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Urszula Szymańska, Marek Wiergowski, Ireneusz Sołtyszewski, Jarosław Kuzemko, Gabriela Wiergowska, Mateusz Kacper Woźniak

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Presence of antibiotics in the aquatic environment in Europe and their analytical monitoring: Recent trends and perspectives

Urszula Szymańska[1], Marek Wiergowski[2], Ireneusz Sołtyszewski[3], Jarosław Kuzemko[4], Gabriela Wiergowska[5,6], Mateusz Kacper Woźniak[7,]*

[1] *Department of International Public Law, Faculty of Law and Administration, Univeristy of Warmia and Mazury in Olsztyn, Poland*

[2] *Department of Forensic Medicine, Faculty of Medicine, Medical University of Gdańsk, Poland*

[3] *Department of Large Animal Diseases with Clinic, Faculty of Veterinary Medicine, Veterinary Research Centre and Center for Biomedical Research, Warsaw University of Life Sciences WULS – SGGW, Nowoursynowska 100, 02-797 Warsaw, Poland*

[4] *Department of Environmental Protection, Municipal Water and Sewage Company S. z o.o., Olsztyn, Poland*

[5] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Poznań University of Medical Science, Poznań, Poland*

[6] *Polfa Tarchomin S.A, Quality Control Department, A. Fleminga 2, 03-176 Warsaw, Poland*

[7] *Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology 11/12 Narutowicza Str., Gdańsk 80-233, Poland*

** Corresponding author: Mateusz Kacper Woźniak, Tel.: +48 58 347 21 10; e-mail address: mateusz.wozniak@pg.edu.pl*

Highlights

- Presence of antibiotics in different types of surface water and wastewater in Poland and Europe.
- Impact on organisms resulting from the presence of antibiotics in the aquatic environment.
- A literature update of analytical methods for quantification of antibiotics based on data from 2009 until today.

Abstract

The presence of antibiotics and their metabolites in the aquatic environment exerts a negative impact on all organisms. Moreover, the easy migration of these substances to drinking water may also have serious consequences for public health, such as drug resistance. Although antibiotics and their metabolites are detected in surface waters and wastewater, there are still no systemic solutions preventing environmental pollution with these substances. The procedure for quantification of antibiotics usually involves solid-phase extraction (SPE) followed by instrumental analysis typically using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS), which provides sensitivity, selectivity and reliability of results. Therefore, it is necessary to take decisive steps aimed at the determination of critical concentrations of antibiotics, which will make it possible to maintain safe values that will not exert a negative impact on the natural environment and human health. This work presents the current state of knowledge based on data from 2009 to 2018 (review of ten years of scientific papers) on the presence of antibiotics and their metabolites in the aquatic environment in Poland and Europe and methods used for the determination of antibiotics in different types of water (surface water and wastewater). The main strategies used for the removal of antibiotics during wastewater treatment processes in the context of antibiotics' concentrations were also presented.

Keywords: Antibiotics, Environmental analysis, LC–MS/MS, Micropollutants, Wastewater

1. Introduction

In Europe, the most frequently used pharmaceuticals still include anti-hypertensives and analgesics, psychoactive substances, substances used in the treatment of diabetes and regulating the level of cholesterol, as well as antibiotics [1–4]. Antibiotic resistance is one of the most important public health problems, which is largely the result of the excessive use of antibiotics. In Poland, as in other countries, the resistance of bacteria to antibiotics is constantly increasing, and this process is closely related to the excessive and often unnecessary use of antibiotics .

Antibiotics are a specific group of pharmaceuticals, and their quantity used in medicine has been growing year by year. Based on the reports, in 2012, the total consumption of antibiotics in outpatient health care (outside hospitals) in EU countries amounted to 3,400 t. The highest consumption of antibiotics was recorded in France (719 t), Italy (622 t), Great Britain (415 t), Spain (321 t) and Germany (297 t). The lowest consumption of antibiotics (below 10 t) was recorded in Cyprus, Estonia, Luxembourg and Iceland (only 2.5 t) (Fig. 1) [5,6]. It should be stressed that on average 50% more antibiotics are used for veterinary purposes. The highest use was recorded in Spain, where the figure almost doubled between 2012 and 2014, and a downward trend was recorded in Germany and Italy. Poland takes the fifth place behind Spain, Italy, Germany, and France (Fig. 1). In comparison with other European countries, the consumption of antibiotics in Poland is relatively high (238.5 t on an active substance basis) and exceeds the European average (130.8 t). In the EU, the most frequently used antibiotics include preparations containing penicillins (62%) and cephalosporins (8%) (Fig. 2) [5,7].

<Insert Fig. 1>

<Insert Fig. 2>

A report published in October 2018 by the European Medicines Agency (EMA) covering 30 countries (27 European Union countries and Iceland, Norway and Switzerland) shows that the most antibacterial substances used in veterinary medicine have been used in Spain, Italy and Germany. On the other hand, the least veterinary medicines were used to treat animals in Iceland, Luxembourg and Slovenia. The structure of antibacterial substances used in the treatment of animals includes, as in previous years, tetracyclines (32.4%), penicillins (25.8%) and sulfonamides (11.5%). Other pharmacologically active substances included in

the treatment belonged to the following classes: macrolides (7.0%), aminoglycosides (5.1%), polymyxins (5.1%), lincosamides (3.0%), pleuromutilins (2.8%) and fluoroquinolones (2.2%). Other groups, including trimethoprims, ampholenols, cephalosporins and other antibiotics account for 5.1% of the total sales of these substances [6].

The consequences of the extensive and often excessive use of antibiotics, not only in medical procedures, but also in the production of foods and feeds, also apply to the natural environment. Particularly important for the natural environment are micropollutants, which are bioaccumulative - they are resistant to biochemical decomposition and disturb biological balance of water, affecting water and wastewater treatment processes. These negative phenomena have a direct impact on aquatic organisms, and also, indirectly, on people. Due to the risks connected with the excessive use of antibiotics, 34 European countries are covered by the European Surveillance of Antimicrobial Consumption (ESAC).

The environmental problem associated with the presence of antibiotics and their metabolites in the environment and methods used for their determination in surface waters and wastewater have been extensively discussed in the review article published in 2009 [8]. However, since this time, many new original papers and reports have been published on this topic. Therefore, this paper is focused mainly on the recent trends (ten-year-review of scientific papers) in:

- global use of antibiotics,
- presence of antibiotics in surface water and wastewater in Poland and European countries in the context of the removal of antibiotics and their metabolites during wastewater treatment plant processes.

Moreover, to the best of our knowledge, there is a lack of new review papers describing literature updates of procedures for the determination of antibiotics and their metabolites in water and wastewater samples. Therefore, this article summarizes also the newest analytical procedures used in the monitoring of antibiotics and their metabolites in surface water and wastewater from 2009 until today. We strongly believe that the proposed article will enrich the existing literature base and will be helpful for researchers whose everyday laboratory practice is focused on environmental analysis.

2. Presence of antibiotics in the aquatic environment

Generally, antibiotics are poorly metabolised in human and animal organisms, and are excreted mainly as the active parent chemical in the faeces and urine, entering the

environment through wastewater and manure [9]. However, after consumption, medicines undergo also a variety of processes in the body in order to have the desired effect and to be finally safely excreted partially in an unchanged form and partially in the form of metabolites. Pharmaceuticals and their metabolites excreted with urine enter urban wastewater. The highest concentration of pharmaceuticals and their metabolites is detected in the wastewater generated by hospitals, outpatient clinics and veterinary clinics. Medical waste (including expired pharmaceuticals which are not properly landfilled), together with leachate may be a potential source of contamination of the aquatic environment. Pharmaceuticals preventively administered to animals, including antibiotics put into water in fish ponds, can also be such a source [10].

Wastewater treatment plants have so far been using technologies aimed primarily at the elimination of compounds of carbon (carbohydrates, proteins and lipids), and, to a smaller extent, biogenic pollutants, and are hardly effective in the elimination of antibiotics, as proved by the concentrations of the substances in treated wastewater in many countries (Table 1). A complete elimination of an antibiotic in the course of a wastewater treatment process was recorded only in a single study, for ofloxacin, despite its high concentration in raw wastewater [11].

<Insert Table 1>

In Poland, the problem of antibiotics in wastewater is hardly monitored, despite the consumption figures. The content of triclosan in wastewater from the treatment plant at Cracow (Poland) amounted to 339 ng/L in raw wastewater, and 430 ng/L in treated wastewater [21]. There are also only a few studies devoted to the presence of antibiotics in surface waters. However, we should add that the environment in the area in which the research was carried out (Poznan, Poland) is heavily polluted with agricultural waste from pig farms. Other antibiotics were also determined in surface waters, which is mainly related to agricultural emissions, but the concentrations did not exceed LOQ determined for the methods used [21,22] (Table 2).

There is a small number of reports of antibiotics being detected in tap water. Segura et al. [23] when reviewing research reports for 24 years since 1984, devoted to the presence of antibiotics in wastewater, surface waters and tap water, discovered that only a few of them concerned tap water, and that the concentration of antibiotics ranged from 0.3 to 5 ng/L

(2 ng/L on average). Research showed that the most effective water treatment methods eliminating antibiotics include adsorption on activated carbon, reverse osmosis and oxidation: chlorination or ozonisation. What is problematic when water treatment plants use reverse osmosis is that they obtain small quantities of concentrated waste with high concentration of micropollutants, including antibiotics and their partial decomposition products, which should be subsequently treated in special conditions (for example incinerated in a plasma furnace), considerably increasing the cost of the entire process. In the case of the oxidation, the process must be very thoroughly controlled so as to avoid uncontrolled synthesis of, for example, precursors to carcinogens during the treatment process.

<Insert Table 2>

Due to agricultural run-off and the discharge of the treated wastewater to rivers, antibiotics and their metabolites as well as degradation products subsequently enter sea waters. Analyses of debris collected along the Polish coast of the Baltic Sea determined residues of nine antibiotics in the concentration of up to 419.2 ng/g of dry matter, and the highest concentration was recorded in the Pomeranian Bay and by river mouths [28]. Thus, marine ecosystems are also exposed to the impact of antibiotics and sea waters should be monitored for micropollutants.

Another problem is posed by the agricultural use of catchment areas of water bodies. Analyses showed contamination of soil with antibiotics originating from slurry and sludge used as fertilisers. It was also determined that some bacteriostatic agents (such as erythromycin and ofloxacin) are excreted in a little changed or unchanged form [11]. It is therefore highly likely that they get to surface waters together with surface run-off, affecting biological and biochemical processes. Moreover, tracing enantiomeric antibacterial agent concentrations in wastewater matrices is vital for the proper understanding of the possible environmental risks. For example, S-(−)-ofloxacin is an enantiomerically pure drug, marketed since 1995 (although on the market as a racemic mixture). The enantiomer S-(−)-ofloxacin has antibacterial activity up to 2 orders of magnitude greater than R-(+)-ofloxacin [29], but no chiral inversion has been reported in the environment [11].

The basic risks both for the natural environment and the human population include bacterial antibiotic resistance, which considerably impedes the treatment of people and animals [9,30–34]. Szczepanowski et al. [35] showed that bacteria isolated from the last stage

of wastewater treatment had 64% out of 192 reference resistance genes and can get to aquatic ecosystems with the treated wastewater. Tests performed on *Escherichia coli* determined that the bacteria's resistance to ampicillin and amoxicillin increased in the course of the process of wastewater treatment [36]. During the last stages of wastewater treatment, the bacteria are resistant to from three [37] to five antibiotics [36], and the treated wastewater gets to surface waters, becoming a source of bacteria resistant for example to β-lactams [37].

Examinations of wild birds from the Polish coast of the Baltic Sea: mallards (*Anas platyrhynchos*) and European herring gulls (*Larus argentatus*), detected antibiotic-resistant strains of *E. coli* [38] in their bodies and excrements. High toxicity of ofloxacin was also detected for the bacteria *Vibrio fischeri*, which is found in marine ecosystems and is also used in environmental pollution tests [39,40]. However, the common duckweed (*Lemna minor*) is sensitive to enrofloxacin, norfloxacin and ciprofloxacin [41].

Sources of drug-resistant bacteria also include fish farms, in which antibiotics are administered for therapeutic purposes and as feed additives. Microbiological testing of the upper River Drwęca (Poland), which receives water from three fish farms, revealed the presence of bacteria resistant to tetracycline [42]. As a part of their research concerning commonly used veterinary antimicrobials, the authors tested 443 samples of the flesh of fish (including common bream *Abramis brama*, roach *Rutilus*, pike *Esoxlucius*, zander *Sander lucioperca* and wels catfish *Silurus glanis*). However, no antibiotics with concentrations exceeding the LOQ were detected, and the concentrations amounted to, respectively: <80-125 for aminoglicosides, <10-50 for β-lactams, <10 for diaminopyrimidines, <5-30 for fluoroquinolones, <20-59 for macrolides, <10 for sulphonamides, and <5-20 for tetracyclines. Nevertheless, this problem requires further studies [24].

Strains of multi-resistant bacteria, including the pandemic clone *E. coli* ST131, get to the environment from urban wastewater treatment plants [43]. In the other study, Riley et al. [44] put forward a hypothesis that the epidemics of human obesity may be a consequence of the extensive use of antibiotics and their metabolites in the natural environment (surface waters, food of animal origin). Moreover, the wide-spread use of antibiotics which started in the 1950s causes changes to human gut microbiota, leading to metabolic dysfunctions.

Biological wastewater treatment methods effectively remove up to more than 90% of the majority of analgesics (such as ibuprofen and aspirin). Antibiotics and hormonal drugs pose a larger problem, since apart from poor biodegradability, they cause many changes to cellular morphology and the functioning of microorganisms living in aquatic environment.

Risks to the aquatic environment include antibiotics - mainly sulfonamides (sulfamethoxazole and trimethoprim), macrolides (erythromycin and clarithromycin) as well as fluoroquinolones (ciprofloxacin, and norfloxacin) [45]. Removal of antibiotics is varied and amounts to 80% for norfloxacin, 75% for levofloxacin, 86% for ciprofloxacin, 69% for azithromycin, 40% for clarithromycin, 20% for trimethoprim, 70% for sulfapyridine, 58% for sulfamethoxazole, 44% for sulfasalazine [12], 20% for erythromycin [46], There are instances when the concentration of a given substance in the treated wastewater is higher than in the raw wastewater reaching the treatment plant. This may result from the split course of the treatment processes, where compounds undergo partial decomposition and secondary reactions may take place. Also, it is very difficult to collect samples of wastewater in such a way as to be sure that the material collected at the beginning and at the end of the treatment process represents the same portion of wastewater [21]. In this type of processes, the competence of staff (technologists) is of key importance, as they should be aware of how long wastewater flows through the particular elements of the treatment plant. There are also instances when the so-called "swelling" of the activated sludge is observed in the course of the wastewater treatment process. In the course of this process, the composition of activated sludge is dominated by filamentous bacteria, which are introduced to the environment from the secondary sedimentation plant together with the outgoing treated wastewater. For this reason, when determining the concentration of pharmaceuticals, it is necessary to make sure that during the collection of samples for analyses wastewater treatment processes function properly. It is also important to monitor sludge containing antibiotics and their metabolites, which is used in agriculture.

The efficiency of the elimination of antibiotics may be increased through the selection of the appropriate wastewater treatment methods. One of the methods consists in the membrane filtration of activated sludge, which may result in a 90% reduction of tetracycline content, while ozonation causes oxidation of many compounds, decreasing their concentration by more than 60% [47]. Bińand Sobera-Madej [48] arrived at similar conclusions concerning the usefulness of ozonation, and pointed out that photolytic methods based on UV radiation are not very effective for the elimination of pharmaceuticals. Biological aerated filters make it possible to decease the concentration of antibiotics by 50% [49].

Another problem discussed in the publications in question (Table 1) is the poor effectiveness of the removal some of antibiotics and their metabolites from wastewater: no considerable differences are determined between concentrations at the inlet of the treatment

plant (in raw wastewater; concentrations range from 5 to 3700 ng/L) and at its outlet (in treated wastewater; from 8 to 2310 ng/L). It should also be pointed out that hospital wastewater contains significantly higher concentrations of antibiotics (100–10000 ng/L) in comparison with surface waters and urban wastewater, which is obvious when taking into account the degree of concentration of this wastewater in relation to the urban wastewater system.

3. Determination of antibiotics in the aquatic environment

The analysis of surface waters (mainly rivers and lakes) and urban wastewater is difficult and time-consuming due to both a complex matrix of sample (the presence of solid particles, lipids, microorganisms etc.), and the diverse physico-chemical properties of analytes (Table 3). The higher the octanol/water partition coefficient (log K_{OW}), and the lower the water solubility of a substance, the more lipophilic and the more active the substance is in the organism, which allows it to easily permeate lipid membranes (bioaccumulation). Log K_{OW} values for pharmacologically active substances ranging between 1 and 3 indicate an average lipophilicity, while log K_{OW} of less than 1 indicates hydrophilic properties. In the environment of wastewater and surface waters, antibiotics marked by average lipophilicity will have a higher affinity to sorb to organic solid particles than hydrophilic antibiotics [21,22].

<Insert Table 3>

In connection with the possibility of adsorption of analytes on solid particles, preliminary testing of samples of surface waters and wastewater must generally involve both liquid and solid phase analysis. The procedure of the sample collection typically begins with a thorough planning of the locations and methods of 24-hour collection, and then the averaged 24-hour samples are collected for analysis. Normally, special automatic collection machines with peristaltic pumps are used, as they collect a defined volume of samples in the defined time to a glass bottle in a programmable way. Alternatively, passive sampling can be used for the collection of averaged samples for a longer monitoring period and for increasing the enrichment of analytes. For this purpose, techniques involving lipid-filled semipermeable membrane devices (SPMDs) and silicone rubber (SR) with polydimethylsiloxane (PDMS) or low density polyethylene (LDPE) are used for non-polar compounds. For polar analytes, passive samplers containing hydrophilic polymers such as Oasis HLB or styrene-

divinylbenzene (SDB) disks sandwiched between poly(ether sulfone) (PES) membranes are used. The correct determination of the velocity of water flow near the sampler, which is usually equal to about 2 x 10^{-4} –75 litres per day, poses a serious problem. Passive samplers with performance reference compounds (PRCs), which do not interfere with analytes and are not naturally found in the environment, are used for the purpose. It is assumed that the velocity of the capture of analytes from water is proportional to the release of PRCs into aquatic environment, and therefore water flow rate variability will be of less importance for the determination of analyte concentrations [50].

The estimation of the uncertainty of determination of analyte concentration in surface waters and wastewater depends not only on the sampling procedure and chemical analysis, but also on many environmental factors, such as temperature, salinity, pH, the presence of microorganisms, bioaccumulation, biomagnification, particle/water partition coefficients, biodegradation and the related stability of compounds (e.g. photochemical degradation of analytes). The correct planning and preparation of analytical procedure also requires the adoption of extraction techniques appropriate for the expected ranges of concentrations of pharmacologically active substances in surface waters and wastewater, which very significantly vary from several hundred pg to several hundred ng/L. Concentrations of antibiotics detected in hospital wastewater are higher in comparison with urban wastewater.

Procedures used for determination of antibiotics in water and wastewater with division into pre-analytical, analytical and post-analytical stages is presented in Figure 3.

<Insert Fig. 3>

Table 4 presents the newest analytical procedures used for the quantitative determinations of antibiotics and their metabolites in waters and wastewater. The volume of water sample used in the analysis typically ranges from 25 to 500 mL [51,52]. Sample preparation step typically involves the filtering of samples on 0.45 um diameter filters or GF/B Whatmann glass fibre, followed by the isolation and enrichment of the analytes using solid phase extraction (SPE) technique. The most frequently used sorbent in SPE extraction is HLB sorbent (hydrophilic-lipophilic balance sorbent), which is selective and effective in the isolation of polar compounds [51]. To increase extraction efficiency, it is suggested that pH be changed through acidification (typically to pH around 3) and an addition of methanol [51,53]. In view of complex matrix of water samples and the expected low concentrations of

analytes (amounting to a few ng/L), extracts are typically analysed utilizing liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). Due to diverse chemical structure of antibiotics, analytes are ionised using electrospray (ESI) both in the positive (ESI+) and the negative (ESI–) mode [54]. Quantitative analysis is performed in the multiply reaction monitoring mode (MRM) using tandem mass spectrometry as detection technique. Analysers typically applied for analysis of antibiotics included: triple quadrupole (QqQ), quadrupole ion trap (Q–TRAP), quadrupole-linear hybrid ion trap mass spectrometry (QqLIT) and Exactive Orbitrap [51,53,55,56].

The development of accurate mass (AM) high resolution mass spectrometry (HRMS) coupled to gas or liquid chromatography (GC or LC) has initiated the new solutions in analytical data processing in the recent years. Therefore, final determinations of antibiotics in aquatic samples may be also performed for screening only (qualitative or non-targeted analysis). Qualitative analysis can be done by library searching and comparing mass spectra with international bases (e.g. NIST base) or with an in-house prepared library based on the standards. The most powerful techniques used for qualitative analysis are time-of-flight (TOF) or quadrupole time-of-flight (Q-TOF) mass spectrometry, typically coupled with LC which allows to obtain high accuracy and resolution in determination of analyte mass (low Δ ppm) and possibility to analyse wide spectrum of compounds. Importantly, qualitative analysis can be performed even when no reference standards are available (thus retention times of analytes are also unknown) while quantitative analysis requires to perform both calibration and validation what is costly and time consuming [57,58].

Another modern analytical technique, ion mobility spectrometry with electrospray ionization (ESI-IMS), was also employed for determination of various environment pollutions in water samples. IMS is a straightforward, low-cost analytical technique, which provides low analysis time and low limits of detection (ppb) based on the drift velocities of ions in the electric field at ambient pressure. A conventional sort of IMS is the time-of-flight (TOF) design, which has a similar principle to Q-TOF [59]. ESI-IMS can be used either as independent measurement technique or coupled with LC or even 2D-LC (two-dimensional liquid chromatography) with Q-TOF detection (IM-Q-TOF-MS). However, in LC-based methods sample preparation step is still required [59,60]. In comparison, Li et al. [61] performed model studies (based on the standards) and developed qualitative and semi-quantitative method for the determination of 18 antibiotics in liquid samples without sample preparation step with detection limits at the level of 0.7 mg/L. Importantly, based on the

possibility of measurements of drift time in IMS-based methods, chromatographic separation in many cases is not required what definitely shorten analysis time and decreases costs. However, in this study only 50% antibiotics in the mixture could be separated and detected. Therefore, the identification of antibiotics in mixtures (thus in real samples) on the basis of reduced mobility is possible but limited without chromatographic separation.

<Insert Table 4>

Summarizing, they are still a lot of analytical problems and challenges concerning the reliable detection of antibiotics in surface waters and wastewaters. The cost of standards and labelled analogues are further complications which means some research groups have reported data without using internal standards. Metabolites may not be commercially available, which further limits current mass spectrometry methods, regardless of whether they are high resolution or not. Nowadays we have been observing better accuracy and lower detection limits of analytical techniques, but the other factors which influencing the assessment of aquatic environment pollution by antibiotics (eg. determination of flow rates, type of sampling at the treatment plants) can change the interpretation of results [68,69]. Many authors have published articles comparing levels of pollution by using measured concentration levels. However this does not reflect the extent of pollution as it fails to consider the flow rates and water volumes. It would also be worth pointing out that some treatment plants are connected to open sewers receiving storm water as well as personal waste, while others are closed from storm water.

4. Conclusion and perspectives

Antibiotic resistance is a global problem, the consequence of which is an increasing number of deaths due to infection with a resistant strain of bacteria. The growing demand for animal products drives an intensification in livestock farming, which in turn leads to the increased use of veterinary antibiotics. Poland has one of the highest rates of total antibiotic consumption among European countries. The structure of antibiotic consumption in Poland is diversified: it is higher than the EU average for open healthcare and below the average for closed care. Of particular importance are integrated activities related to the correct prescription of antibiotics by doctors and dentists, as well as changes in the provisions on the prescription of antibiotics in open healthcare [70].

Since the publication of review article by Moreno-Bondi et al. [8] in 2009, some new findings related to the problem were published. Therefore, we consistently point to the increasing quantity of antibiotics in the environment, which indicates an absence of systemic solutions effectively preventing the pollution of the natural environment with these substances. It is particularly important to determine critical concentrations of toxic substances, and especially antibiotics, which will make it possible to maintain safe values, which will not have a negative impact on the natural environment, and, consequently, on human health.

What is noteworthy, according to the publications under discussion, liquid chromatography continues to be the most frequently used analytical technique, although it is now coupled with new detection techniques, mainly based on tandem mass spectrometry, time-of-flight analysers and high-resolution mass spectrometry. The use of these new detection techniques has considerably improved the sensitivity and selectivity of analytical procedures. However, the preparation of samples for analysis has not been changed significantly and is still based on the SPE technique. Despite the fact that analytical techniques based on LC-MS/MS and SPE for the simultaneous detection of many residues have been extensively studied over the last decade, the optimization of analytical methods for different environmental aqueous samples remains a challenge due to their different properties. Although SPE provides effective sample clean-up and facilitates high recovery of analytes, it does not fit into the dynamically developing green chemistry trend. For this reason the future research should be focused on the introduction of microextraction-based methods, which could shorten extraction time and decrease the quantity of toxic solvents used at the stage of the preparation of samples for analysis. However, microextraction-based protocols pose some difficulties, including low recoveries of analytes and in polar physiochemical properties of antibiotics what results in problems associated with the selection of extraction solvent or fiber type. That is why further improvement of wastewater treatment methods and development of more efficient and effective methods of the removal of antibiotics from wastewater should be considered a key issue with a view to the halting of the spread of these substances to the environment. Therefore, it is important in the near future to develop standard analytical methods for highly different classes of antibiotic residues in order to effectively monitor antibiotics in the aquatic environment and to provide the possibility of comparing the effectiveness of the removal of antibiotics during processes taking place in sewage treatment plants.

5. Compliance with ethical standards

8.1. Conflict of interest

The authors declare that they have no conflict of interest.

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Figures captions

Fig. 1. Consumption of antibiotics in the primary care sector in 2012 (t) and in animal production in 2012 (data for the production of food producing animals including horses); tablet antibiotics have not been taken into account [5,6].

Fig. 2. Consumption (in tonnes and percentages of active ingredient) of antimicrobials authorised for human medicine (presented according to the ATC classification) by UE in 2012 [5,7].

Fig. 3. Pre-analysis, analysis and post-analysis stages typically performed for determination of antibiotics and their metabolites in surface waters and wastewater samples.

Table captions

Table 1 Antibiotic concentrations in wastewater before and after treatment processes.

Table 2 Antibiotic concentrations in surface waters.

Table 3 Classification and physico-chemical characterization of selected antibiotics [22].

Table 4 LC-based procedures employed for determination of antibiotics and their metabolites in water samples of different origin.

Tables

Table 1 Presence of antibiotics and their concentrations in wastewater before and after treatment processes.

Antybiotics	Concentration (ng/L)		Treatment type	Country, region	Ref.
	Raw sewage (inlet)	Sewage treated (outlet)			
Azithromycin	14–510 (M 140)	8–220 (M 50)	CWWTP	Czech Republic, České Budějovice City	[12]
	80–860 (M 410)	8–190 (M 65)	CWWTP	Czech Republic, České Budějovice City	
	310–3090 (M 1480)	210–2310 (M 930)	CWWTP	Czech Republic, České Budějovice City	
	(M 351)	(M 202)	CWWTP	UK, 45 CWWTPs	[13]
	ND-437	ND-592	CWWTP	Spain, Girona City, 3 WWTPs	[14]
	10–33 (A 13)	7–18 (A 13)	HWWTP	Italy, North region	[15]
	79.7–295 (A 186±79)	93.7–297 (A 171±68)	HWWTP	Portugal, Coimbra	[16]
Chloramphenicol	13-24 (A 19)	ND	HWWTP	Italy, North region	[15]
1S,2S-(+)-Chloramphenicol, 1R,2R-(−)-Chloramphenicol	ND	ND	CWWTP	North and Middle Europe	[11]
Ciprofloxacin	A 320±10	A 31,5±3,0	TSTP	Sweden, Kristianstad City	[17]
	(M 861)	(M 147)	CWWTP	UK, 45 CWWTPs	[13]
	185-613	ND-147	CWWTP	Spain, Girona City, 3 WWTPs	[14]
	1100-3700 (A 2200)	290-1100 (A 640)	HWWTP	Italy, North region	[15]
	107-330 (A 221±88)	127-1396 (A 369±455)	HWWTP	Portugal, Coimbra	[16]
Clarithromycin	25-133 (M 71)	NA (M 22)	CWWTP	Croatia, Zagreb City	[18]
	185-632	172-229	CWWTP	Spain, Girona City, 3 WWTPs	[14]
	(M 953)	(M 400)	CWWTP	UK, 45 CWWTPs	[13]
	110-780 (A 310)	260-310 (A 280)	HWWTP	Italy, North region	[15]
	ND-52.3 (A 22.2±17.8)	12.0-14.0 (A 22.4±11.4)	HWWTP	Portugal, Coimbra	[16]
Clindamycin	14-37	18-57	CWWTP	Spain, Girona City, 3 WWTPs	[14]
Enoxacin	81-130 (A 100)	30-100 (A 61)	HWWTP	Italy, North region	[15]
Enrofloxacin	ND-58	ND-52	CWWTP	Spain, Girona City, 3 WWTPs	[14]
Erythromycin	20–300 (M 77)	30–350 (M 110)	CWWTP	Czech Republic, České Budějovice City	[12]
	(M 733)	(M 350)	CWWTP	UK, 45 CWWTPs	[13]
	10–72 (A 45)	10–33 (A 16)	HWWTP	Italy, North region	[15]
	9.64–220 (A 92.7±77.9)	20.4–134 (A 71.2±40.6)	HWWTP	Portugal, Coimbra	[16]
Josamycin	ND–7 (A 20)	ND	HWWTP	Italy, North region	[15]
Levofloxacin	5–69 (M 22)	4–18 (M 6)	CWWTP	Czech Republic, České Budějovice City	[12]

	130–1330 (M 550)	20–250 (M 83)	CWWTP	Czech Republic, České Budějovice City	[12]
Marbofloxacin	ND-39	ND-16	CWWTP	Spain, Girona City, 3 WWTPs	[14]
Metronidazole	28-316	17-83	CWWTP	Spain, Girona City, 3 WWTPs	[14]
	28-56 (A 42)	13-41 (A 28)	HWWTP	Italy, North region	[15]
	BQL–113 (A 51.1±49.8)	19.4–83.5 (A 51.1±21.1)	HWWTP	Portugal, Coimbra	[16]
Metronidazole-OH (metabolite)	ND-145 (A 62.9±69.0)	64.7-158 (A 102±33)	HWWTP	Portugal, Coimbra	[16]
Nifuroxazide	19-76 (A 52)	10-22 (A 13)	HWWTP	Italy, North region	[15]
Norfloxacin	(A 18,0±2,5)	ND	TSTP	Sweden, Kristianstad City	[17]
	ND-385	ND-149	CWWTP	Spain, Girona City, 3 WWTPs	[14]
	150-310 (A 200)	140-170 (A 150)	HWWTP	Italy, North region	[15]
Ofloxacin	(A 22,5±2,5)	(A 10,0±1,0)	TSTP	Sweden, Kristianstad City	[17]
	73-524	63-101	CWWTP	Spain, Girona City	[14]
	450-2200 (A 1000)	220-520 (A 390)	HWWTP	Italy, North region	[15]
	51.9-498.6 (A 94.6±179.0)	110-366 (A 233±79)	HWWTP	Portugal, Coimbra	[16]
S-(−)-Ofloxacin	539 000	ND	CWWTP	North and Middle Europe	[11]
R-(+)-Ofloxacin	ND	ND	CWWTP	North and Middle Europe	
Roxithromycin	ND-140 (A 63)	13-53 (A 29)	HWWTP	Italy, North region	[15]
Spiramycin	ND-150 (A 61)	19–53 (A 29)	HWWTP	Italy, North region	[15]
Sulfadiazine	13-26 (A 22)	10-21 (A 17)	HWWTP	Italy, North region	[15]
Sulfamethazine	10–33 (A 18)	10–15 (A 11)	HWWTP	Italy, North region	[15]
Sulfamethoxazole	119-544 (M 323)	NA (M 220)	CWWTP	Croatia, Zagreb City	[18]
	NA	9,14–53,3	CWWTP	Portugal, Douro River estuary	[19]
	43–490 (M 220)	31–260 (M 90)	CWWTP	Czech Republic, České Budějovice City	[12]
	367.9–2170.4 (A 904.2)	ND–55.9 (A 23.7)	CWWTP	Greece, Ioannina City (urban and industrial)	[20]
	122.2–2626.3 (A 1464.5)	37.0–481.3 (A 205.7)	HWWTP	Greece, Ioannina hospital	
	12.7–224.1 (A 112.2)	BQL–34.4 (A 21.8)	CWWTP	Greece, Arta City (urban)	
	36.3–213.3 (A 119.5)	BQL–27.9 (A 15.9)	CWWTP	Greece, Preveza City (urban)	
	ND–41.3 (A 16.4)	ND–16.1 (BQL)	CWWTP	Greece, Agrinio City (urban)	
	ND–323.3 (A 132.8)	ND–28.4 (A 11.0)	CWWTP	Greece, Grevena City (urban)	
	90.6–532.5 (A 227.2)	12.1–25.9 (A 21.8)	CWWTP	Greece, Kozani City (urban)	
	38.9–280.9 (A 140.9)	BQL–72.9 (A 30.6)	CWWTP	Greece, Veroia City (urban)	

	43-528	19-198	CWWTP	Spain, Girona City, 3 WWTPs	[14]
	280-740 (A 440)	170-240 (A 210)	HWWTP	Italy, North region	[15]
	529–1662 (A 912±391)	340–1679 (A 950±460)	HWWTP	Portugal, Coimbra	[16]
Sulfapyridine	18–660 (M 200)	14–200 (M 55)	CWWTP	Czech Republic, České Budějovice City	[12]
Sulfasalazine	29–730 (M 100)	17–830 (M 50)	CWWTP	Czech Republic, České Budějovice City	[12]
Tetracycline	BQL-32.3 (A 12.1±12.7)	BQL–22.8 (BQL)	HWWTP	Portugal, Coimbra	[16]
Tilmicosin	21-460 (A 250)	ND-81 (A 36)	HWWTP	Italy, North region	[15]
Triclosan	339	430	CWWTP	Poland, Cracow City	[21]
	NA	3,89-15,7	CWWTP	Portugal, Douro River estuary	[19]
	120–530 (M 320)	83–440 (M 250)	CWWTP	Czech Republic, České Budějovice City	[12]
Trimethoprim	36.7–180.3 (A 132.1)	BQL–111.2 (A 59.8)	CWWTP	Greece, Ioannina City (urban and industrial)	[20]
	31.7–1866.2 (A 621.8)	BQL–533.2 (A 186.7)	HWWTP	Greece, Ioannina hospital	
	BQL –54.5 (A 23.1)	BQL–27.4 (A 11.6)	CWWTP	Greece, Arta City (urban)	
	BQL–42.1 (A 16.2)	BQL–23.1 (A 8.0)	CWWTP	Greece, Preveza City (urban)	
	BQL–39.1 (A 16.7)	ND–20.0 (A 8.3)	CWWTP	Greece, Agrinio City (urban)	
	ND–19.6 (A 7.9)	ND–BQL	CWWTP	Greece, Grevena City (urban)	
	BQL–72.9 (M 33.7)	BQL–7.0 (BQL)	CWWTP	Greece, Kozani City (urban)	
	BQL–39.4 (M 22.9)	ND–18.6 (M 10.0)	CWWTP	Greece, Veroia City (urban)	
	BQL-178	BQL-108	CWWTP	Spain, Girona City, 3 WWTPs	[14]
	39-72 (A 58)	36-51 (A 40)	HWWTP	Italy, North region	[15]
	ND-360 (A 124±131)	66.6-299 (A 167±77.8)	HWWTP	Portugal, Coimbra	[16]

A – the average value, M - the median value, NA- not analysed, BQL – below quantification limit, ND – not detected, CWWTP - conventional wastewater treatment plant, HWWTP - hospital wastewater treatment plant, TSTP - tertiary sewage treatment plant

Table 2 Antibiotic concentrations in surface waters.

Antibiotics	ng/L	Country. region	Ref.
Aminoglicosides	<1000–10000	Poland, six rivers (Vistula, Warta, Odra, Brda, Wkra, and Dunajec) and three lakes (Lanskie, Maroz, and Rybnik power station reservoir)	[24]
Azithromycin	32.15–35.66	Portugal, Montego River, Tagus River	[25]
B-lactams	<20–10000	Poland, six rivers (Vistula, Warta, Odra, Brda, Wkra, and Dunajec) and three lakes (Lanskie, Maroz, and Rybnik power station reservoir)	[24]
	24.8–39.1	Portugal, Montego River, Tagus River	[25]
Cinoxacin	0.42-44.70 (A 9.50)	Portugal, Tejo River (estuary)	[26]
Ciprofloxacin	1.56-7.14 (A 3.91)	Portugal, Tejo River (estuary)	[26]
Diaminopyrimidines	<50	Poland, six rivers (Vistula, Warta, Odra, Brda, Wkra, and Dunajec) and three lakes (Lanskie, Maroz, and Rybnik power station reservoir)	[24]
	32.9–38.8	Portugal, Montego River, Tagus River	[25]
	11–21	UK, Taff	[27]
Doxycycline	1.73-128.00 (A 36.74)	Portugal, Tejo River (estuary)	[26]
Flumequine	0.24-37.70 (A 6.08)	Portugal, Tejo River (estuary)	[26]
Fluoroquinolones	<20	Poland, six rivers (Vistula, Warta, Odra, Brda, Wkra, and Dunajec) and three lakes (Lanskie, Maroz, and Rybnik power station reservoir)	[24]
Lincosamides	<20	Poland, six rivers (Vistula, Warta, Odra, Brda, Wkra, and Dunajec) and three lakes (Lanskie, Maroz, and Rybnik power station reservoir)	[24]
Macrolides	<50–5000	Poland, six rivers (Vistula, Warta, Odra, Brda, Wkra, and Dunajec) and three lakes (Lanskie, Maroz, and Rybnik power station reservoir)	[24]
Pleuromutilins	<20	Poland, six rivers (Vistula, Warta, Odra, Brda, Wkra, and Dunajec) and three lakes (Lanskie, Maroz, and Rybnik power station reservoir)	[24]
	53.3	Portugal, Douro	[19]
Sufadoxine	0.10-38.80 (A 12.94)	Portugal, Tejo River (estuary)	[26]
Sulfaquinoxaline	1.68-8.53 (A 3.98)	Portugal, Tejo River (estuary)	[26]
Sulfathiazole	0.38-49.80 (A 7.76)	Portugal, Tejo River (estuary)	[26]
Sulfisoxazole	0.55-4.99 (A 2.14)	Portugal, Tejo River (estuary)	[26]
Sulphonamides	<50	Poland, six rivers (Vistula, Warta, Odra, Brda, Wkra, and Dunajec) and three lakes (Lanskie, Maroz, and Rybnik power station reservoir)	[24]
Tetracyclines	<20–50	Poland, six rivers (Vistula, Warta, Odra, Brda, Wkra, and Dunajec) and three lakes (Lanskie, Maroz, and Rybnik power station reservoir)	[24]
	15.7	Portugal, Douro	[19]
	30–120	UK, Taff	[27]
Trimethoprim	2.25-7.76 (A 5.00)	Portugal, Tejo River (estuary)	[26]

A – the average value, M - the median value

Table 3 Classification and physico-chemical characterization of selected antibiotics [22].

Group	Substance	Molar mas [g/mol]	pK_a	log K_{OW}	Solubility in water [mg/L]
sulfonamides	sulfamethoxazole	253.28	5.81	0.48	610
	sulfametizole	270.33	2.10	0.41	1050
	sulfamerazine	264.31	-	-0.79	14.9
macrolides	erythromycin	733.95	8.88	2.48	1.437
	roxithromycin	837.07	-	2.75	0.019
fluoroquinolones	ciprofloxacin	331.35	6.09	0.28	30000
	sparfloxacin	385.36	-	1.07	1139
	ofloxacin	361.38	-	-0.2	28260
tetracyclines	oxytetracycline	460.44	3.27	-2.8	0.31
	tetracycline	444.45	3.3	-1.3	231
	doxycycline	444.45	4.5	-1.36	630

Table 4 LC-based procedures employed for determination of antibiotics and their metabolites in water samples of different origin.

Analytes/Group of analytes	Type and volume of sample	Sample preparation	LC conditions		Detection		LOD/LOQ [ng/L]	Ref.
			Column	Mobile phase	Type	Ionization		
Sulfomethaxazole	Water (25 mL)[1]	Filtration through a 0.45 μm polyvinylidene fluoride membrane filters, dilution in methanol:water (10:90, v/v) and SPE using Oasis® HLB cartridges (60 mg, 3 ml)	Acquity BEH C18 (50 mm x 2.1 mm i.d., 1.7 μm)	ACN and 0.1% FA in water (gradient)	QqLIT (MRM mode)	ESI+	n/a	[51]
Clarithromycin, fluoxetine, norfluoxetine, carbamazepine	Wastewater (100 mL) and surface water (250 mL)	Samples were acidified to pH 3 with sulphuric acid, SPE using Oasis® HLB Cartridges (150 mg, 6 mL)	Kinetex™ XB-C18 (100 mm x 2.1 mm i.d., 1.7 μm)	EtOH/water 50/50, v/v (gradient of flow rate)	QqQ (MRM mode)	ESI+	0.01-0.20/-	[53]
Phenicol antibiotics: thiamphenicol, florfenicol	Water[1]	SPE using Oasis® HLB cartridges (200 mg, 6 mL)	BEH C18 (50 mm x 2.1 mm i.d., 1.7 μm)/ C18 column (150 mm × 2.1 mm i.d. 5 μm)	ACN/water 10/90, v/v (isocratic) and ACN/water+0.05% FA (gradient)	DAD/QqQ (MRM mode)	ESI+	n/a	[62]
Amoxicillin, Azithromycin, Benzylpenicillin, Ciprofloxacin, Metronidazole, Sulfamethoxazol, Trimethoprim	Surface water (500 mL)	Filtration through a 1.0 μm GF/B Whatmann glass fibre and through a 0.45 μm Whatmann nylon filter, pH adjustment to 7 with NaOH solution and 5% (w/v) Na_2EDTA solution and SPE using Oasis® HLB cartridges (200 mg, 6 mL)	Phenomenex Luna C18(2) (150 x 2.0 mm i.d., 3 μm)	MeOH and water/0.1% FA (gradient)	HRMS (SCAN and MRM mode)	ESI+	<50/-	[52]
Chloramphenicol, ciprofloxacin, levofloxacin, metronidazole, nalidixic acid, sulfadoxin, sulfamethazine, sulfamethoxazole, trimethoprim	Wastewater, surface and ground water (100 mL)	pH adjustment with 5 M NaOH and 10% FA solution, filtration through a 1.0 μm GF/B Whatman glass fiber filter and then through a 0.45 μm Whatman nylon membrane and SPE using Oasis® HLB (200 mg, 6 mL) cartridges	Phenomenex Luna C18 (150 x 2.0 mm i.d.,3 μm)	MeOH and water both with 0.1% FA (ESI+)/ACN and water (ESI-) (gradient)	HRMS (MRM mode)	ESI+/ESI-	<50/-	[54]
Tetracycline (TC) and metabolites 4-	Wastewater and surface	Filtration through Whatman glass microfiber filter (0.7 μm), pH adjustment to 3 with HCl and	ACQUITY UPLC	ACN and water/0.1% FA	Q–TRAP (MRM	ESI+	-/ 920 (TC), 990 (ETC),	[56]

epitetracycline (ETC), 4-epianhydrotetracycline (EATC), and anhydrotetracycline (ATC)	water (150 mL)	SPE using Oasis® HLB (500 mg, 6 mL) cartridges followed by Oasis MAX (60 mg, 3 mL) cartridges	BEH C18 column (100 x 2.1 mm i.d., 1.7 µm)	(gradient)	mode)		1320 (EATC), and 1950 (ATC)	
Penidline, quinolone, tetracycline, sulfonamide antibiotics	Wastewater (100 mL effluent or 50 mL influent)	pH adjustment with 5 M NaOH and 10% FA solution, filtration through a 1.0 µm GF/B Whatman glass fiber filter and then through a 0.45 µm Whatman nylon membrane and SPE using Oasis® HLB (200 mg, 6 mL) cartridges	Phenomenex Luna C18 (150 x 2.0 mm i.d.,3 µm)	MeOH and water both with 0.1% FA (ESI+)/ACN and water (ESI-) (gradient)	HRMS (MRM mode)	ESI+/ESI-	-/-	[63]
Organic pollutants including antibiotics (screening procedure)	Surface water, ground water and effluent wastewater (100 mL)	Sample centrifugation and SPE using Oasis® HLB (60 mg, 3 mL)	Acquity UPLC BEH C18 (100 x 2.1 mm i.d., 1.7 µm)	Water/0.01% FA and MeOH/0.01% FA (gradient)	Q–TOF–MS (SCAN and MRM mode)	ESI+/ESI-	0.1-1/0.1-1 (LOI/SDL)	[55]
Pharmaceuticals and fungicides including sulfonamides tetracyclines, macrolides, penicillines anticiotics (screening procedure and confirmation by UPLC-MS/MS)	Surface and groundwater (100 mL)	pH adjustment to 3 with AA and SPE using Strata-X (200 mg, 6 mL) cartridges	Acquity U-HPLC C18 (100 x 2.1 mm i.d., 1.8 µm)	Water/2 mM ammonium formate/160 µL FA and MeOH/2 mM ammonium formate/160 µL FA (pH 3.5) (gradient)	Exactive Orbitrap MS (SCAN and MRM mode)	ESI+/ESI-	10-50/-	[64]
Sulfapyridine (SULF), p-nitroanisole (PNA) and pyridine (PYR) (degradation study)	Water (3–5 mL)[1]	SPE using Oasis® HLB (60 mg, 3 mL)	SULF: Symmetry C18, (150 x 4.6 mm i.d., 3.5 µm) PNA and PYR: Zorbax HILIC plus (100 x 2.1 mm i.d., 3.5 µm)	Water (A) and ACN/0.05% FA (10 mM, pH 3) (B) SULF: 70:30 A:B PNA and PYR: 80:20 A:B (isocratic)	UV-DAD	-	-	[65]

Macrolide antibiotics (azithromycin, erythromycin, clarithromycin and roxithromycin) and metabolites	Wastewater and surface water (1-250 mL)	Filtration through glass-fiber filters, pH adjustment with FA to 7–7.5 and SPE using Oasis® HLB (200 mg/6 mL) and Strata SAX (100 mg/3 mL)	ACE C18 PFP (150 x 3 mm i.d., 3 µm)	Water/0.1% FA and ACN (gradient)	QqQ (MRM mode)	ESI+	2-30/-	[66]
Metronidazole, sulfamethoxazole, trimethoprim, ceftazidime, ciprofloxacin, ofloxacin, spiramycin	Wastewater (100 mL)	Filtration through filter membrane (0.45 µm), pH adjustment with FA to 3.0, and SPE using Waters Sep-Pak C18	X Terra MS C18 (250 mm x 4.6 mm i.d., 5 µm)	Water and ACN/AA pH 3 (ACN and ammonium acetate (60:40) for spiramycin)	QqQ (MRM mode)	ESI+	0.0001-0.12/0.0003-0.4 (µg/L)	[67]

[1] degradation model study - real samples were not analysed

AA acetic acid; *ACN* acetonitrile; *DAD* diode array detector *ESI* electrospray ionization; *FA* formic acid; *HRMS* high resolution mass spectrometry; *LOD* limit of detection *LOI* limit of identification; *LOQ* limit o quantification; *MeOH* methanol; *MRM* multiply reaction monitoring mode; *MS* mass spectrometry; *n/a* not available; *QqLIT* quadrupole-linear hybrid ion trap mass spectrometry; *QQQ* triple quadrupole mass spectrometry; *Q–TOF* quadrupole time of flight; *Q–TRAP* quadrupole ion trap; *SDL* screening detection limit; *SPE* solid phase extraction

Highlights

- Presence of antibiotics in different types of surface water and wastewater in Poland and Europe.
- Impact on organisms resulting from the presence of antibiotics in the aquatic environment.
- A literature update of analytical methods for quantification of antibiotics based on data from 2009 until today.

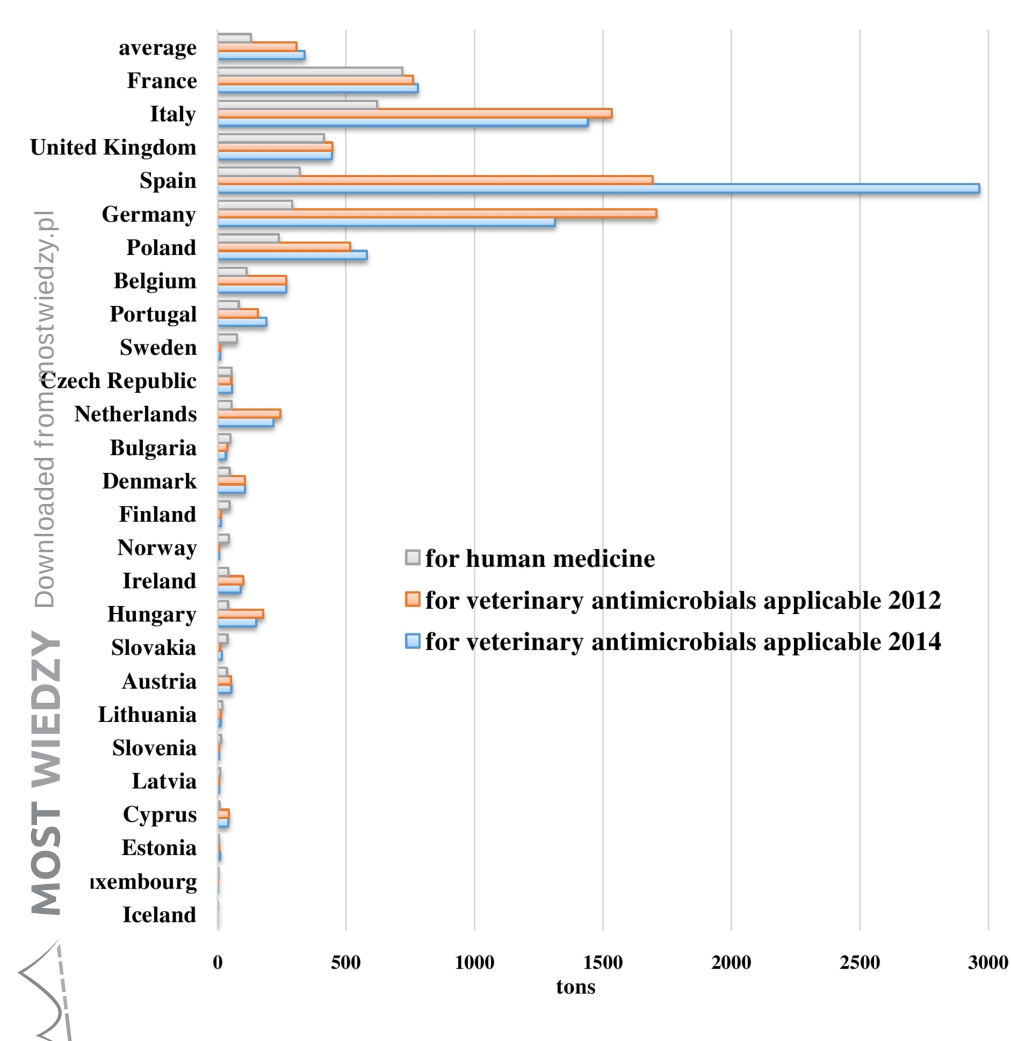

Figure 1

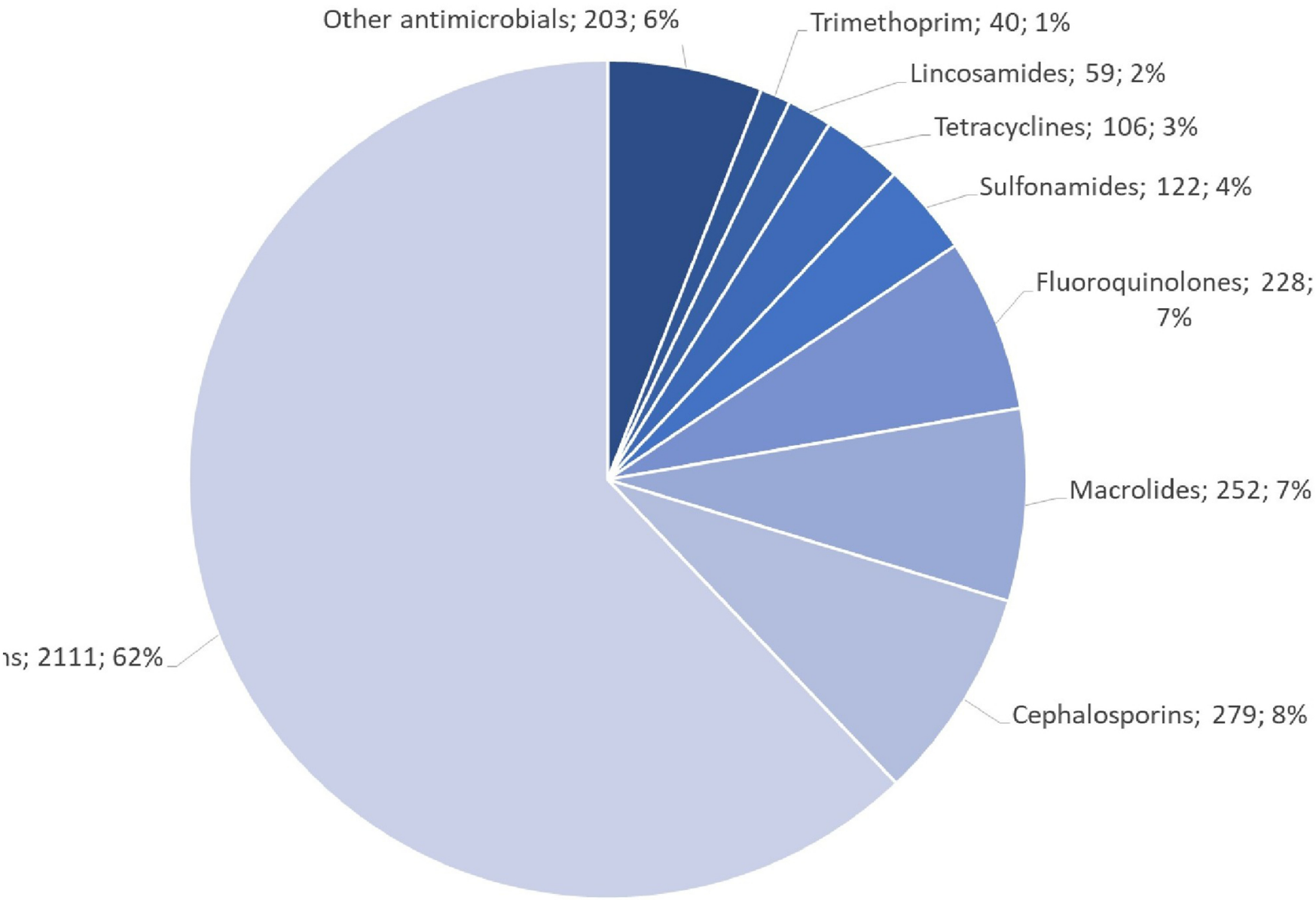

Figure 2

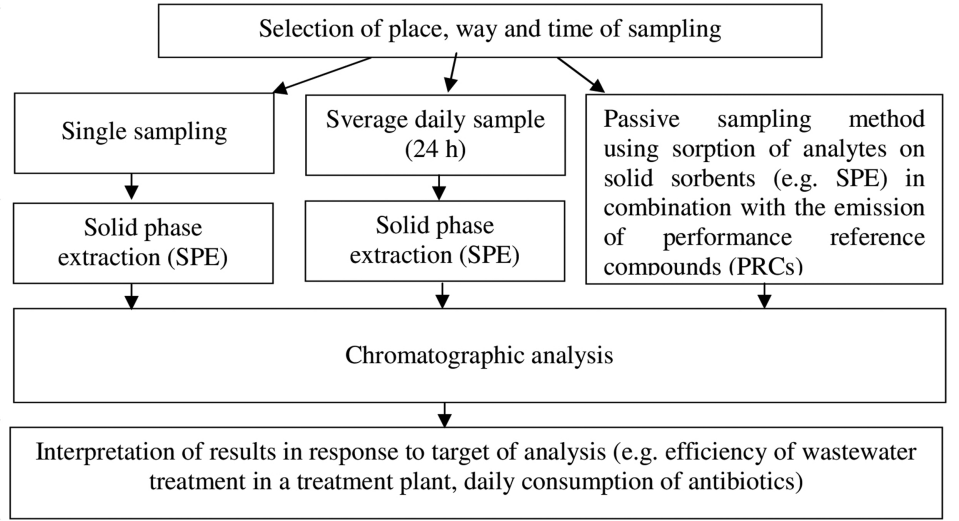

Figure 3