Analysis of antibiotic residues in fish

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Introduction

Aquaculture is one of the worldwide strategic development fields and its importance is evident in its significant worldwide growth in the last decades. It exhibits a faster growth than any other animal production sector. It plays a significant goal in any country's economic development plan because of its worldwide growth. The rapid transition from a capture species model to a culture and production model was a necessary response to the market needs. Aquaculture has the possibility of producing larger quantities of products in reduced space than the wild capture of species. The fast growth of these productions has resulted in concerns over fish quality and safety. Similar to other sectors of animal production, fish production adopts intensive and semi-intensive practices. These practices lead to a higher concentration of animals in small spaces and substantially increase the risk of disease. This growth is associated with the implementation of intensive and semi-intensive production methods, with the use of antibiotics in order to prevent the emergence and spread of infectious diseases in fish.

Fluoroquinolones, tetracyclines, and sulphonamides among others, are widely used for this purpose. This practice constitutes a real public health concern, not only due to the presence of antimicrobial residues in edible tissues, which can cause allergic reactions in hypersensitive individuals, but also due to the emergence of bacterial resistance. Food safety, as well as its consequences on human health, has become an extremely important topic for consumers and for public health authorities. In particular, there have been numerous events involving large-scale contamination of foods of animal origin. As of now, there are no antibiotics specifically designed for aquaculture; therefore, authorized products developed for other areas of veterinary medicine are used.

Two major concerns arise from these practices related to their effect on consumers' health: a) The presence of antimicrobial residues in edible tissues of treated animals. In persistent low doses, they become part of the consumers' diet and b) The emergence of antimicrobial resistance, which represents a huge threat to public health worldwide according to health professionals, governments, WHO and other non-governmental international agencies. The inappropriate, and frequently abusive, use of antibiotics affects human health. It is also evident that the public health hazards related to antimicrobial use in aquaculture include the development and spread of antimicrobial resistant bacteria and resistance genes. The greatest potential risk to public health associated with antimicrobial use in aquaculture is the development of a reservoir of transferable resistance genes in bacteria, and in aquatic environments. These genes can be disseminated by horizontal gene transfer to other bacteria and ultimately reach human pathogens.

Antimicrobials are chemical substances that either destroy (bactericidal) or inhibit the growth of microorganisms (bacteriostatic). Although the term "antibiotic" refers to the group of these substances that are produced by microorganisms. The monitoring of antimicrobial residues in fish tissues requires sensitive and selective analytical methodologies to verify the accomplishment of the legal framework and reach the desirable high standards of quality and food safety. For each group of antibiotics, the analytical determination depends on the extraction and purification step for determination using different methods.

a) Aminoglycosides: Determination of AG can be performed either directly, e.g., by spectrophotometric, immunochemical, or microbiological methods, or after liquid chromatography (LC) separation. Regarding the LC-based methods, there is an important challenge to be considered, related with the molecular structures of AG.

b) Amphenicols: The most representative amphenicol is chloramphenicol (CAP). Gas chromatography (GC) was the analytical tool previously used to determine CAP, florfenicol, and thiamphenicol levels in fish and shrimp samples. Currently, LC-MS/MS without derivatization is the technique of choice to determine antibiotic residues. This hyphenation of liquid chromatography and mass spectrometry enables the detection and quantification, without derivatization, of polar non-volatile analytes, such as CAP.

c) Beta-lactam antibiotics: β-lactam antibiotics are antibiotic agents that contain a β-lactam ring in their molecular structure and include penicillin derivatives, cephalosporins, monobactams, carbapenems and β-lactamase inhibitor. The β-lactam family can be divided into two main groups: penicillins and cephalosporins. LC has become the analytical method of choice for the identification and quantification of these drugs. Recent advances in LC and LC-MS/MS analysis enables easy detection of penicillin residues in food products. The use of LC-MS/MS allowed for the characterization of amoxicillin's degradation products at trace levels.

d) Macrolides: Macrolides are highly potent antimicrobials used in veterinary practices against a wide variety of Gram-positive and Gram-negative bacteria. They consist of macrocyclic lactone rings with 14 (erythromycin, roxithromycin and clarithromycin), 15 (azithromycin) or 16 (spiramycin, tylosin and tilmicosin) carbons linked to the carbohydrate molecules. The molecular structure of macrolides contains chromophores, which allows them to be analysed by UV and fluorometric detection. However, the improved sensitivity and specificity of MS has replaced UV and fluorometric methods in detection and quantification of macrolides in different biological matrices.

e) Nitrofurans: Nitrofurans (furazolidone, furaltadone, nitrofurazone, nifursol, nifurpirinol and nitrofurantoin) are a group of synthetic antibacterial agents that were widely used in food-producing animals. Nifurpirinol and nitrofurazone are effective against many fish pathogens. However, they are carcinogenic and mutagenic, and it is illegal to use them in fish intended for consumption in many countries. LC-MS/MS is the current tool for the detection of nitrofuran tissue-bound side-chain metabolites. It is used throughout the world in animal tissue and other matrices.

f) Quinolones: Quinolones represent a group of synthetic antibiotics used in both human and veterinary medicine. They are used in the treatment of septicaemia or skin diseases in fish. The introduction of the fluorinated quinolones provided important therapeutic advantages because this antibiotic group has higher antibacterial activity than the parent compounds and is highly active against both Gram-positive and Gram-negative strains. HPLC is the most widely used analytical method for these compounds with UV or fluorescence detection. LC coupled with MS detection has become the preferred analytical method for quantification.

g) Sulfonamides: The sulfonamide family includes sulfadiazine, sulfamethizole, sulfamethoxazole, sulfasalazine, sulfisoxazole and various high-strength combinations of three sulfonamides. GC-MS methods are considered to be an inappropriate option as they require a previous derivatization step, because of the high polarity and low volatility of these compounds. Several methods for SA determination, based on HPLC, have been reported but, nowadays, these methods are being replaced by MS/MS methods with the advantage of achieving more sensibility and specificity.

h) Tetracyclines: Tetracycline antibiotics (TC) are intensively used in therapy and prophylactic control of bacterial infections in human and veterinary medicine. They are also used as food additives for growth promotion in the farming industry. Their widespread use has caused antibiotic resistance among bacterial species, including resistance against TC. There are several different analytical methods that determine TC in products of animal origin including immunoassays and capillary electrophoresis. Liquid chromatography is the preferred method. Recent LC-MS/MS methods detect the epimers along with the tetracycline molecule.

i) Multi-residue and multi-class techniques: There is a trend toward the development of cost-effective methodologies that detect drug residues in food and maintain efficient screening technologies that prevent false positive and negative results. Multi-residue and multi-class techniques are important because they simultaneously detect numerous analytes of the identical family and different chemical classes in a single run. The desired efficiency is being achieved by multi-detection methods based on liquid chromatography technology coupled with tandem mass spectrometry and time of flight mass spectrometry. UHPLC also offers short running times and higher resolution and sensitivity.

New trends on the development of analytical methodologies concerning antimicrobial residues in aquaculture

The recent and recurrent episodes, involving large scale contamination of food products, especially with antimicrobial drug residues, has grown the consumer's awareness and the need to develop simpler, faster and, still, very sensitive and selective techniques for residues monitoring and control. On the other hand, the cost-effectiveness of analytical procedures is becoming a major issue for all laboratories involved in residue analysis, as the reagents and equipment are very expensive. The multiresidue and multi-class UHPLC–MS/MS methodology is the most powerful measurement tool, mainly with ToF. However, matrix effects could be observed when mass spectrometry is used. Ion suppression or increase of signal detection is frequently achieved. These phenomena need to be studied in order to know the real impact on final results. Thus, and if the final detection could be considered up-to-date to current knowledge, the different chemical structures of the different antibiotics, as well as their different physicochemical properties, implies that substantial improvements are still needed in the sample pre-treatment step. Last but not least, it is important to consider the concerns of antibiotic residues in causing adverse effects in the environment. In fact, antibiotics are “designed” to change specific biochemical pathways in target species but, when they are released into the environment they still have the potential to induce the same effects in non-target organisms or to promote other different and unknown actions, even in trace concentrations. Due to the need of monitoring natural ecosystems, it also becomes important to develop analytical methodologies that can be applied to environmental matrices, i.e., matrices not intended directly for human consumption but that can influence the presence of antibiotic residues in the food chain. The development, optimization and validation of

UHPLC–MS/MS multiresidue and multi-class antibiotic residue methods applied to multi-matrices could be a priority in a nearly future.

Public health concern of antibiotic use

Illegal use of antibiotics for veterinary purposes has become a matter of public concern. Antibiotics are used in aquaculture as prophylactics, as growth promoters and for treatment of diseases. They are usually administered in feeds and most commercial shrimp feeds contain antibiotics. The feeding of antibiotics as growth promoters is associated with decrease in animal gut mass, increased intestinal absorption of nutrients and energy sparing. But inappropriate and frequently abusive, use of antibiotics can affect human health. The two major concerns are the presence of antimicrobial residues in edible tissues and the emergence of antimicrobial resistance, which represents a huge threat to public health worldwide

The greatest potential risk to public health associated with antimicrobial use in aquaculture is the development of a reservoir of transferable resistance genes in bacteria of aquatic environments. The antibiotics lose their efficacy over time because of the emergence and dissemination of resistance among bacterial pathogens.

EU implemented "zero tolerance policy" regarding antibiotic residue. Using LCMSMS method EU laboratories are equipped to detect traces of prohibited carcinogenic antibiotics like chloramphenicol up to 0.3 ppb and nitrofuran up to 1 ppb levels. Many of the antibiotics are listed as prohibited substance in fish and fishery products. In India the tolerance limit has been set only for the following antibiotics.

Antibiotic	**MRL (ppm)**
Tetracycline	0.1
Oxytetracycline	0.1
Trimethoprim	0.05
Oxolinic Acid	0.3

The monitoring of antimicrobial residues in fish tissues requires sensitive and selective analytical methodologies to verify the accomplishment of the legal framework and reach the desirable high standards of quality and food safety. The methods can be microbiological, immunochemical or physico-chemical. European council directive 96/23/EC, 1996 gives direction on measures of monitoring residues in live and animal products.