The One Health concept in the prevention of β-lactam antibiotic residues in milk and the environment

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Abstract

The One Health concept emerged with the aim of balancing the lives of humans, animals, and plants on Earth, while also preserving the environment. Its aim is to improve overall living conditions on Earth and one of these issues is the overuse of antibiotics for therapeutic purposes and the use of antibiotics to promote livestock breeding on farms, which has led to a rapid spread of antibiotic resistance among microorganisms. Additionally, residual antibiotics are found in food of animal origin, and in the environment where they pollute soil and water. The most widely used group of antimicrobial agents are the β-lactam antibiotics. Due to their powerful antimicrobial effect and low toxicity, they are used to treat bacterial infections in both human and animal medicine. β-lactam antibiotics are major antibiotics used for treating mastitis, the most common infection occurring in milk-producing cows. Across EU Member States, testing and identifying the residues of β-lactam antibiotics in milk samples is included in national antibiotic residue monitoring plans for food of animal origin. The development of sophisticated methods with high sensitivity and precision, based on liquid chromatography coupled to mass spectrometry, enables the simultaneous control of different antibiotic classes for the official control of residues in foods of animal origin. According to the literature, β-lactam antibiotics are frequently detected antibiotic residues in EU Member States, with penicillin G as the most common compound.

Key words: *One Health, antibiotics; β-lactams; residues; screening methods; confirmatory methods*

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Introduction

The Food and Agriculture Organization of the United Nations (FAO) has set new global goals for sustainable development for the period up to 2030, the most important of which are reducing hunger in the world and reducing food losses during harvesting in the fields. After five years of stagnating food shortages in the world, the emergence of the COVID-19 pandemic led to an increase in global hunger from 8.4 to 10.4% in just one year. At the same time, the percentage of food lost during cultivation on farms and in transportation, storage and processing was 13.8% worldwide. Since then, food safety controls have been strengthened (OECD/FAO, 2021).

Milk is a food rich in proteins, which are crucial as sources of amino acids and nitrogen for all physiological processes in the body. Studies have been conducted showing an association between the consumption of dairy products and markers for the risk of cardiovascular metabolic disorders and diseases. Increased consumption of fermented dairy products has a favourable effect on body biomass index (BMI), which is attributed to the metabolic activity of the intestinal microbiota, reduced caloric effect of certain macronutrients, and influence on the appetite (Trichia et al., 2020). The consumption of meat, milk and eggs as the main sources of dietary protein depends on the standard of living, dietary habits, development of animal husbandry, and macroeconomic security. According to the latest report, meat consumption in the European Union is 14,438 kg per inhabitant per year, while milk consumption is expressed as the mass of milk powder per year and equals 105,550 kt (OECD/FAO, 2021). The largest milk producers are the European Union, the United States of America, and India, while the leading European countries are Germany and France (US Department of Agriculture, 2023).

The human population is exposed to antimicrobial substances directly through treatment, but also through the consumption of food of animal origin that may contain residues of veterinary drugs used in animal breeding or due to environmental contamination (Varenina et al., 2022). Therefore, this paper reviews the literature dealing with the application of the One Health concept in preventing the presence of veterinary drug residues in milk, and determining and identifying residues of β-lactam antibiotics as the most frequently detected group of antibiotics in milk samples using screening and confirmatory analytical methods, such as biosensors and ultra-high performance liquid chromatography with mass spectrometry (UHPLC-MS/MS).

ONE HEALTH concept

The coexistence between humans, animals and the environment requires a relationship that should take into account the needs of all stakeholders in order to be sustainable and safe for all. Concern for food safety begins on the farm. Therefore, the health and well-being of animals is crucial in ensuring food safety. Maintaining health and preventing animal diseases is important for preserving human health, maintaining animal husbandry, ensuring economic aspects, and protecting the environment. The father of modern epidemiology, Dr. Calvin Schwabe, first used the term "One Health" in 1964, emphasising the importance of the principle of One Medicine, in which the treatment of humans and animals is based on the same medical principles and is not possible without the availability of clean water, healthy food and hygienic living conditions (Garcia et al., 2019). The joint task of physicians, veterinarians and scientists is to improve the health of humans, animals and ecosystems through interdisciplinary work so that everyone benefits (Figure 1). One Health is therefore a complex approach that seeks solutions in the context of the occurrence of numerous zoonoses. This is a group of infectious diseases that occur in humans and animals and can be transmitted from animals to humans and vice versa (Bhatia, 2021). One of the global health problems that links all three areas of the One Health concept is antibiotic resistance.

β-lactams in human and veterinary medicine

The use of β-lactam antibiotics, starting with penicillin in the 1930s, enabled the effective treatment of bacterial infectious diseases, so it is not surprising that the discovery of penicillin is one of the most important scientific discoveries of the last 100 years. The annual value of β-lactam antibiotics on the global market is around USD 15 billion, and accounts for 65% of the total antibiotics market.

β-lactam antibiotics are among the most frequently prescribed groups of drugs in the hospital sector. Due to their broad microbiological spectrum of activity, efficacy over decades and their rela-

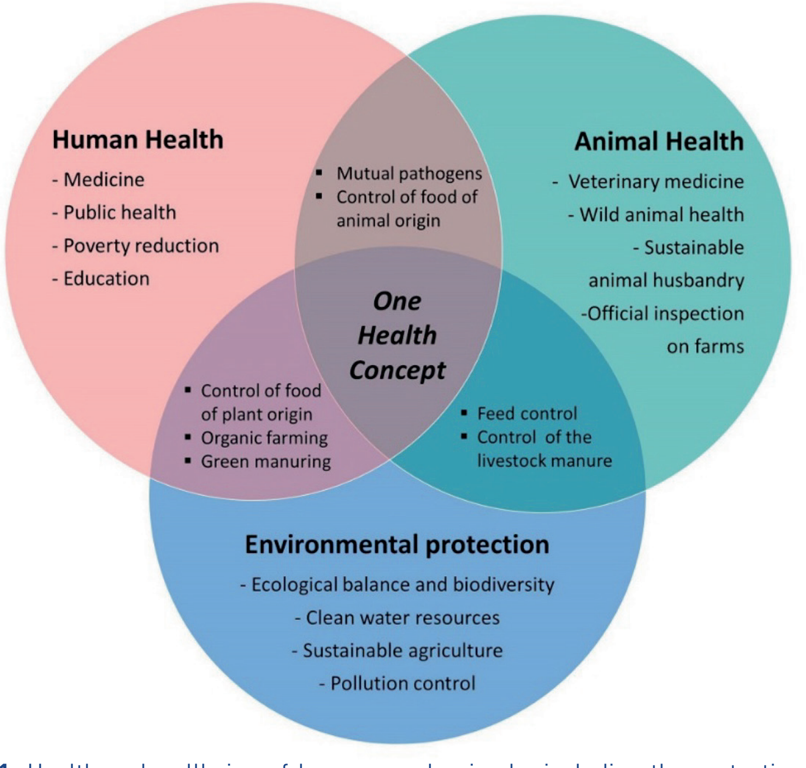

Figure 1. Health and wellbeing of humans and animals, including the protection of the environment, as a basis for sustainable development, summarised as the concept of One Health (Modified and adapted from Garcia et al., 2019)

tively low incidence of side effects, they play a key role in the prevention and treatment of infections (Fratoni et al., 2021).

This group of antibiotics contains a β-lactam ring in its basic chemical structure, which is highly reactive and responsible for its antimicrobial effect; the most important subgroups are:

a) Penicillins − the structure contains 6-animopenicillanic acid (β-lactam ring and thiazolidine ring). This group includes natural penicillins, β-lactamase-resistant agents, aminopenicillins, carboxypenicillins and ureidopenicillins.
b) Cephalosporins − the structure contains 7-aminocephalosporanic acid (dihydrothiazine and β-lactam ring). Five generations of cephalosporin antibiotics have been developed to date.
c) Carbapenems − the structure contains a carbapenem linked to a β-lactam ring that provides protection against the action of most β-lactamases. However, antibiotic resistance to these compounds is also a major problem and occurs mainly in Gram-negative pathogens (e.g., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*), which produce various β-lactamases, called carbapenemases.
d) Monobactams − the structure contains a β-lactam ring that is not connected to another ring.
e) B-lactamase inhibitors − they have no antimicrobial effect, with exception of sulbactam, but they inactivate serine β-lactamases, enzymes that hydrolyse and inactivate the β-lactam ring (especially in Gram-negative bacteria). Representatives of the first generation are clavulanic acid, sulbactam and tazobactam, while avibactam and vaborbactam were developed later, which are active against carbapenemases, such as the carbapenemase produced by *Klebsiella pneumoniae* (Pandey & Cascella, 2022).

Resistance to β-lactam antibiotics is a particular problem, and above all it refers to Gram-positive pathogenic bacteria *Streptococcus pneumoniae* and Gram-negative pathogenic bacteria such as *Pseudomonas aeruginosa*. With the emergence of antibiotic resistance, the mechanisms of this resistance have been investigated to aid in deciding which drugs should be prescribed to overcome resistance. Although bacterial resistance to β-lactams is mainly expressed through the production of β-lactamases, other mechanisms are also involved, such as: reduced penetration to the target site of action in the pathogen's cell (e.g., resistance in *Pseudomonas aeruginosa*), change of the target site of action (penicillin-binding proteins) in the pathogen cell (e.g., penicillin resistance in pneumococci), and efflux from the periplasmic space through specific mechanisms of antibiotic expulsion from the pathogen cell (Pandey & Cascella, 2022).

Recent research has indicated new types of enzymes in isolates of clinical strains of Gram-negative bacteria: extended-spectrum β-lactamases, β-lactamases with reduced sensitivity to β-lactamase inhibitors, plasmid-mediated β-lactamases and β-lactamases that hydrolyse carbapenems (Tooke et al., 2019).

In response to the rapid spread of microbial resistance to antimicrobials, the main role is played by the β-lactamase inhibitors that enhance the antimicrobial effect of β-lactam antibiotics. New combinations of β-lactam antibiotics and β-lactamase inhibitors (ceftolozane/tazobactam, ceftazidime/avibactam, meropenem/vaborbactam, imipenem/cilastatin/relebactam, aztreobactamoconam, aztreonam), new cephalosporins (cefiderocol) and new monobactams conjugated with siderophores have been developed. They are intended for the treatment of complicated infections caused by resistant pathogenic bacteria, particularly in intensive care units (Schalk, 2018). In 2023, the US FDA approved the use of a drug containing two β-lactamase inhibitors, sulbactam and durlobactam, for the treatment of bacterial pneumonia caused by *Acinetobacter baumannii–calcoaceticus* complex MDR strains (FDA News Release, https://www.fda.gov/news-events/press-announcements/fda-approves-new-treatment-pneumonia-caused-certain-difficult-treat-bacteria).

The use of different antimicrobial drugs in human and veterinary medicine leads to the contamination of the ecosystem through direct application, improper disposal of used packaging, unused drugs or treated animal feed, use of manure from treated animals, and directly from production. Phytotoxicity of veterinary drugs is rarely mentioned in the published research, although this knowledge can be used in predicting the impact of pollutants on biodiversity and can influence the development and application of phytoremediation (Bártíková et al., 2016).

Numerous scientific papers have demonstrated the prevalence of antibiotic resistance in the environment. In Tunisia, strains of *Enterobacteria* that produce extended- spectrum betalactamase (ESBL) were detected in 8–16% of tested samples of vegetables, soil and irrigation water (Ben Said et al., 2015).

Research has been performed on antibiotic residues detected in manure, agricultural land and river sediments. Groundwater pollution is a major problem because it is not subject to the same controls as freshwater sources. Although numerous studies have been published in the past decade on contaminants in surface and waste water, the number of studies on residues of antimicrobial substances in water is significantly lower. Kaczala & Blum (2015) published a review of studies on residues of veterinary medicinal products in wastewater and surface waters, and in particular detected sulphonamides, tetracyclines, macrolides and coccidiostats in wastewater samples from pig and livestock farms.

When using antibiotics for therapeutic purposes or as growth promoters, it is important to determine the concentration below which there is no impact on the development of resistance in bacteria, as well as the specific antimicrobial concentrations in feed that will have an impact on growth promotion (Koutsoumanis et al., 2021).

Residues of β-lactam antibiotics in milk

Intensive animal husbandry on farms leads to the excessive use of antibiotics and other veterinary medicines as growth promoters and for therapeutic purposes. Residues of pharmacologically active substances that are proven or suspected to be harmful to human health are found in food of animal origin and also in milk. They can cause toxic and allergic reactions in consumers or cause global problems by affecting the entire ecosystem through the induction of antibiotic resistance in bacteria (Manyi-Loh et al., 2018).

According to the report of the European Food Safety Authority (EFSA, 2023), of the total 488,631 milk samples analysed in 2020, 44 samples were detected to have a concentration of veterinary drug residues above the maximum permitted concentration, with antimicrobial substances detected in seven samples (Table 1). The largest proportion of positive findings was recorded in the group of non-ste-

Table 1. Results of non-compliant milk samples from veterinary drug residue monitoring in Europe in 2020 (EFSA, 2023)

Substance group	Compliant Results	Non-compliant Results
A6 Prohibited substances	26,859	7
B1 Antibacterial substances, sulphonamides, quinolones	243,053	7
B2a Anthelmintics	80,398	5
B2b Anticoccidials, nitroimidazoles	5471	0
B2c Carbamates and pyrethroids	3074	0
B2d Sedatives	104	0
B2e NSAIDs	86,049	21
B2f Other pharmacologically active substances	3961	0

roidal anti-inflammatory drugs (21 samples), as also described in other reports (Božić Luburić et al., 2022).

The next group with the highest proportion of discordant analyses are antibacterial substances, where seven non-compliant findings were recorded, including ampicillin (2), benzylpenicillin (3), cephapirin (1) and tetracycline (1).

Many published papers in EU Member States have described the highest occurrence of β-lactam residues in milk (Bilandžić et al., 2020). In Italy, 0.08% of suspect samples from 2018–2022 were detected as part of a monitoring plan. β-lactam antibiotics were most frequently detected, with penicillin G as the most common compound (Butovskaya et al., 2023).

Methods for the detection of β-lactam antibiotics residues in milk samples

For screening methods, the most important factors are simplicity, possibility of on-site use, and high sample throughput. This is especially important for analysis in production lines where the presence of antibiotics can negatively affect fermentation processes in the production of dairy products. Numerous papers have described the use of simple, fast and sensitive screening methods for the immediate detection of antibiotics in different matrices.

Initially, the development of screening methods was based on microbiological tests or molecular biology tests with a specific antigen-antibody. The latest developments have been focused on biosensor technology with simple preparation and high sample throughput (Ribeiro et al., 2022). Microbiological methods are time consuming due to the long incubation time and low specificity. Methods are based on tube test, plate test, or microbiological receptor tests. Usually, such methods are specific for one or several groups of antibiotics and for some analytes, the sensitivity is not satisfactory. An extensive study was published describing the validation of the screening method based on five-plate test for the screening of the residues of 10 different antibacterial groups (macrolides, aminoglycosides, tetracyclines, penicillins, cephalosporins, lincosamides, quinolones, phenicols and polypeptides). Among the 62 tested antimicrobials, satisfactory inhibition zones were obtained for only 21 substances for concentrations below or at MRL values (Gaudin et al., 2004).

Immunoassays are selective biochemical methods based on the specific antigen–antibody interactions with radioisotope, enzyme, fluorescence, or chemical labelling agents. The reaction is catalysed by labelling enzymes and substrate degradation to form the coloured product for visual and spectrophotometric observation. Many of the available immunochemical methods are available in kit format and are easy to use, but the disadvantage is that such tests are specific for only some of main representatives of an antibiotic group. This disables the screening of entire antibacterial groups and the scope of monitoring antibiotic residues in food samples is less than satisfactory. Research papers describing ELISA tests for β-lactams in milk are rather scarce. Production of sensitive antisera against the active forms of penicillins is demanding because of the chemical reactivity of the β-lactam ring (Grubelnik et al., 2010). A specific and sensitive enzyme immunoassay (EIAs) for the analysis of benzylpenicillin and cloxacillin has been developed using antibodies specific for the hydrolysed forms of antibiotics. This method showed good reproducibility and sensitivity at concentrations below the established MRL (Grubelnik et al., 2010). Ecke & Schneider (2021) concluded that the choice of antibody is crucial to obtain the best analytical results. It was shown the use of commercial anti-AMX antibody exhibits a higher affinity not only towards a hydrolysis product of amoxicillin but also to other penicillins and their hydrolysis products. The latest published method described the ELRA (enzyme-linked receptor assay) which was used to detect 40 β-lactams antibiotics in various animal derived foods. The method principle is based on a signal transducing membrane protein positioned in the extracellular region of the bacteria and acts as a drug binding site for various β-lactam antibiotics. The limits of detection were lower than the maximum residue limits (MRLs) set by the EU for all analytes. Simple sample preparation, high sensitivity, and efficiency makes this method suitable for detecting β-lactam antibiotic residues in animal derived foods and milk samples (Ahmed et al., 2020).

Biosensors are based on a bioreceptor or biorecognition element that recognises the target analyte, and a transducer that converts this reaction into a measurable signal. The development of biosensor analysers is aimed to achieve an inexpensive, fast, easy to use and specific method with high sample throughput, with detection limits in line with the regulatory limits (Gaudin, 2017).

In recent years, many electrochemical sensors and biosensors have been developed for the monitoring of aminoglycosides, amphenicols, β-lactams, fluoroquinolones, sulfonamides, and tetracyclines in milk samples (de Faria et al., 2021). Electrochemical methods are simple, inexpensive and allow a high frequency of the tested samples. Development of such novel techniques was investigated in detail, and some key challenges highlighted: the appearance of different antimicrobials with a similar chemical structure can cause false positive results, while for some complex biosensing techniques the removal of fat and proteins is crucial and therefore on-site analysis is not applicable (Singh et al., 2023). Application of electrochemical biosensors in the detection of β-lactams was described for substances showing electro activity either by their potential of chemical structures, or after an oxidative reaction that allowed a measurable signal (de Faria et al., 2021). It was reported that the use of enzymes immobilised by covalent bonds to carbon, gold or platinum plates can evoke hydrolysis of the β-lactam ring and formation of penicillinoic acid. Transfer of electrons between this reaction product and the electrode can provide a signal and high sensitivity (Prado et al., 2015). Electrochemical methods based on gold nanoparticles have been published for amoxicillin in milk and serum with minimal sample preparation for removing matrix and adjusting pH (Essousi et al., 2020).

The latest reported development of biosensors moved forward with the receptor-based assays with the ability to recognise 22 different β-lactam antibiotics in milk. Peptide-based molecules highly specific to β-lactams have been self-assembled to gold nanoparticles, thus improving the signal intensity of an electrochemical immunosensor and developing a highly sensitive sensor for simultaneous identification of 22 β-lactam antibiotics in milk (Mao et al., 2023).

In case of suspect findings, a follow-up step to screening involves confirmatory methods for the selective identification and quantitative determination of the concentration in relation to MRL values. According to Commission Implementing Regulation 2021/808, analytical techniques that can be used in confirmatory purposes include liquid chromatography, gas chromatography or supercritical fluid chromatography coupled to DAD, FLD, or the most representative mass spectrometry (EC, 2021). The identification points define the suitability of the selected acquisition modes and evaluation criteria. A minimum of four or five identification points is required depending on the authorisation of the analysed substance. Further, specific performance criteria for mass spectrometry should be respected, including separation technique requirements, response characteristics and mass spectra parameters (EC, 2021).

Recent studies have been focused on the development of multi-methods that cover up to ten different antibacterial classes (Varenina et al., 2022; Kenjeric et al., 2024). The most commonly used analytical platform for this analysis is a liquid chromatography coupled to tandem mass spectrometry, which allows for high sensitivity, selectivity, reproducibility of the results and robustness. The influence of the matrix on the analyte signal and its percentage of suppression or enhancement is an important characteristic that can be influenced by the sample preparation conditions. The optimisation of a multiresidue method for different pharmacologically active substances is difficult given the broad spectrum of chemical and physical properties of those substances. Due to the complexity of the milk matrix containing different proteins and fats, and its ability to chemically react with other substances, extraction techniques are based on a number of different cleanup principles.

The most frequent extraction techniques are: simple extraction with ACN:H_2O (80:20, v/v) (Kenjeric et al., 2024), novel magnetic solid-phase extraction procedure using magnetic hyper-crosslinked polystyrene (HCP/Fe_3O_4) (Melekhin et al., 2022), extraction using acetonitrile containing 3.35% formic acid (v/v) followed by the clean-up step with primary secondary aminesorbent (PSA) and octadecyl silica (C18) that provides optimal results in the purification of samples with significant fat content (Izzo et al., 2022). Another developed method described extraction by using quick and easy extraction with acetate buffer at pH 5.2 and acetonitrile (20/80, v/v), followed by C18 dispersive Solid Phase Extraction (dSPE) bulk sorbent (Igualada et al., 2022). A successful extraction technique for quinolones and β-lactams was achieved using ultrasound-assisted extraction (UAE) followed by a clean-up step using a dispersive solid-phase extraction (d-SPE) sorbent (Dorival-García et al., 2016).

In a LC-MS/MS multi-method for more than 140 compounds published by Kenjeric et al. (2024), the LOQ was below the MRL for 42% of antimicrobial and antiparasitic compounds, whereas method sensitivity was not satisfactory for most β-lactams. The multiclass liquid chromatography high resolution mass spectrometry method (UHPLC-HRMS) based on the QuEChERS (quick, easy, cheap, effective, rugged, and safe) approach was developed for the simultaneous determination of mycotoxins and veterinary drug residues (n = 45) occurring in infant milk formulas, but included only several β-lactams (amoxicillin, ampicillin, ceftiofur and procaine benzyl penicillin) and for which LOQ values were below the MRL (Izzo et al., 2022). In the validation of the screening UHPLC-HRMS method for the determination of β-lactams, lincosamides, macrolides, quinolones, sulfonamides, and tetracyclines in milk, satisfactory CCβ was achieved at 25% or 50% of the maximum residue limit (MRL) for all analytes (Igualada et al., 2022). A selective and accurate UHPLC–MS/MS analysis for 17 quinolone and 14 β-lactam antibiotic residues in raw cow milk was validated with the LOQ values from 0.3 to 2.0 ng/g g, and within-laboratory reproducibility under 7.1% (Dorival-García et al., 2016).

Conclusions

Milk is globally the most consumed food, characterised as a highly nutritional food that describes the social and economic status of the country. Milk serves as a starting raw material for many products, mostly in the process of fermentation. The distribution of antibiotic residues between fatty and aqueous fractions depends on the lipophilicity of the antibiotic substance. B-lactams, due to their high solubility in water, remain in whey, which is why residues are rarely found in cheese. A fast, accurate and precise screening method is necessary in milk production lines to be able to avoid inhibition of the fermentation process. In recent years, the most promising methods concerning sensitivity and applicability to different antibiotic substances have been biosensor assays.

Another global problem concerning the use of antibiotics is the emergence of antibiotic resistance in pathogenic bacteria. National monitoring plans for the control of veterinary drug residues have been laid down by Council Directive 96/23/EC or since 2022 by Commission Delegated Regulation (EU) 2022/1644, where each European county should fulfil the minimum requirement for sampling frequency according to the size of milk production (EC, 1996, 2022). In the last decade, the development of methods based on liquid chromatography coupled to mass spectrometry has covered a wide range of veterinary drugs, thus enabling the simultaneous control of different antibiotic classes. Although such methods require large financial investments and experience in working with such sophisticated methods, because of their high sensitivity and precision, they remain the reference methods for the official control of foods of animal origin.

In order to avoid further spreading of antimicrobial resistance and to ensure high quality milk products for consumers, systematic controls are required throughout the process; from increasing awareness of the correct use of the drugs, to milking at the farm, and production in the dairy industry. This is a task that requires the cooperation of different professions and scientific fields, which is ultimately defined by the term *One Health* as the key to the health of people, animals and ecosystem sustainability.

References

1. AHMED, S., J. NING, D. PENG, T. CHEN, I. AHMAD, A. ALI, Z. LEI, M. ABU BAKR SHABBIR, G. CHENG and Z. YUAN (2020): Current advances in immunoassays for the detection of antibiotics residues: a review. Food Agric. Immunol. 31, 268-290. 10.1080/09540105.2019.1707171
2. BÁRTÍKOVÁ, H., R. PODLIPNÁ and L. SKÁLOVÁ (2016): Veterinary drugs in the environment and their toxicity to plants. Chemosphere 144, 2290-2301. 10.1016/J.CHEMOSPHERE.2015.10.137
3. BEN SAID, L., A. JOUINI, N. KLIBI, R. DZIRI, C. A. ALONSO, A. BOUDABOUS, K. BEN SLAMA and C. TORRES (2015): Detection of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in vegetables, soil and water of the farm environment in Tunisia. Int. J. Food Microbiol. 203, 86-92. 10.1016/J.IJFOODMICRO.2015.02.023
4. BHATIA, R. (2021): Addressing challenge of zoonotic diseases through One Health approach. Indian J. Med. Res. 153, 249-252. 10.4103/IJMR.IJMR_374_21
5. BILANDŽIĆ, N., I. VARENINA, B. SOLOMUN KOLANOVIĆ, Đ. BOŽIĆ LUBURIĆ and I. VARGA (2020): Praćenje farmakološki aktivnih tvari u mlijeku u Europskoj uniji . Vet. stn. 51, 19-31. 10.46419/vs.51.1.3
6. BOŽIĆ LUBURIĆ, Đ., N. BILANDŽIĆ, I. VARENINA, I. VARGA, B. SOLOMUN KOLANOVIĆ, D. VRATARIĆ and I. RADOJČIĆ REDOVNIKOVIĆ (2022): The analysis of acidic and basic non-steroidal anti-inflammatory drugs in milk and muscle samples: a comprehensive analytical approach using UHPLC-MS/MS. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 39, 256-271. 10.1080/19440049.2021.1994157
7. BUTOVSKAYA, E., L. GAMBI, A. GIOVANETTI and G. FEDRIZZI (2023): Screening of antibiotic residues in raw bovine milk in Lombardy, Italy: Microbial growth inhibition assay and LC-HRMS technique integration for an accurate monitoring. Heliyon 9(4), e15395. 10.1016/J.HELIYON.2023.E15395
8. EC (1996): Council Directive 96/23/EC of 29 of April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. Off. J. Eur. Commun. L 125, 10-32.
9. EC (2021): Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC. Off. J. Eur. Commun. L 180, 1-84.
10. EC (2022): Commission Delegated Regulation (EU) 2022/1644 of 7 July 2022 supplementing Regulation (EU) 2017/625 of the European Parliament and of the Council with specific requirements for the performance of official controls on the use of pharmacologically active substances authorised as veterinary medicinal products or as feed additives and of prohibited or unauthorised pharmacologically active substances and residues thereof. Off. J. Eur. Commun. L 248, 3-17.
11. DE FARIA, L. V., T. P. LISBOA, N. DA S. CAMPOS, G. F. ALVES, M. A. C. MATOS, R. C. MATOS and R. A. A. MUNOZ (2021): Electrochemical methods for the determination of antibiotic residues in milk: A critical review. Anal. Chim. Acta. 1173, 338569. 10.1016/J.ACA.2021.338569
12. DORIVAL-GARCÍA, N., A. JUNZA, A. ZAFRA-GÓMEZ, D. BARRÓN and A. NAVALÓN (2016): Simultaneous determination of quinolone and β-lactam residues in raw cow milk samples using ultrasound-assisted extraction and dispersive-SPE prior to UHPLC−MS/MS analysis. Food Control 60, 382-393. 10.1016/J.FOODCONT.2015.08.008
13. ECKE, A. and R. J. SCHNEIDER (2021): Pitfalls in the immunochemical determination of β-lactam antibiotics in water. Antibiotics 10, 298. 10.3390/antibiotics10030298
14. EFSA (European Food Safety Authority), BROCCA, D. and S. SALVATORE (2023): Report for 2021 on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products. EFSA supporting publication 2023: 20(2):EN-7886 111. 10.2903/sp.efsa.2023.EN-7886
15. ESSOUSI, H., H. BARHOUMI, S. KARASTOGIANNI and S. T. GIROUSI (2020): An electrochemical sensor based on reduced graphene oxide, gold nanoparticles and molecular imprinted over-oxidized polypyrrole for amoxicillin determination. Electroanalysis 32, 1546-1558. 10.1002/elan.201900751

16. FRATONI, A. J., D. P. NICOLAU and J. L. KUTI (2021): A guide to therapeutic drug monitoring of β-lactam antibiotics. Pharmacotherapy 41, 220-233. 10.1002/phar.2505
17. GARCIA, S. N., B. I. OSBURN and J. S. CULLOR (2019): A one health perspective on dairy production and dairy food safety. One Health 7, 100086. 10.1016/J.ONEHLT.2019.100086
18. GAUDIN, V., P. MARIS, R. FUSELIER, J. L. RIBOUCHON, N. CADIEU and A. RAULT (2004): Validation of a microbiological method: The STAR protocol, a five-plate test, for the screening of antibiotic residues in milk. Food Add. Contam. 21, 422-433. 10.1080/02652030410001667575
19. GAUDIN, V. (2017): Advances in biosensor development for the screening of antibiotic residues in food products of animal origin – A comprehensive review. Biosens. Bioelectron. 90, 363-377. 10.1016/j.bios.2016.12.005
20. GRUBELNIK, A., C. PADESTE and L. TIEFENAUER (2010): Highly sensitive enzyme immunoassays for the detection of β-lactam antibiotics. Food Agric. Immunol. 13, 161-169. 10.1080/09540100120075817
21. IGUALADA, C., J. GIRALDO, G. FONT and V. YUSÀ (2022): Validation of a multi-residue UHPLC-HRMS method for antibiotics screening in milk, fresh cheese, and whey. J. Food Compos. Anal. 106, 104265. 10.1016/J.JFCA.2021.104265
22. IZZO, L., A. NARVÁEZ, L. CASTALDO, A. GASPARI, Y. RODRÍGUEZ-CARRASCO, M. GROSSO and A. RITIENI (2022): Multiclass and multi-residue screening of mycotoxins, pharmacologically active substances, and pesticides in infant milk formulas through ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry analysis. J. Dairy Sci. 105, 2948-2962. 10.3168/JDS.2021-21123
23. KACZALA, F. and S. E. BLUM (2015): The Occurrence of Veterinary Pharmaceuticals in the Environment: A Review. Curr. Anal. Chem. 12, 169-182. 10.2174/1573411012666151009193108
24. KENJERIC, L., M. SULYOK, A. MALACHOVA, B. GREER, T. KOLAWOLE, B. QUINN, C. T. ELLIOTT and R. KRSKA (2024): Development and validation of an LC-MS/MS multi-class method for the determination of 15 different classes of veterinary drug residues in milk and poultry feed. Food Chem. 138834. 10.1016/J.FOODCHEM.2024.138834
25. KOUTSOUMANIS, K., A. ALLENDE, A. ALVAREZ-ORD, et al. (2021): SCIENTIFIC OPINION Role played by the environment in the emergence and spread of antimicrobial resistance (AMR) through the food chain EFSA Panel on Biological Hazards. EFSA J. 19, 6651. 10.2903/j.efsa.2021.6651
26. MANYI-LOH, C., S. MAMPHWELI, E. MEYER and A. OKOH (2018): Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. Molecules, 23, 795
27. MAO, Y., Y. FAN, R. YANG, et al. (2023): A highly sensitive electrochemical sensor derived from peptide amphiphilic inspired self-assembled, ordered gold nanoparticles for determination of 22 β-lactams. Electrochim. Acta. 461, 142669. 10.1016/J.ELECTACTA.2023.142669
28. MELEKHIN, A. O., V. V. TOLMACHEVA, N. O. GONCHAROV, V. V. APYARI, , S. G. DMITRIENKO, E. G. SHUBINA and A. I. GRUDEV (2022): Multi-class, multi-residue determination of 132 veterinary drugs in milk by magnetic solid-phase extraction based on magnetic hypercrosslinked polystyrene prior to their determination by high-performance liquid chromatography – tandem mass spectrometry. Food Chem. 387, 132866. 10.1016/J.FOODCHEM.2022.132866
29. OECD/FAO (2021): OECD-FAO Agricultural Outlook 2021-2030, OECD Publishing, Paris, https://doi.org/10.1787/19428846-en.
30. PANDEY, N. and M. CASCELLA (2023): Beta-Lactam Antibiotics. StatPearls. Treasure Island (FL): StatPearls Publishing.
31. PRADO, T. M. D., M. V. FOGUEL, L. M. GONÇALVES and M. D. P. T. SOTOMAYOR (2015): β-Lactamase-based biosensor for the electrochemical determination of benzylpenicillin in milk. Sensors Actuators B: Chem. 210, 254-258. 10.1016/J.SNB.2014.12.108
32. RIBEIRO, B. V., L. F. FERREIRA and D. L. FRANCO (2022): Advances in biosensor development for the determination of antibiotics in cow's milk - A review. Talanta Open 6, 100145. 10.1016/J.TALO.2022.100145
33. SCHALK, I. J. (2018): Siderophore–antibiotic conjugates: exploiting iron uptake to deliver drugs into bacteria. Clin. Microbiol. Infect. 24, 801-802. 10.1016/J.CMI.2018.03.037
34. SINGH, B., A. BHAT, L. DUTTA, K. R. PATI, Y. KORPAN and I. DAHIYA (2023): Electrochemical Biosensors for the Detection of Antibiotics in Milk: Recent Trends and Future Perspectives. Biosensors 13, 867. 10.3390/bios13090867
35. TOOKE, C. L., P. HINCHLIFFE, E. C. BRAGGINTON, C. K. COLENSO, V. H. A. HIRVONEN, YTAKEBAYASHI and J. SPENCER (2019): β-Lactamases and β-Lactamase Inhibitors in the 21st Century. J. Mol. Biol. 431, 3472-3500. 10.1016/J.JMB.2019.04.002
36. TRICHIA, E., R. LUBEN, K.-T. KHAW, N. J. WAREHAM, F. IMAMURA and N. G. FOROUHI (2020): The associations of longitudinal changes in consumption of total and types of dairy products and markers of metabolic risk and adiposity: findings from the European Investigation into Cancer and Nutrition (EPIC)–Norfolk study, United Kingdom. Am. J. Clin. Nut. 111, 1018-1026. 10.1093/ajcn/nqz335
37. VARENINA, I., N. BILANDŽIĆ, Đ. BOŽIĆ LUBURIĆ, B. SOLOMUN KOLANOVIĆ and I. VARGA (2022): High resolution mass spectrometry method for the determination of 13 antibiotic groups in bovine, swine, poultry and fish meat: An effective screening and confirmation analysis approach for routine laboratories. Food Control 133, 108576. 10.1016/j.foodcont.2021.108576

38. US DEPARTMENT OF AGRICULTURE. (2023): Major producers of cow milk worldwide in 2023, by country (in million metric tons). Statista. Statista Inc. Accessed: March 21, 2024. https://www.statista.com/statistics/268191/cow-milk-production-worldwide-top-producers/

39. U.S. Food & Administartion (2023): FDA Approves New Treatment for Pneumonia Caused by Certain Difficult-to-Treat Bacteria. FDA News Release. Accessed: March 21, 2024. https://www.fda.gov/news-events/press-announcements/fda-approves-new-treatment-pneumonia-caused-certain-difficult-treat-bacteria

Koncept "Jedno zdravlje" u prevenciji pojave ostataka β-laktamskih antibiotika u mlijeku i okolišu

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Zbog brige oko očuvanja suživota ljudi, životinja i biljaka na zemlji, istovremeno vodeći računa i o očuvanju okoliša, razvijen je koncept "jedno zdravlje" (engl. „One Health) čija primjena za cilj ima globalno poboljšanje uvjeta života na Zemlji. Jedan od problema koji se javlja je pretjerana uporaba antibiotika u terapijske svrhe te kao promotora rasta životinja na farmama, što je prouzročilo naglo širenje antibiotičke rezistencije među mikroorganizmima. Javlja se i problem ostataka antibiotika u hrani životinjskog podrijetla, kao i u okolišu, gdje dolazi do zagađenja tla i vode. Najčešće primjenjivana skupina antibiotika su β-laktamski antibiotici koji predstavljaju najrašireniju skupinu antimikrobnih lijekova. Zbog vrlo snažnog antimikrobnog djelovanja i vrlo niske toksičnosti, koriste se, kao i u humanoj, tako i u veterinarskoj medicini, za liječenje bakterijskih infekcija. β-laktamski antibiotici su glavni antibiotici u terapiji mastitisa, najčešće infekcije koja se javlja u krava namijenjenih proizvodnji mlijeka. Ispitivanje i pouzdana identifikacija ostataka β-laktamskih antibiotika u uzorcima mlijeka je u zemaljama članicama Europske unije obuhvaćena Državnim monitoringom rezidua antibiotika u hrani životinjskog podrijetla. Razvoj inovativnih metoda visoke osjetljivosti i preciznosti tehnikama temeljenim na tekućinskoj kromatografiji s masenom spektrometrijom omogućava istodobnu analizu različitih grupa antibiotika u svrhu službene kontrole rezidua u hrani životinjskog podrijetla.

Ključne riječi: *Jedno Zdravlje, antibiotici, β-laktami, rezidue, orijentacijske metode, potvrdne metode*