloramphenicol, nitroimidazoles, lincosamides, and macrolides) in eggs, milk, chicken, and freshwater fish is proposed in the article [17]. The developed method is based on ultra-performance liquid chromatography and MS/MS combined with the solid-phase extraction. The target analytes were separated on a chromatographic column at a column temperature of 40 °C and a flow rate of 0.4 ml/min. As mobile phases, the gradient elution was performed with methanol and 0.1% aqueous formic acid solution. The developed method provides low detection limits from 0.05 to 0.50 pg/kg and quantitative detection limits from 0.2 to 1.5 pg/kg.

Y. Duana et al. proposed a method for identifying chloramphenicol in animal products using a specific aptamer and fluorescent quantitative real-time PCR. The method's essence consists of hybridizing the chloramphenicol-specific aptamer with a complementary probe modified with biotin and its further immobilization on magnetic beads conjugated with streptavidin. When chloramphenicol presents in the test sample, the resulting aptamer binds explicitly to it, forming a hairpin structure. It is then released from the magnetic beads and detected by the real-time PCR. To determine the optimal conditions for the chloramphenicol detection, the influence of such factors as the length of the probe chain, the concentration of aptamer, the NaCl concentration, and the incubation time was studied. Under optimal conditions, the developed technique was distinguished by high sensitivity and made it possible to determine chloramphenicol from 0.1 to 20.0 ng/ml. Also, this technique has shown high selectivity with structural analogs of chloramphenicol, such as thiamphenicol and florfenicol [18].

Scientists from Iran have presented a fluorescent method for detecting traces of chloramphenicol in milk and honey using an optical sensor based on a nanostructured polymer with a molecular imprint applied to a luminescent zirconium

metal-organic framework. This method allows determining the chloramphenicol concentration in the range from 0.16 to 161.56 $\mu\text{g/L}$, with a detection limit of 0.013 $\mu\text{g/L}$ [19].

Tetracyclines

Tetracyclines are inexpensive broad-spectrum antibiotics against gram-positive and gram-negative bacteria; therefore, they are widely applied in medicine and veterinary medicine. However, their residual traces in food and the environment have raised serious concerns recently. In this regard, various methods of identifying and preparing samples for the analysis of tetracycline residues in food products are being developed.

A highly selective method for the determination of the tetracycline traces in food is presented in the article [20]. The authors of the article prepared an aptamer attached to the tetrahedral nanostructured functionalized DNA magnetic beads (Apt-tet MB) as a probe to detect tetracycline. If tetracycline traces are found, the DNA primer is released from the Apt-tet MB. The separated DNA primer triggers a reaction of the rolling circle amplification (RCA) and generates a long tandem single-stranded sequence. The fluorescence signal is then captured by detection probes using the SYBR Green I fluorescent dye by hybridizing the RCA product. This method allows determining the concentration of tetracycline in the range from 0.001 to 10 ng/mL.

H. Sereshti et al. developed a method that makes it possible to determine target analytes within the detection limits of 0.32–1.01 and 2.42–7.59 $\mu\text{g/L}$ in water and milk samples, respectively [21].

In the study [22], a method was proposed to obtain organometallic frameworks for dispersive solid-phase extraction to determine tetracycline traces in honey. The most effective adsorption-extraction was found with a combination of MIL-101 (Cr), MIL-100 (Fe), and MIL-53 (Al) and a component ratio of 7:1:2. This method allowed determining the concentration of oxytetracycline, tetracycline, chlortetracycline, and doxycycline in the range from 0.239 to 1.449 ng/g.

A. Kumar et al. described a method developed to determine the residual amounts of

oxytetracycline and amoxicillin in cow's milk using HPLC with a diode array detector. The detection limits of the desired analytes were 1.4 and 0.9 $\mu\text{g/kg}$ for oxytetracycline and 2.5 and 1.5 $\mu\text{g/kg}$ for amoxicillin [23].

Sulfonamides

Sulfonamides are synthetic antibacterial drugs, derivatives of the pair (π)-aminobenzenesulfonamide. They can be rapidly assimilated, so they are widely used in animal husbandry as medicines and growth stimulants. Currently, sulfonamide residues are increasingly entering soil and water bodies. Therefore, they are potentially dangerous for the environment.

In article [24], a spherical mesoporous covalent organic framework was synthesized as an adsorbent for solid-phase extraction for the supersensitive determination of sulfonamides in food and water samples by liquid chromatography and MS/MS. The developed method provides low detection limits (0.5–1.0 ng/L) and a wide measurement range of sulfonamides (5–1000 ng/L).

A method for identifying residual traces of antibiotics in goat milk is presented in [25]. The method is based on extraction on a PriME HLB column combined with ultra-HPLC and high-resolution quadrupole / electrostatic field orbital mass spectrometry. The developed method makes it possible to determine simultaneously up to 62 veterinary antibiotics to six different classifications. The limit of quantitative determination of antibiotics was from 0.5 to 100 $\mu\text{g/L}$.

A. Mehl et al. [26] developed a high-throughput planar solid-phase extraction method to rapidly screen nine different classes of antibiotics (sulfonamides, diaminopyrimidines, lincosamides, pleuromutilins, macrolides, cephalosporins, penicillins, amphenicols and nitroimidazoles). Meat, cow's milk and chicken eggs were used as research objects. The analysis time was 7 minutes per sample, which is 5 times faster than conventional modern technologies. According to 2002/657/EC, the test method is approved for one antibiotic of each class. The authors suggest that the analysis method developed by them will increase food safety by studying samples [26].

Aminoglycosides

Aminoglycosides are a group of natural and semi-synthetic antibiotics with a similar chemical structure, including the presence in the molecule of an amino sugar linked by a glycosidic bond to an amino-cyclic ring. Antibiotics of this group have a broad spectrum of action and bactericidal activity against aerobic bacterial infections. When accumulated in the human body, they can negatively affect and have ototoxicity and nephrotoxicity. Due to the potential health risks, many countries have established maximum limits for the content of residual traces of aminoglycosides in animal products.

Y. Yu *et al.* developed a susceptible method for determining aminoglycosides in food using capillary electrophoresis, ionization, and tandem electrospray mass spectrometry. Capillary electrophoresis was used to separate aminoglycosides; quantification was performed using mass spectrometry. The detection limit for aminoglycosides was 0.67 µg/kg [27].

Y.R. Kim and H.-S Kang presented a method for determining twenty aminoglycoside residues in animal products using liquid chromatography and MS/MS. Separation was carried out on a C18 column with a reverse phase and was eluted with acetonitrile containing an ion-pair reagent - heptafluorobutyric acid. Compared with analogs, the developed method is less laborious and more economically due to d-SPE purification [28].

Tylosin

Tylosin is a broad-spectrum macrolide antibiotic produced by *Streptomyces fradiae* strains. It belongs to the group of antibacterial drugs for curing diseases of bacterial etiology. Tylosin tartrate granulate is used as a growth stimulant (feed antibiotic) for pigs, miniature and cattle, and poultry. Residual traces of tylosin can cause allergic reactions, disturb the intestinal microflora and have carcinogenic, mutagenic, and hepatotoxic effects [29].

Determination of tyrosine residues in milk using Fourier Transform Infrared Spectroscopy (FTIR) in combination with chemometrics is presented in [30]. The authors used the total reflection of the attenuated coupled FTIR with the multilayer network of perceptrons (MLP) and partial least

squares (PLS). MLP allows identifying milk samples contaminated with tylosin, and PLS predicts very low deficient concentrations (0.1-100 µg/L) of tylosin residues in milk when analyzing FTIR data. This method is positioned as an effective and inexpensive quantitative method for the analysis of tylosin residues in milk.

To determine the antibiotics lincomycin and tylosin in food products, a double immunochromatographic test system was developed by Russian, Bulgarian, and Chinese scientists. Immuno-chromatographic analysis was carried out in an indirect competitive format using anti-species antibodies conjugated to gold nanoparticles as a label. Under optimal conditions, the detection limits for tylosin and lincomycin were 0.090 ng/mL and 0.008 ng/mL, respectively, and the duration of the analysis took 10 min. The developed test system made it possible to determine the residues of tylosin and lincomycin in milk, honey, and eggs [31].

The article [32] describes a method for detecting tylosin traces using FTIR. The authors note that the proposed technique is highly efficient, fast in analysis, and low in cost.

β-lactam antibiotics

β-lactam antibiotics are widely used in veterinary practice; they inhibit the bacterial cell wall synthesis, especially gram-positive ones. β-lactam antibiotics include compounds with a β-lactam ring in their structural structure - penicillins and cephalosporins.

V.G. Amelin, D.S. Bolshakov and I.V. Podkolzin developed a method for rapid screening of residues of 19 β-lactam antibiotics in food using ultra-HPLC - quadrupole-time-of-flight mass spectrometry high resolution. Acetonitrile was used to extract analytes. Identification was carried out according to the exact masses of analyte ions, retention time, and the pattern of isotopic distribution of ions (mSigma). This method allows determining the content of analytes in the range (1-10) -200 ng/g (R₂≥0.99), the detection limit is 0.05-5.00 ng/g and the duration of sample analysis is 20-30 minutes [33].

The article [34] describes selective chromatographic separation to analyze 32

residues of β -lactam antibiotics in milk samples. Chromatographic studies were performed using a binary gradient water/acetonitrile with formic acid and ammonium acetate. The extraction of the β -lactams' residues from milk was carried out with a solution of water and acetonitrile, then purified by dispersive solid-phase extraction with C18 followed by concentration in nitrogen and determination ultra-HPLC with MS/MS. This method allows the detection of β -lactam antibiotics within the detection range from 0.0090 to 1.5 $\mu\text{g}/\text{kg}$ and quantitative determination in the range from 0.03 to 5.00 $\mu\text{g}/\text{kg}$.

Quinolones

Quinolones are a group of widely used synthetic antimicrobials, also including fluoroquinolones. The mechanism of action of quinolones is to inhibit bacterial enzymes DNA gyrase, topoisomerases II and IV, which leads to a violation of the DNA replication. Inhibition of the DNA gyrase causes bacterial death. Quinolones are used to treat acute gastrointestinal infections as first-line drugs in humans because of their high activity against intestinal pathogens and in cases where the causative pathogen is not yet known. Due to their broad spectrum of action, quinolones are also used in veterinary medicine. However, it was noted that the introduction of quinolones into veterinary practice contributed to a significant increase in bacterial resistance to antibiotics of this group.

Fluoroquinolones

Enrofloxacin belongs to the group of fluoroquinolones. It is effective against all types of mycoplasma, gram-negative and gram-positive microorganisms.

In the paper [35], Argentine scientists proposed a method for determining residual traces of enrofloxacin in chicken eggs using HPLC in combination with a fast scanning fluorescence detector.

Lithuanian scientists have developed a method for detecting quinolones in poultry meat using a Fourier transform ion cyclotron resonance. Sample preparation has been simplified and reduced to the stage of extraction and freezing. The chromatographic separation step was also

eliminated, and the mass spectrometric parameters were optimized. The developed method makes it possible to determine the presence of ten quinolone compounds in poultry meat, including ciprofloxacin and enrofloxacin [36].

Kanamycin

Kanamycin is an aminoglycoside antibiotic produced by *Streptomyces kanamyceticus* or other related microorganisms. It is widely used to treat gram-positive and gram-negative bacterial infections in human and veterinary medicine. The mechanism of action of kanamycin is based on interaction with ribosomal RNA, which interferes with the synthesis of bacterial protein. Residual traces of kanamycin in excess can cause allergic reactions, hearing loss and nephrotoxicity.

The article [37] describes a biosensor based on aptamers to determine kanamycin residues in samples of agricultural products. It is a flow-through aptamer biosensor, signal changes in the sensor are monitored using surface plasmon resonance measurements based on the specific interaction of the aptamer with the antibiotic. This biosensor allows determining the kanamycin concentration in the range from 1 to 100 mmol/L.

Ceftiofur sodium

Ceftiofur sodium belongs to a third-generation cephalosporin antibiotic. It is widely used to prevent and treat bacterial infections of the respiratory tract of animals or as a feed additive to stimulate growth [38,39].

The paper [40] presented a susceptible, selective method for determining sodium ceftiofur. Scientists have synthesized an environmentally friendly molecular imprinted polymer which is used for solid-phase extraction as an adsorbent. The analysis was performed using HPLC with an ultraviolet detector. The developed method was successfully used to identify traces of sodium ceftiofur in milk, chicken, pork, and beef samples.

Conclusions

In many countries, antibiotics in agriculture exceeds their use in medicine. Excessive, uncontrolled use of antibiotics in veterinary medicine, coupled with non-adherence to drug intake rules, withdrawal periods, and strict

adherence to food safety rules, poses a significant threat to public health and the ecosystem. In addition to the various adverse health effects that can arise from exposure to antibiotic residues, antibiotic resistance of microorganisms is considered the main threat to human health in the future. Through environmental objects, contact with animals, and food, people can gain resistance to antibiotics. Antibiotic resistance has no ecological, geographic, sectoral or biological boundaries. Thus, the use of antibiotics in one country or industry affects the spread of antibiotic resistance in other countries and industries. Controlling and reducing the negative impact of residual traces of antibiotics on the human body and the environment is a top priority worldwide. Following all of the above, the development of methods for identifying residual traces of antibiotics in food products remains an urgent task, and new, more effective methods of their analysis are being developed.

Conflict of Interest

The authors declare no conflict of interest.

Authorship Criteria

The authors were equally involved in the writing of the manuscript and are equally responsible for plagiarism.

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