Amoxicillin in the Aquatic Environment, Its Fate and Environmental Risk

Armando Elizalde-Velázquez, Leobardo Manuel Gómez-Oliván, Marcela Galar-Martínez, Hariz Islas-Flores, Octavio Dublán-García and Nely SanJuan-Reyes

Abstract

Amoxicillin is a broad-spectrum antibiotic widely used for treating both human and animal diseases, and it belongs to a group that are excreted unchanged within urine and faeces; therefore, it is possible to find traces of this drug or its degradation products in environmental water bodies. In water, it is rapidly degraded by biotic and abiotic factors, yielding different intermediate products; these are suspected of being more resistant to degradation, and potentially more toxic, than the parent compound. In the water bodies, these compounds may produce toxic effects on the aquatic organisms from different trophic levels and produce an ecological imbalance. Amoxicillin may bioaccumulate in fish muscle tissues, with the possibility of the occurrence of these drugs in food, leading to a passive consumption of this antibiotic resulting in undesirable effects on consumer health such as immunoallergic responses. However, the main problem related with the presence of this antimicrobial compounds in fish tissues is the possibility of inducing bacterial resistance genes. At present, the available scientific knowledge is less than what is needed to fully assess the risks that amoxicillin pose to the environment, and it is still necessary to conduct large amount of research works before a thorough understanding of this severe environmental issue.

Keywords: Amoxicillin, risk, toxicology, occurrence, analytical methods
1. Introduction

In the recent decades, the term “emerging pollutants” have been used widely to refer to a variety of chemical compounds without regulatory status in the environment and its impact on the environment and human health are poorly understood. Within the category of emerging pollutants, the antibiotics are one of the most significant groups. Antibiotics are among the most successful drugs used for human therapy; however, these drugs are also recognized by its use and benefits in many different activities such as agriculture, aquaculture, bee keeping and livestock as growth promoters. Wise in 2002, estimated that the consumption in the market of antibiotics at worldwide level, was between 100,000 and 200,000 tonnes annually; however, the World Health Organization states that the amount of antibiotics is not precisely known because only a few countries have national statistics of the use, amounts and patterns of antibiotics [1–9].

Due to the extensive use in human and veterinary medicine, it is a reality that antibiotic compounds may be found in different environmental compartments. Indeed, in the last few years, antibiotics have been detected and reported by several researchers around the world in the μg/L range in municipal sewage, hospital effluents, sewage treatment plants effluents, surface water, ground water, marine water and drinking water [10–23]. The excretion of incompletely metabolized antibiotics by human and animals is the primary source of antibiotics in the environment, and other sources include the disposal of unused antibiotics and waste from pharmaceutical manufacturing process; residential (private residences, dormitories, hotels and residential care facilities) and commercial facilities (including hospitals) are also known as contributors of antibiotics to municipal wastewater. Other potential contributors of antibiotics to surface and groundwater are effluents from wastewater treatment plants and industrial facilities, and surface runoff from concentrated animal feeding operations [22, 24].

The occurrences of these compounds in different water bodies represent a potential threat to the environment, since these drugs are designed to have a pharmacological effect and to be persistent; moreover, the greatest concern about the presences of antibiotics in the environment is the emergence and dissemination of antibiotic resistance genes [1, 4, 12, 13, 16, 25–28].

In addition, antibiotics are of concern due to their high toxicity to algae and bacteria at low concentrations, potential genotoxic effects, disruption of aquatic ecology, promotion of antibiotic resistance and possibly even increased human health risks [20, 22, 29].

As a result, Sanderson et al. in 2004 classifies the antibiotics as pollutants of high priority for measuring environmental risk due to the probability of potential effects on human and environmental health, even more than sex hormones, cardiovascular drugs and antineoplastics [30].

Risk analysis is the scientific methodology used internationally to assess toxic effects on the environment [31–33]. In 2002, Jones et al. reported that the risk quotient (RQ) for amoxicillin in the United Kingdom exceeded the 1.00 unit, as well as, Park and Choi in 2008 reported that
Amoxicillin has a risk quotient (RQ) in Korea of 1.62, suggesting the need for further research to this antibiotic as an ongoing and future environmental monitoring toxicological investigations [34, 35].

Likewise in 2004, Boxall et al. scored the amoxicillin as “high priority” in environmental monitoring and prioritization of toxicological studies [34–36].

2. Amoxicillin

Originally introduced in the early 1970’s for oral use in United Kingdom, this drug has found gradually a regular place as a broad-spectrum antibiotic. In 1981, SmithKline Beecham patented amoxicillin/clavulanate potassium tablets, and first sold the antibiotic in 1998 under the trade names of amoxicillin, amoxil and timox [37–39].

The amoxicillin is a semi-synthetic drug, which belongs to a class of antibiotics called the penicillins (β-lactam antibiotics). This drug has been shown to be effective against a wide range of infections caused by gram-positive and gram-negative bacteria and is used for the treatment and prevention of respiratory, gastrointestinal, urinary and skin bacterial infections due to its pharmacological and pharmacokinetics properties [37, 40, 41]. Besides its use in human medicine, amoxicillin is also used for treating and preventing animal diseases as well as it is used as growth promoters for many domestic and food animals, including dogs, cats, pigeons, horses, broiler chickens, pigs, goats, sheep, pre-ruminating calves, cattle and fishes [42, 43]. It is well absorbed from gastrointestinal tract. The apparent volume of distribution of amoxicillin is approximately 0.26-0.31L/Kg and widely distributed to many tissues, including liver, lungs, prostate muscle, bile, ascetic, pleural and synovial fluids, and ocular fluids, accumulates in the amniotic fluid and crosses the placenta but penetrates poorly into the central nervous system unless inflammation is present. It is approximately 17–20% bound to human plasma proteins, primarily to albumin [44, 45].

Amoxicillin is very closely related to ampicillin with the same spectrum of activity and potency but is much better absorbed when given orally, achieving blood concentrations approximately twice as high as those obtained with ampicillin [37].

It acts binding to penicillin binding protein (PBP-1A) located inside the bacterial cell wall, the amoxicillin acylate the penicillin-sensitive transpeptidase C-terminal domain by opening the lactam ring causing inactivation of the enzyme, prevents the formation of a cross-link of two linear peptidoglycan strands, inhibiting the third and last stage of bacterial cell wall synthesis, which is necessary for cell division and cell shape and other essential processes, producing as a consequence the lysis of the bacteria cells [37, 46–49]. The two major metabolites of amoxicillin are amoxicilloic acid and piperazine-2, 5-dione (diketopiperazine). These metabolites have lost antibacterial activity of the parent component, but the amoxicilloic acid could have potential allergic properties [50–52].
The drug’s terminal half-life of elimination is 1 to 1.5 hours. Excretion of amoxicillin is predominantly renal, more than 80% of the original drug is recovered unchanged in the urine, leading to very high urinary concentrations and is also secreted in milk [53–55].

3. Amoxicillin sales and occurrence in water bodies

Amoxicillin acts against a broad spectrum of gram-positive and gram-negative microorganisms; therefore, it is used as first-line antibiotic both in human medicine and in veterinary medicine in many different countries [56–59]. Table 1 is listed numbers of data reported of sales of amoxicillin in different countries around the globe.

| Country | Amount (kg) | Reference |
|---------|-------------|-----------|
| Italy   | 209, 500 in 2001 | [60] |
| UK      | 71, 467     | [34] |
| Korea   | 106, 673    | [61] |
| Australia | 959,000     | [62] |
| Australia | 4 million scripts filled for amoxicillin which equates to approximately 40, 000kg of this antibiotic | [63] |
| UK      | 170, 432    | [64] |
| Brazil  | In Brazil, it is the most commonly prescribed antibiotic and have been sold 6 billions units of amoxicillin between 2007–2011 | [65] |
| China   | In China, Amoxicillin and Penicillin are the two most prescribed antibiotics | [18] |
| Nederland | Reported that amoxicillin and ampicillin are the two most consumed antimicrobial drugs in 71 countries between 2000–2010 | [25] |

Table 1. Sales of amoxicillin in different countries around the world.

Besides to the numbers of sales and use of this drug that have been mentioned above, amoxicillin belongs to a group of drugs that are excreted unchanged in urine and faeces at high rates; therefore, it is likely to find amoxicillin concentrations traces in the environment. Table 2 is listed the occurrence and quantity of amoxicillin that have been found in different water bodies of different countries around the world.

| Country | Water body                              | Concentrations (ng L⁻¹) | Reference |
|---------|-----------------------------------------|-------------------------|-----------|
| Italy (Cagliari) | Effluents of sewage treatment plants | 7.40                    | [66] |
| Italy (Cosenza) |                                         | 1.80                    |           |
| Italy (Palermo) |                                         | 120.35                  |           |
| Italy (Roma)   |                                         | 15.20                   |           |
| Italy (Napoli) |                                         | 1.80                    |           |
| Country | Water body | Concentrations (ng L\(^{-1}\)) | Reference |
|---------|------------|---------------------------------|-----------|
| Italy (Torino) | Effluents of sewage treatment plants | 120 | [67] |
| Italy (Latina) | Surface waters | 198–245 | [68] |
| Italy (Varese Olona) | Raw sewage | 280 | [69] |
| Australia | Hospital effluent | 900 | [20] |
| Australia (Brisbane) | Effluent of conventional treatment plant | 30 | |
| Australia | Wastewater treatment Plant influent | 6940 | |
| Australia | Wastewater treatment Plant effluent | 50 | |
| Australia | Surface waters | 200 | |
| Italy | Wastewater of sewage treatment plants | 622 | [26] |
| Spain | Surface water | 200 | [70] |
| Spain | Hospital effluent | 900 | |
| Spain | Urban effluent | 1670 | |

**Table 2.** Occurrence of amoxicillin in different water bodies around the world.

Although amoxicillin is an antibiotic drug highly consumed for human and veterinary medicine, and therefore is expected to be found at a relatively high concentration in wastewater and surface water, it is worth noting that there is no much information available in the literature regarding with its occurrence in environmental compartments. This can be explained by the clear fact that the chemical structure of this compound has an unstable β-lactam ring causing it readily undergo hydrolysis shortly after excretion; moreover, this might be the consequence of the incapability of the analysis to assess the presence of all antibiotic compounds presents in the different samples or even that its concentration in the aquatic media is lower than the limits of detection and quantification of the analytical methods.

**3.1. Methods of analysis for amoxicillin**

British, India and US Pharmacopoeia recommended liquid chromatography and potentiometric methods for the analysis of pure amoxicillin in pharmaceutical dosage forms (tablet, capsule, oral suspension and injection) [71–73].

However, the extensive literature survey showed that there are several methods, which can be used for assaying amoxicillin in drug substances, formulation products, biological fluids and environmental water samples, that is ultraviolet spectroscopy methods, colorimetric
methods, bienzimetic UV-spectrophotometric methods, polarography methods, spectrofluorometric methods, microbiological assays, enzyme linked immunosorbent assays (ELISA), and high-performance liquid chromatography methods (HPLC) [37].

The literature are reported several high-performance liquid chromatography methods with fluorescence, UV or mass spectrometry detection for the determination of amoxicillin residues in edible tissues of cattle, pig, sheep and goat as well as for cow and sheep milk [75–78]. Table 3 is summarized methods to measure amoxicillin residues in animal tissues that are reported in the literature.

| Tissue                                      | Method     | Limit of detection and limit of quantification | Reference |
|---------------------------------------------|------------|-----------------------------------------------|-----------|
| Catfish and salmon tissues                  | HPLC       | LOQ: 1.2ng/g                                  | [79]      |
| Pig, cattle and chicken muscle              | HPLC       | LOQ: 5 μg/Kg                                  | [80]      |
|                                             | Fluorescence|                                               |           |
| Pig liver, kidney, muscle and fat           | LC-MS/MS   | LOD: 2.3–12 μg/Kg                             | [81]      |
|                                             |            | LOQ: 25 μg/Kg                                 |           |
| Chicken muscle, kidney, liver, fat          | LC-MS/MS   | CC α: 51.6–57.0 μg/Kg                         | [82]      |
|                                             |            | CC β: 72.4 μg/Kg                              |           |
| Pig kidney, liver, fat and muscle           | LC-ESI-MS/MS| LOD: 1.5–3.5 ng/g                            | [83]      |
|                                             |            | LOQ: 25ng/g                                   |           |
| Sheep serum and tissue cage fluid           | HPLC       | LOD: 0.06–0.10μg/mL                           | [84]      |
|                                             | Fluorescence| LOQ: 0.10–0.20 μg/mL                         |           |
| Bovine milk                                 | UHPLC-MS/MS| LOD: 1.0ng/mL                                 | [85]      |
|                                             |            | LOQ: 5ng/mL                                   |           |
| Bovine muscle                              | LC-MS/MS   | CC α: 61.2 μg/Kg                              | [86]      |
|                                             |            | CC β: 72.4 μg/Kg                              |           |
| Chicken muscle, kidney and liver            | HRMS       | LOD: 10 μg/Kg                                 | [87]      |
|                                             |            | LOQ: 15–25μg/Kg                               |           |
| Eggs chicken                               | LC-MS/MS   | LOD: 0.6 μg/Kg                                | [88]      |
|                                             |            | LOQ: 1.8 μg/Kg                                |           |

Notes: CC α: Decision limit; CC β: Detection capability

Table 3. Methods to measure amoxicillin residues in food animal tissues.

The literature are also reported a few high-performance liquid chromatography methods with fluorescence, UV or mass spectrometry detection for the determination of amoxicillin in effluents and environmental water samples [60, 67]. Table 4 summarises some methods to measure amoxicillin and its degradation products in water samples.

The chromatographic methods for amoxicillin analysis in animal tissues and environmental water samples play a significant role in the regulation of the use of this drug in livestock as
well as in the creation of future regulations and monitoring programs for this drug in effluents from wastewater treatment plants, hospitals and households. Regulatory agencies rely on data generated from these methods to establish regulatory actions. Therefore, it is necessary to develop sensitive, accurate and reliable methods to support regulatory programs.

4. Environmental degradation of amoxicillin

Several processes can affect the fate and transport of organic compounds in the environment including 1) sorption, 2) biotic transformation and 3) abiotic transformation. The knowledge of the chemical properties and structures of compounds can allow preliminary estimation of their fate and persistence in the environment [91].

Despite that it is well known that β-lactam drugs contain a lactam ring, which is unstable and easily opened by β-lactamases (a widespread enzyme in bacteria) [24] as well as have shown thermal degradation with the hydrolytic cleavage and ultimate mineralisation to CO$_2$ and water [20], there was no detailed information regarding to the transformation of this antimicrobial compound in the environment until recent years.

In 2013, Gozlan et al., suggested a full degradation pathway of amoxicillin in aqueous medium. This starts with the opening of the four-membered β-lactam ring by hydrolysis to yields the intermediate AMX-penicilloic acid, which contains an extra free carboxylic acid group. Also it was reported that various metal ions such as mercury, zinc, cadmium, cobalt and copper might catalyse the degradation of the β-lactam ring. Subsequently depending of the pH of the medium, this intermediate compound could yield two different more stable compounds [89, 92, 93].
At high pH, the lone pair electrons on the amine group of the AMX-penicilloic acid are available for nucleophilic attack on the carbonyl group to yield a six membered stable diketopiperazine ring and the AMX diketopiperazine degradation product [89]. In the other hand, at low pH, the AMX-penicilloic acid suffers a decarboxylation process yielding the AMX penicilloic acid degradation product [89].

Another metabolite of amoxicillin is yield under sunlight irradiation merely as an indirect photolysis process enhanced by the presence of natural photo-sensitisers like humic acids, which activate the oxygen dissolved in water to oxidise the amoxicillin, forming the AMX-S-oxide degradation product; also this product is obtained under ozonation process. It is worth noting that the presence of this compound in aquatic environments is of great concern, because the AMX-S-oxide β-lactam ring is still active and may lead to the development of resistant bacteria and even cause other possible health hazards to human and wild and domestic animals [93].

To sum up, it is important to remark that these degradation products are suspected of being more resistant to degradation, and potentially more toxic, than the parent compound [36, 66, 70, 93].

5. Bioaccumulation and bacteria resistance genes

Antibiotics like amoxicillin are used not only for treating human health issues; they are also wide used in livestock farming and fish farming for treating diseases. The improper administration of these pharmaceutical compounds may lead to the occurrence of these drugs in food supplies [4, 78, 94]. Over the last decades, the occurrence of antibiotics in tissues of aquatic organisms has raised the interest of different organisations around the globe, particularly the FDA in USA. It has been reported that continuous exposure to these pharmaceutical compounds may result in accumulation of the parent compound, their metabolites or both in tissues of aquatic organisms [95].

Due to the risk associated with direct and indirect effects on human health due to both passive and active consumption of antibiotics has lead to set regulations on the use of some antibiotics and the establishment of maximum residue limits. The US official limit tolerance for amoxicillin residues is 0.01ppm (10 ppb o 10ng/g) in milk and uncooked cattle tissue; however, no tolerance has been established for amoxicillin residues in fish tissues at the present time [78, 95, 96].

It is important to remark that the presence of these drugs in animal tissues can have undesirable effects on consumer health such as allergies; however, the main problem related with the presence of this antimicrobial compounds in fish tissues is the non-controlled ingestion of antibiotics, possibly inducing resistance in bacterial strains [4, 66, 94, 95].

It has been recently shown an exchange of genes for resistance to antibiotics between bacteria in the aquaculture environment and bacteria in the terrestrial environment, including bacteria of animals and human pathogens [4, 97–99]. Therefore, the presence of antibiotics in the aquatic
environment can result in the appearances of resistance among human pathogens forming part of its microbiota [4, 100–102].

To sum up the unrestricted use of antibiotics like amoxicillin for any purpose in any country has the potential to affect human and animal health on a global scale; hence, this problem should be dealt through unified local and global preventive approaches [4, 103, 104].

6. Immuno-allergic potential

In the literature, it is reported that antibacterial drugs like amoxicillin are not considered as important toxics substances, even at high concentrations; however, an frequent use or exposure to this drugs may produce allergic reactions. Currently, the widely prescribed β-lactam antibiotics are among the drugs most frequently eliciting allergic reactions in human populations [105–108].

Protein haptenation plays a key role in immunological reactions to amoxicillin. The haptenation process occurs through the nucleophilic opening of the β-lactam ring by the attack of free amino groups in proteins, particularly modelling molecular studies found that the most reactive residues towards to amoxicillin is the Lysine, favouring the amoxicilloyl-protein adduct formation, which is able to elicit an immune response [109–112].

Clinically, drug allergy is characterized by a spectrum of immune reactions ranging from mild skins rashes to angio-oedema or life-threatening anaphylaxis, meaning a serious and potentially life-threatening problem, causing injury to tissues throughout the body. Moreover, diagnosis is complicated and requires a careful medical history, laboratory studies and in many cases oral food challenge to confirm a diagnosis. Particularly, some people have hypersensitive immune systems that overreact to these types of drugs, and without immediate medical treatment, allergic reactions may be fatal [113, 114]. Residues of many antibiotics and antibacterial agents, or haptenised macromolecules, for example penicilloylated proteins in meat and other dietary products from food animals and farmed fish might be responsible for hypersensitivity reactions in human population. Up to 7–10% of the general population in the world has true allergic sensitivity to penicillin and their related compounds due to prior medical treatment. However, in the literature, only a very small number of cases of hypersensitivity reactions related to food containing residues of penicillin have been reported [115–117]. Even though these cases are very rare, they remark the continuing need to control antibiotic residues vigilantly [117].

7. Toxicological risk of amoxicillin

In aquatic environments, few studies have reported the effects of amoxicillin in fish, probably because it is not described as a toxic substance important in the scientific literature reporting that the LC$_{50}$ at 96 hours for *Oryzias latipes* was 1000 mg / L [35].
However, Andreozzi et al., in 2004, reported a high toxicity of amoxicillin in the blue green algae *S. leopoliensis* (NOEC = 0.78μg / L; LOECs = 1.56μg / L; EC50 = 2.22μg / L) at 96 hours in concentrations between of 50 ng/L and 50 mg/L [66].

Furthermore, in 2013 at the University of Aveiro Portugal, Oliveira et al., measured the effects of amoxicillin in embryos and adults of *Danio rerio*; the effects found in embryos exposed with amoxicillin were premature hatching, malformations such as edema and deformities in the tail as well as abnormal development of fish. In the other hand, in adults exposed to amoxicillin, they found that amoxicillin produce the inhibition on the activity of the enzyme catalase (CAT) in certain tissues such as gills and brain, as well as produce an induction of the activity of the enzyme glutathione transferase (GTS) in tissues such as muscle, gills and brain, indicating that this antibiotic is capable of modifying the normal enzyme activity in fish [118].

In addition, Liu et al., in 2015, reported that the exposure of the photosynthetic cyanobacteria *Microcystis aeruginosa* to different concentrations of amoxicillin produced a significant increase in reactive oxygen species (ROS) and an increase in the activity of superoxide dismutase (SOD), peroxidase (POD) and glutathione-S-transferase (GST) as well as an increase in the content of glutathione (GSH) and malondialdehyde (MDA) [119].

Similarly, Li, et al., in 2007 reported that amoxicillin has the potential to produce genomic injuries in human deoxyribonucleic acid (DNA), possibly by intracellular induction of reactive oxygen species (ROS) [120].

Although the full extent and consequences of antibiotics in the aquatic environment are still largely unknown, their biological activity and toxicology is of concern [70]. However, despite being an issue of global concern, research regarding toxicological testing and biological activity is still insufficient and necessary.

9. Conclusion

Due to the lack of information regarding with environmental occurrence, ecotoxicity tests and it unstable structure, β-lactam drugs like amoxicillin are not generally thought could be of concern as environmental pollutants, however, amoxicillin is one of the most consumed antibiotics in the world, hence this pharmaceutical compound could be present at concentrations of concern by continual infusion in to the environment.

In the literature, there have been found traces of this antimicrobial compound in different water bodies; furthermore, some toxicological tests reported toxic effects in algae and fishes as well as the calculation of the risk quotient (RQ) in UK and Korea suggest that amoxicillin should be consider as a pollutant of high priority in the environment.

However, at present, the available scientific knowledge is less than what is needed to fully assess the risks that amoxicillin pose to the environment; therefore, future works will need to focus on more detailed ecotoxicity testing, using a wide range of aquatic organisms, in order to fully understand the environmental toxicity of this antimicrobial product and how may
affect both the aquatic and terrestrial environments and indicate possible remediation strategies; future works are needed in the development of new analytical methods that are more sensitive, accurate and reliable in order to assess the occurrence of the amoxicillin and its metabolites in different water bodies.

A large amount of research work is necessary before a thorough understanding of this severe environmental issue.

Author details

Armando Elizalde-Velázquez¹, Leobardo Manuel Gómez-Oliván¹*, Marcela Galar-Martínez², Hariz Islas-Flores¹, Octavio Dublán-García¹ and Nely SanJuan-Reyes¹

*Address all correspondence to: lgolivan74@gmail.com

1 Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México. Paseo Colón intersección Paseo Tollocan s/n. Col. Residencial Colón, 50120 Toluca, Estado de México, , México.

2 Laboratorio de Toxicología Acuática, Sección de Graduados e Investigación, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. Plan de Ayala y Carpio s/n, 11340 México D.F., México.

References

[1] Wise, R., 2002 Antimicrobial resistance: priorities for action. Journal of Antimicrobial Chemotherapy. 49, 585–586.

[2] Gothwal, R. and Shashidhar, T., 2014 Antibiotic Pollution in the Environment: A Review. Clean Soil Air Water. doi: 10.1002/clen.201300989.

[3] MartinezJ.L., 2009 Environmental pollution by antibiotics and by antibiotic resistance determinants. Environmental Pollution. 157, 2893–2902.

[4] Cabello, F.C., 2006 Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environmental Microbiology, 8, 1137–1144.

[5] Kümmerer, K, 2003 Significance of antibiotics in the environment Journal of Antimicrobial Chemotherapy. 52, 5–7.

[6] Singer, R.S., Finch, R., Wegener, H.C., Bywater, R., Walters, J., Lipstich, M., 2003 Antibiotic resistance: the interplay between antibiotic use in animals and human beings. Lancet Infectious Disease. 3, 47–51.
[7] McManus, P.S., Stockwell, V.O., Sundin, G.W., Jones, A.L., 2002 Antibiotic use in plant agriculture. Annual Review Phytopathology, 40, 443–465.

[8] Smith, D.L., Harris, A.D., Johnson, J.A., Sildberg, E.K., Morris, G.J., 2002 Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. Proceedings of the National Academy of Science. 99, 6434–6439.

[9] WHO, 2002. Overcoming Antibiotic Resistance, World Health Organization Report in Infectious Diseases. WHO, Geneva. Fact sheet No. 268.

[10] Johnson, A.C., Keller, V., Dumont, E., Sumpter, J.P., 2015 Assessing the concentrations and risks of toxicity from the antibiotics ciprofloxacin, sulfamethoxazole, trimethoprim and erythromycin in European rivers. Science of the Total Environment. 511, 747–755.

[11] Ma, Y., Li, M., Wu, M., Li, Z., & Liu, X., 2015 Occurrences and regional distributions of 20 antibiotics in water bodies during groundwater recharge. Science of the Total Environment. 518–519C, 498–506.

[12] Rodriguez-Mozaz, S., Chamorro, S., Marti, E., Huerta, B., Gros, M., Sanchez-Melsio, A., Borrego, C.M., Barcelo, D., and Balcazar, J.L., 2015 Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Research. 69: 234–242.

[13] Birosova, L., Mackulak, T., Bodik, I., Ryba, J., Skubak, J., Grabic, R. 2014 Pilot study of seasonal occurrence and distribution of antibiotics and drug resistant bacteria in wastewater treatment plants in Slovakia. Science of the Total Environment. 490, 440–444.

[14] Chen, C., Li, J., Chen, P., Ding, R., Zhang, P., & Li, X., 2014 Occurrence of antibiotics and antibiotic resistances in soils from wastewater irrigation areas in Beijing and Tianjin, China. Environmental Pollution. 193, 94–101.

[15] Gibs, J., Heckathorn, H. A., Meyer, M. T., Klapinski, F. R., Alebus, M., & Lippincott, R. L. 2013. Occurrence and partitioning of antibiotic compounds found in the water column and bottom sediments from a stream receiving two wastewater treatment plant effluents in northern New Jersey. Science of the Total Environment. 458–460, 107–116.

[16] Huerta, B., Marti, E., Gros, M., Lopez, P., Pompeo, M., Armengol, J., Barcelo, D., Balcazar, J.L., Rodriguez-Mozaz, S., Marce, R. 2013 Exploring the links between antibiotic occurrence, antibiotic resistance, and bacterial communities in water supply reservoirs. Science of the Total Environment. 456–457, 161–170.

[17] Na, G., Fang, X., Cai, Y., Ge, L., Zong, H., Yuan, X., Yao, Z., Zhang, Z. 2013 Occurrence, distribution, and bioaccumulation of antibiotics in coastal environment of Dalian, China. Marine Pollution Bulletin. 69(), 233–237.(1–2)
[18] Zhang, R., Tang, J., Li, J., Zheng, Q., Liu, D., Chen, Y., Zou, Y., Chen, X., Luo, C., Zhang, G. 2013 Antibiotics in the offshore waters of the Bohai Sea and the Yellow Sea in China: occurrence, distribution and ecological risks. *Environmental Pollution*, 174, 71–77.

[19] Behera, S. K., Kim, H. W., Oh, J. E., & Park, H. S., 2011 Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea. *Science of the Total Environment*. 409(20), 4351–4360.

[20] Watkinson, A. J., Murby, E. J., Kolpin, D. W., & Costanzo, S. D., 2009 The occurrence of antibiotics in an urban watershed: from wastewater to drinking water. *Science of the Total Environment*. 407(8), 2711–2723.

[21] Tamtam, F., Mercier, F., Le Bot, B., Eurin, J., Tuc Dinh, Q., Clement, M., Chevreuil, M., 2008 Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. *Science of the Total Environment*. 393(1), 84–95.

[22] Brown, K. D., Kulis, J., Thomson, B., Chapman, T. H., Mawhinney, D. B., 2006 Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the Rio Grande in New Mexico. *Science of the Total Environment*. 366(2–3), 772–783.

[23] Karthikeyan, K. G., and Meyer, M. T., 2006 Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. *Science of the Total Environment*. 361(1–3), 196–207.

[24] Hirsch R, Ternes T, Haberer K, Kratz K L., 1999 Occurrence of antibiotics in the aquatic environment. *Science of the Total Environment*. 225(1–2): 109–18.

[25] Van Boeckel, T. P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B. T., Levin, S. A., Laxminarayan, R., 2014 Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *The Lancet Infectious Diseases*. 14(8): 742–750.

[26] Zuccato E, Castiglioni S, Bagnati R, Melis M, Fanelli R., 2010 Source, occurrence and fate of antibiotics in the Italian aquatic environment. *Journal of Hazardous Materials*.179: 1042–1048.

[27] Kümmerer K. 2009a Antibiotics in the aquatic environment — a review — part I. *Chemosphere*. 75: 417–34.

[28] Kümmerer K. 2009b Antibiotics in the aquatic environment — a review — part II. *Chemosphere*. 75: 435–41.

[29] Daughton C, Ternes T. 1999 Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environmental Health Perspective*. 107: 907–38.

[30] Sanderson, H., Brain, R.A., Jhonson, D.J., Wilson, C.J., Solomon, K.R., 2004 Toxicity classification and evaluation of four pharmaceuticals classes: antibiotics, antineoplastics, cardiovascular, and sex hormones *Toxicology*, 203, 27–40.
[31] Van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003 Fish bioaccumulation and bio-markers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology, 13, 57–149.

[32] División de Protección Pecuaria, 2011 Evaluación de riesgo ambiental de productos farmaceuticos de usos veterinario. Boletín Veterinario Oficial, XIV, 1–9.

[33] Suter, G.W., 1993 Ecological Risk Assessment. Lewis Publishers, Boca Raton, FL, USA, 538.

[34] Jones, O.A.H., Voulvoulis, N., Lester, J.N., 2002 Aquatic environmental assessment of the top 25 English prescription pharmaceuticals. Water Research, 36, 5013–5022.

[35] Park, S., Choi, K., 2008. Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems. Ecotoxicology 17, 526–538.

[36] BoxallABA, SinclairCJ, FennerK, KolpinD, MaundSJ. 2004. Peer reviewed: when synthetic chemicals degrade in the environment. Environmental Science & Technology; 38: 368A–75A.

[37] Rao, R., Kaur, P.S., Nanda, S., 2011 Amoxicillin: a broad-spectrum antibiotic. International Journal of Pharmacy and Pharmaceutical Sciences, 3(3), 30–37.

[38] Subhas, C.M., Harsha, R., Dinesha, R., THammanna, G.S.S., 2010 Antibacterial activity of Coleus aromatics leaves. International Journal of Pharmacy and Pharmaceutical Sciences, 2(3), 63–66.

[39] Garau, J., 2005 Role of the β-lactam agents in the treatment of community-acquired pneumonia. European Journal of Clinical Microbiology and Infectious Diseases, 24, 83–99.

[40] Soussa, J.C., 2005 Manual de antibióticos antibacterianos. Fernando Pessoa University, Oporto, Portugal. See, 219–221.

[41] Bush, K., β-lactam antibiotics: Penicillin, and other β-lactam antibiotics. In: Finch, R.G., Greenwood, D., Norrby, S.R., and Whitley, R.J., Antibiotic and Chemotherapy: anti-infective agents and their use in therapy. 8th ed. Philadelphia (USA): Churchill Livingstone, an imprint of Elsevier Science Limited; 2003, p. 224–278.

[42] Pfizer, 2004 Clavamox® for cats and dogs. See: http://animalhealth.pfizer.com/sites/pahweb/US/EN/Products/Documents/AILF0504022.pdf Accessed July 2015.

[43] Schering-Plough, 2007 Amoxi-Mast, lacting cow formula. Intramammary infusion. Union, NJ07083, USA. Available at: http://www.merck-animal-health-usa.com/binaries/Amoxi-Mast_tcm130-164276.pdf Accessed July 2015.

[44] Eppes, S.C., Childs, J.A., 2002 Comparative study of cefuroxime axetil versus amoxicillin in children with early Lyme disease. Pediatrics, 109(6), 1173–1177.
[45] Shah, S.H., Shah, I.S., Turnbull, G., Cunningham, K., 1994 Cefuroxime axetil in the treatment of bronchitis: comparison with amoxicillin in a multicentre study in general practice patients. The British Journal of Clinical Practice, 48(4), 185–189.

[46] Torres, R.F., Consentino, M.O., Lopez, M.A.B., Mochon, M.C., 2010 Simultaneous determination of 11 antibiotics and their main metabolites from four different groups by reversed phase high-performance liquid chromatography-diode array-fluorescence (HPLC-DAD-FLD) in human urine samples. Talanta, 81, 871–880.

[47] Dousa, M., Hosmanova, R., 2005 Rapid determination of amoxicillin in premixes by HPLC. Journal of Pharmaceutical and Biomedical Analysis, 37, 373–377.

[48] Nagralli, B.S., Seetharamappa, J., Melwanki, M.B., 2002 Sensitive spectrophotometric methods for the determination of amoxicillin, ciprofloxacin, and piroxicam in pure and pharmaceutical formulations. Journal of Pharmaceutical and Biomedical Analysis, 29, 859–864.

[49] Amin, A.S., El-Ansary, A.L., Issa, Y.M., 1994 Colorimetric determination of amoxicillin in pure form and in pharmaceutical preparations. Talanta, 41(5), 691–694.

[50] Fujiwara, K., Shin, M., Miyazaki, T., Maruta, Y., 2011 Immunochemistry for amoxicillin and its use for studying uptake of the drug in the intestine, liver, and kidney of rats. Antimicrobial agents and chemotherapy, 55(1), 62–71.

[51] Reyns, T., De Boever, S., Schauvliege, S., Gasthuys, F., Meissonnier, G., Oswald, I., De Backer, P., Croubels, S., 2009 Influence of administration route on the biotransformation of amoxicillin in the pig. Journal of Veterinary Pharmacology and Therapeutics, 32(3): 241–248.

[52] Reyns, T., De Boever, S., De Baere, S., De Backer, P., Croubels, S., 2008a Tissue depletion of amoxicillin and its major metabolites in pigs; influence of the administration route and simultaneous dosage of clavulanic acid. Journal of Agricultural and Food Chemistry, 56, 448–454.

[53] Brodie, D.P., Griggs, J.V., Cunningham, K., 1990 Comparative study of cefuroxime axetil suspension and amoxicillin syrup in the treatment of acute otitis media in general practice. Journal of International Medical Research, 18(3), 235–239.

[54] Dattwyler, R.J., Volkman, D.J., Conaty, S.M., Platkin, S.P., Luft, B.J., 1990 Amoxicillin plus probenecid versus doxycycline for treatment of erythema migrans borreliosis. Lancet, 336 (8728), 1404–1406.

[55] Paavonen, J., Roberts, P.L., Stevens, C.E., Wolner-Hanssen, P., Brunham, R.C., Hillier, S., 1989 Randomized treatment of mucopurulent cervicitis with doxycycline or amoxicillin. American Journal of Obstetrics & Gynecology, 161(1), 128–135.

[56] Calvo, J., Martínez, L., 2009 Mecanismos de acción de los antimicrobianos Enfermedades infecciosas y microbiología clínica. 27, 44–52.
[57] Iannacone J., Alvárino L., 2009 Evaluación del riesgo acuático de siete productos farmacéuticos sobre Daphnia magna. Ecología Aplicada, 8(2), 71–80.

[58] Suárez, C., Gudiol, F., 2009 Antibióticos betalactámicos. Enfermedades infecciosas y microbiología clínica., 27(2), 116–129.

[59] Valdés Lozano, D., Muguercia Larrondo, H., Herrera Torres Ma. L., Arias Rivero, E., Marín Zamora, R., Praderes Araujo, J.L., 1998 Penicilinas. ACTA MEDICA, 8(1), 28–39.

[60] Calamari, D., Zuccato, E., Castiglioni, S., Bagnati, R., Fanelli, R., 2003 Strategic, survey of therapeutic drugs in the rivers Po and Lambro in Northern Italy. Environmental Science and Technology, 37, 1241–1248.

[61] Lee, Y., Lee, S., Lee, D., Kim, Y., 2008 Risk assessment of human antibiotics in Korean aquatic environment. Environmental Toxicology and Pharmacology, 26. 216–221.

[62] Therapeutic Goods Administration (TGA). 2003. Import volumes of antibiotics into Australia for human, veterinary and feed application 1992–2003. Canberra: Therapeutic Goods Administration.

[63] Australian Bureau of Statistics (ABS). 2004. Highest volume PBS drugs by generic name, year ending: June 2004. Canberra: Australian Bureau of Statistics; p. 3.

[64] Sarmah, A. K., Meyer, M. T., & Boxall, A. B. 2006 A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere, 65(5), 725–759.

[65] Magalhaes, J.L., Borschiver, S., 2012 Amoxicillin and Ampicillin: Import trends and increasing use in Brazil. Chemistry Today; 30 (5): 91–93.

[66] Andreozzi, R., Caprio, V., Ciniglia, C., De Champdoré, M., Lo Giudice, R., Marotta, R., Zucatto, E., 2004 Antibiotics in the Environment: Occurrence in Italian STPs, fate, and preliminary assessment on algal toxicity of amoxicillin Environmental Science and Technology. 38, 6832–6838.

[67] Castiglioni, S., Bagnati, R., Calamari, D., Fanelli, R., & Zucatto, E. 2005 A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewaters. Journal of Chromatography A, 1092(2), 206–215.

[68] Kazprzyk-Horden, B., Dinsdale, R., Guwy, A., 2007 Multiresidue method for the formation of basic/neutral pharmaceuticals and illicit drugs in surface water solid-phase extraction and ultra performance liquid chromatography-positive Electro spray ionization tandem mass spectrometry. Journal of Chromatography Association, 1161, 132–145.

[69] Watkinson, A., Murby, E., Constanzo, S., 2007 Removal of antibiotics in conventional and advance wastewater treatment: Implications for environmental discharge and wastewater recycling. Water Research, 41, 4164–4176.
[70] Fatta-Kassinos, D., Meric, S., Nikolau, A., 2011 Pharmaceutical residues in environmental waste and wastewater: current state of knowledge and future research. Analytical and Bioanalytical Chemistry, 399, 251–275.

[71] British Pharmacopoeia, 2009 ed. Vol I and II. London: British Pharmacopoeia Commission, 2009, 353–367.

[72] United States Pharmacopoeia-30 and National Formulary-25: The Official Compendia of Standards. Rockville (US): United States Pharmacopoeia Convention; 2007, 1402–1407.

[73] Indian Pharmacopoeia 5th ed. Vol. 2. Ghaziabad (INDIA): Indian Pharmacopoeia Commission; 2007, 100–107.

[74] Adam, G., Rorberts, S., 2007a. Report No. 1541N-03-04-165. Depletion of tissues residues following i.m. administration of clamoxyl TRU (amoxicillin) to sheep. Unpublished report submitted to FAO by Pfizer Animal Health.

[75] Adam, G., Roberts, S., 2007b. Report No. 1820N-03-04-219. Development and validation of an analytical method for the determination of amoxicillin in swine liver, kidney, muscle and skin with fat. Unpublished reports submitted to FAO by Pfizer Animal Health.

[76] Doran, A., Adam, G., 2005 Report No. 1840N-03-03-154. Development and validation of an analytical method for the determination of amoxicillin in ovine liver, kidney, muscle, fat and milk. Unpublished report submitted to FAO by Pfizer Animal Health.

[77] Neely, M., Conolly, P., 2004. Report No. 1830N-60-03-405. Validation of analytical methods for the determination of amoxicillin in bovine tissues (liver, kidney, muscle, fat) and milk. Unpublished report submitted to FAO by Pfizer Animal Health.

[78] Ang, W.Y.C., Liu, F.F., Lay, O.J., Luo, W., McKim, K., Ghering, T., Lochmann, R., 2000. Liquid chromatography analysis of incurred amoxicillin residues in catfish muscle following oral administration of the drug. Journal of Agriculture and Food Chemistry, 48. 1673–1677.

[79] Ang, C.Y.W., Luo, W., Hansen, E.B., Freeman, J.P., Thompson, H.C., 1996 Determination of amoxicillin in catfish and salmon tissues by liquid chromatography with precolumn formaldehyde derivatization. Journal of AOAC International, 79(2), 389–396.

[80] Luo, W., Ang, C.Y.W., 2000 Determination of amoxicillin residues in animal tissues by solid phase extraction and liquid chromatography with fluorescence detection. Journal of AOAC International, 83, 20–25.

[81] De Baere, S., Cherlet, M., Baert, K., De Backer, P., 2002 Quantitative analysis of amoxicillin and its major metabolites in animal tissues by liquid chromatography combined with electrospray ionization tandem mass spectrometry. Analytical Chemistry, 74(6), 1393 -1401.
[82] De Baere, S., Wassink, P., Croubels, S., De Boever, S., Baert, K. & De Backer, P., 2005. Quantitative liquid chromatographic-mass spectrometric analysis of amoxycillin in broiler edible tissues. Analytica Chimica Acta, 529, 221–227.

[83] Reyns, T., De Boever, S., De Baere S., De Backer, P., Croubels, S., 2008b Rapid method for the quantification of amoxicillin and its major metabolites in pig tissues by liquid chromatography-tandem mass spectrometry with emphasis on stability issues. Journal of Chromatography, B, 861, 108–116.

[84] Delis, G., Batzias, G., Kounenis, G., & Koutsoviti-Papadopoulou, M., 2009 Application and validation of a LC/fluorescence method for the determination of amoxicillin in sheep serum and tissue cage fluid. Journal of Pharmaceutical and Biomedical Analysis, 49(2), 375–380.

[85] Liu, C., Wang, H., Jiang, Y., & Du, Z., 2011 Rapid and simultaneous determination of amoxicillin, penicillin G, and their major metabolites in bovine milk by ultra-high-performance liquid chromatography-tandem mass spectrometry. Journal of Chromatography. B, Analytical technologies in the biomedical and life sciences, 879(7–8), 533–540.

[86] Lugoboni, B., Gazzotti, T., Zironi, E., Barbarossa, A., & Pagliuca, G., 2011 Development and validation of a liquid chromatography/tandem mass spectrometry method for quantitative determination of amoxicillin in bovine muscle. Journal of Chromatography. B, Analytical technologies in the biomedical and life sciences 879(21), 1980–1986.

[87] Hermo, M. P., Saurina, J., Barbosa, J., & Barron, D., 2014 High-resolution mass spectrometry applied to the study of metabolome modifications in various chicken tissues after amoxicillin administration. Food Chemistry, 153, 405–413.

[88] Sun, L., Jia, L., Xie, X., Xie, K., Wang, J., Liu, J., Wang, J., Cui, L., Zhang, G., Dai, G., Wang, J., 2015 Quantitative Analysis of Amoxicillin, Its Major Metabolites and Ampicillin in Eggs by Liquid Chromatography Combined with Electrospray Ionization Tandem Mass Spectrometry. Food Chemistry. Accepted manuscript.

[89] Gozlan, I., Rotstein, A., Avisar, D., 2013 Amoxicillin degradation products formed under controlled environmental conditions: Identification and determination in the aquatic environment. Chemosphere, 91, 985–992.

[90] Gbylik-Sikorska, M., Posyniak, A., Sniegocki, T., Zmudzki, J., 2015 Liquid chromatography-tandem mass spectrometry multiclass method for the determination of antibiotics residues in water samples from water supply systems in food-producing animal farms Chemosphere, 119, 8–15.

[91] HuangCH, RenewJE, SmebyKL, PinkerstonK, SedlakDL., 2001 Assessment of potential antibiotic contaminants in water and preliminary occurrence analysis. Water Resources Update. 120:30–40.
[92] Lamm, A., Gozlan, I., Rotstein, A., Avisar, D., 2009 Detection of amoxicillin-diketopiperazine-2',5' in wastewater samples. Journal of Environmental Science and Health Part. 44, 1512–1517.

[93] Gozlan, I., Rotstein, A., Avisa, D., 2010 Investigation of an amoxicillin oxidative degradation product formed under controlled environmental conditions. Environmental Chemistry. 7, 435–442.

[94] Tittlemier, S. A.; Van de Riet, J.; Burns, G.; Potter, R.; Murphy, C.; Rourke, W.; Pearce, H.; Cao, X. L.; Dabekai, R. W.; Dufresne, G., 2007 Analysis of veterinary drug residues in fish and shrimp composites collected during the Canadian Total Diet Study, 1993–2004. Food Additives and Contaminants, 24 (1), 14–20.

[95] Fernandez-Torres, R., Bello Lopez, M. A., Olias Consentino, M., Callejon Mochon, M., Perez-Bernal, J. L., 2010 Application of enzymatic probe sonication extraction for the determination of selected veterinary antibiotics and their main metabolites in fish and mussel samples. Analytica Chimica Acta, 675(2), 156–164.

[96] CFR. U.S. Code of Federal Regulations, Part 21. δ 556.38, Amoxicillin; U.S. GPO: Washington, DC, 1991.

[97] Sørum, H., and L’Abée-Lund, T.M., 2002 Antibiotic resistance in food related bacteria a result of interfering with the global web of bacterial genetics. International Journal of Food Microbiology, 78: 43–56.

[98] Schmidt, A.S., Bruun, M.S., Dalsgaard, I., Pedersen, K., and Larsen, J.L., 2000 Occurrence of antimicrobial resistance in fish pathogenic and environmental bacteria associated with four Danish rainbow trout farms. Applied Environmental Microbiology 66: 4908–4915.

[99] Angulo, F.J., Nargund, V.N., and Chiller, T.C., 2004 Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. Journal of Veterinary Medicine 51: 374–379.

[100] Cabello, F.C., 2003 Antibiotics and aquaculture: An analysis of their potential impact upon the environment, human and animal health in Chile. Fundación Terram. Análisis de Políticas Publicas, 17, pp. 1–16. See: http://www.terram.cl/docs/App17_Antibioticos_y_Acuicultura.pdf Accessed in July 2015.
[104] Cabello, F.C., 2004 Antibiotics and aquaculture in Chile: implications for human and animal health. Revista Medica Chilena 132: 1001–1006.

[105] ArizaA., GarzonD., AbanádesD.R., Vivian de los Rios., Vistoli G., Torres M.J., Carini M., Aldini G., Perez-Sala D., 2012 Protein hapetanation by amoxicillin: High resolution mass spectrometry analysis and identification of target proteins in serum. Journal of Proteomics, 77, 504–520.

[106] AlpízarOlivares Y., 2000 La penicilina y sus derivados como agentes desencadenantes de la respuesta immune. Revista Cubana de Hematología, Immunología y Homoterapia, 16(2), 99–104.

[107] Vega, J.M., Blanca, M., Garcia, J.J., Carmona, M.J., Miranda, A., Perez-Estrada, M., Fernandez, S., Acebes, J.M., Terrados, S., 1994 Immediate allergic reactions t amoxicil- lin. Allergy, 49, 317–322.

[108] Anderson, J.A., Adkinson, N.F., 1989 Reacciones alergicas a fármacos y agentes biológicos. Compendio de Enfermedades alérgicas e inmunológicas, Washington DC, 82–95.

[109] ArizaA., MontañezM.I., Pérez-SalaD., 2011 Proteomics in immunological reactions to drugs” Current Opinion in Allergy and Clinical Immunology. 11, 305–312.

[110] Meng, X., Jenkins, R.E., Berry, N.G., Maggs, J.L., Farrell, L., Lane, C.S., ., 2011 Direct evidence for the formation of diasteroisomeric benzylpenicilloyl haptons from benzilpenicillin and benzilpenicillenic acid in patients. Journal of Pharmacology and Experimental Therapeutics, 338, 841.849.

[111] Yvon, M., Wal, J.M., 1988 Identification of lysine residue 199 of human serum albumin as binding site for benzilpenicilloyl groups. FEBS Letters, 239, 23–240.

[112] Yvon, M., Anglade, P., Wal, J.M., 1990 Identification of the binding sites of benzyl penicilloyl, the allergenic metabolite of penicillin on the serum albumin molecule. FEBS Letters, 263, 237–240.

[113] Woodward, K., 2012 Toxicological Effects of Veterinary Medicinal Products in Humans. Royal Society of Chemistry.

[114] Sicherer, S.H., Sampson, A.H, 2010 Food allergy. Primer on Allergic and Immunologic Disease, 125(2), S116-S125.

[115] Graham, F., Paradist, L., Bégin, P., Paradis, J., Babin, Y., Des Roches, A., 2014 Risk of allergic reactions an sensitization to antibiotics in foods. Annals of Allergy, Asthma and Immunology, 113(3), 329.

[116] Dayan, A.D., 1993 Allergy to antimicrobial residues in food: assessment of the risk to man. 35(3–4), 213–226.

[117] Dewdney, J.M., Maes, L., Raynaud, J.P., Blanc, J.P., Scheid, T., Jackson, T., Lens, S., Verschueren, C., 1991 Risk assessment of antibiotic residues of β-lactams and macro-
lides in food products with regard to their immuno-allergic potential. Food and Chemical Toxicology, 29(7), 477–483.

[118] Oliveira R., McDonough S., Ladewig C.L., Soares M.V.M.A., Nogueira J.A.A., Domingues I. 2013. Effects of oxytetracycline and amoxicillin on development and biomarkers activities of zebrafish (Danio rerio). Environmental Toxicology and Pharmacology, 36. 903–912.

[119] Liu, Y., Wang, F., Chen, X., Zhang, J., Gao, B., 2015 Cellular responses and biodegradation of amoxicillin in Microcystis aeruginosa at different nitrogen levels. Ecotoxicology and Environmental Safety. 111, 138–145.

[120] Li, P.Y., Chang, Y.C., Tzang, B.S., Chen, C.C., Liu, Y.C., 2007, Antibiotic amoxicillin induces DNA lesions in mammalian cells possibly via the reactive oxygen species. Mutation Research. 629 (2007) 133–139.
