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Review on Antibiotic Residue in Animal Food, their Detection Methods and Public Health Impact

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Review Article

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ABSTRACT

Antibiotics are used in therapeutics, and prophylaxis of infectious diseases or as a production aid in the food animals. After their administration to animals, such treatments leave residues in the tissues of these animals and the foods derived from them which results in public health impact. Generally, this review emphasizes on detection methods of veterinary drug residue in food of animal origin and its public health impact. Screening and confirmatory analyses of drug residue are needed to ensure the safety of animal derived food. The most frequently used screening methods for antimicrobial are microbiological inhibition assays, immunoassays and biosensor tests. The microbiological methods used for detecting antimicrobial residues in foodstuffs are based on inhibiting microbial growth, microbial receptor activity and enzymatic reactions. The immunological techniques work on the principle of antigen antibody interactions and it is usually very specific and helps in detecting residues from in food producing animals and ELISA is the most common method. Biosensors have been developed in recent years as an alternative approach to screen veterinary drugs residues in animal derived food. Confirmatory methods are methods that provide full or complementary information enabling the substance to be unequivocally identified and if necessary quantified at the level of interest. Liquid Chromatograph Mass Spectrometry (LC-MS) is a very sophisticated, technique used as quantitative as well for confirmation of veterinary drug residues in animal derived foods products. Coupling of HPLC with Mass Spectrometry (MS-MS) has resulted in substantial reduction of analysis time for confirmation in presumed positive samples after initial screening. The presence of drug residues in foods of animal origin, combined with failure to comply with the instructions for their use or poor livestock production practices, can have serious consequences for consumer health. Therefore, residue control strategy is based on the detection of residues using sensitive tests with a low rate of false negatives followed by confirmation, requiring quantification against the MRL and identification with a low rate of false positives is very crucial.

Keywords: Drug residue; Detection; Animals food; Public health; Mass spectrometry

INTRODUCTION

The safety of human food is threatened by various veterinary drugs used in food producing animals. Antibiotics are natural products of a micro-organism, identical synthetic products, or similar semi-synthetic products that inhibit the growth or

destroy microorganisms and the most commonly used antibiotics in food animals include the beta-lactams, tetracyclines, aminoglycosides, macrolides and sulphonamides [1].

Drug residues could result from chemotherapeutic or chemo prophylactic use of drugs in food animals. The presence of this residue in meat and offal may result from incorrect usage of drugs. This occurrence of residue in animal food products has received enormous worldwide attention from some local, international and public health agencies.

In most countries, veterinary medicine is allowed to use only those agents that are officially registered and approved. In drugs which are registered for use with food producing animals, protection periods are prescribed during which the quantity of residues in foodstuffs of animal origin (milk, meat, and eggs) should be reduced to a level not threatening the consumer's health.

Occurrences of veterinary drug residues pose the broad range of health consequences in the consumers. The main risks are exemplified by allergic reactions, regularly associated with β-lactams antibiotics; genotoxic and carcinogenic responses, often related to chloramphenicol, sulfamethazine, nitro furans, malachite green and gentian violet; and the development of antibiotic resistant strains of bacteria, with possible transference of resistance to other susceptible microorganisms.

Detection of drug residues from tissues and other animal products could be quite an expensive, time consuming and laborious venture. Today, many screening and confirmatory methods have been developed to detect veterinary drug residues in animal derived foods. Microbiological inhibition assays, immunoassays, and biosensor tests are the most common antimicrobial screening methods. Confirmatory procedures are those that offer complete or supplementary information that allows the substance to be recognized definitely and, if required, measured at the level of interest. The main confirmatory method used for veterinary drug residue analysis were Liquid Chromatography Mass Spectrometry (LC-MS) with UV detection, Diode Array Detection (DAD), and Fluorescent Detection (FLD), and Gas Chromatography (GC) with flame ionisation detection and electron capture detection [2].

To avoid veterinary drug residue, Internationally recognized organizations such as the World Health Organization (WHO), Food and Agriculture Organization (FAO), Veterinary Medicine Directorate (VMD) of the European Union (EU) as well as the Food and Drug Administration in the USA (FDA) has set Parameters such as an Acceptable Daily Intake (ADI), the No-Observed Adverse Effect Level (NOAEL), Maximum Residue Limits (MRLs), and the Withdrawal Periods (WPs) for each drug used in livestock European Commission Council regulation (EEC). As described above the presence of veterinary drug residues in animal derived foods remains a public health concern and for this reason, high efficiency detection technology plays an important role in food safety. Therefore, the objectives of this seminar paper are:

General objective

To describe the antibiotic residue in animal food, with their detection methods and public health impact respectively.

Specific objectives

- To evaluate the detection methods of veterinary drug residues in food of animal origin.
- To highlight the occurrence and risk factors of veterinary drug residue.
- To describe public health impact of veterinary drug residue in food of animal origin.
- To establish control and prevention strategy of drug residue in food of animal origin.

Antibiotic residue in animal food

Antibiotics were first used in the veterinary industry shortly after they were used to treat bacterial illnesses in people.

Antibiotics are primarily used in animals for three purposes: therapeutic use to treat sick animals such as mastitis, arthritis, respiratory diseases, gastrointestinal infections, and other infectious bacterial diseases, prophylactic use to prevent infection in animals, and as growth promoters to improve feed utilization and production [3]. Because of their growth promoting properties, antibiotics are routinely used as animal feed additives at sub-therapeutic levels (Table 1).

Table 1. Commonly used antibiotics in animal food.

Antibiotics in animal food	Class	Examples of drugs
1	Penicillins	Benzylpenicillin, procaine penicillin, amoxicillin, ampicillin, cloxacillin
2	Cephalosporins	Ceftriaxone, ceftiofur, cefotaxime, ceftizoxime, cefoperazone, cephalexin
3	Aminoglycosides	Streptomycin, dihydrostreptomycin, gentamicin, neomycin, amikacin
4	Tetracyclines	Oxytetracycline, tetracycline, chlortetracycline, doxycycline
5	Macrolides	Erythromycin, tylosin, azithromycin
6	Sulphonamides	Sulfadimidine, sulfamethoxazole, sulfadiazine, sulfanilamide
7	Fluoroquinolones	Enrofloxacin, ciprofloxacin, marbofloxacin, ofloxacin, levofloxacin
8	Other	Chloramphenicol, metronidazole, polymyxin B, colistin, tiamulin, bacitracin

Antibiotics are extremely essential and are thus frequently employed in veterinary medicine for medicinal, prophylactic, and growth promotion objectives. These compounds hinder disease causing or infectious bacteria' DNA topoisomerases, protein synthesis, cell division and development, and/or cell wall formation [4].

Antibiotics have recently been used to improve growth, particularly in broilers and feedlot animals. Antibiotics improve growth rate by thinning mucous membranes in the gut (which facilitates absorption); altering gut motility, which improves assimilation; producing favourable conditions for beneficial gut microbes (by destroying harmful bacteria); and partitioning of proteins for muscle growth *via* cytokine suppression. Antibiotics also promote growth by lowering immune system activity, minimizing nutrient waste, and reducing toxin creation. However, in most situations, only young developing animals and poultry respond to antibiotic mediated health maintenance. This strategy is truly troublesome since these feed additives are typically used without a prescription and for extremely long periods of time, in both big and small amounts, resulting in contamination.

A residue, defined in the simplest terms, results when a drug or pesticide is deliberately applied to a food producing animal or plant. Residues of veterinary drugs include the parent compounds and/or their metabolites in any edible portion of the animal product and include residues of associated impurities of the veterinary drug concerned. The concept of drug residues in food was developed over the second half of the 20th Century, resulting in the definition of a 'NOEL, an ADI and an MRL in food [5].

Antibiotic residues are found in food mostly as a result of therapeutic treatment for animals or augmentation of animal feed. Small quantities of these are caused by the use of antibiotics in the preservation of milk, meat, fish, and poultry. Antibiotic resistance develops as a result of repeated intake of tiny amounts of contaminated food [6]. prophylactics and growth promoters, in these two cases, antibiotics are used at lower concentrations than therapeutic concentrations for a longer period of time, which is a potentially dangerous practice because it is one of the strongest selective pressures leading to the emergence of antibiotic resistance strains of bacteria, the induction of allergic reactions in humans, and technological problems with fermented meat products.

Occurrence of antibiotic residue in animal food

Presently, approximately 80% of food animals receive antibiotics for part or most of their lifetime which may lead to residue accumulation in animal products. Penicillin (including ampicillin), tetracycline (including chlortetracycline and oxytetracycline), neomycin, gentamicin, streptomycin, flunixin, arsenicals, and sulfonamides are the most probable drugs to be discovered in meat (including sulfadimethoxine and sulfamethazine and sulfamethoxazole). Penicillin and sulfonamide medicines were found at the highest amounts in pigs and cattle. Neomycin and gentamicin were also found in certain animals, notably calves. Tilmicosin, flunixin, and tetracycline were among the other medicines found in cattle and swine [7].

Tetracycline was found in the bones of hens fed only 5 ppm for no more than three days. Continuous feeding of tetracycline 100 g/ton in conjunction with penicillin 50 g/ton and sulfamethazine 100 g/ton for 14 weeks resulted in less than 1 ppm chlortetracycline in all tissues on day 0 of withdrawal in swine.

The most often mentioned reasons for antibiotic residues are the use of intra-mammary medications and errors in milk withholding periods. Antibiotic residues infiltrate the milk supply when treated cows are returned to the milking herd too soon or when a cow has antibiotic residues in her system for an unusually long period of time. The most prevalent way penicillin contaminates milk is by intramammary infusion in the treatment of cow mastitis. Penicillin given *via* other means, on the other hand, can result in milk contamination. Dairy processors may protect the milk supply by testing each shipment for penicillin, sulfa medicines, and other antibiotics before accepting it [8].

On chicken farms, sulfonamide medications are most widely utilized. These medications are quickly absorbed and dispersed throughout the chicken's body, accumulating in various tissues and being transferred into their products. If the specified withdrawal periods are not followed prior to slaughtering of the treated animals, the products derived from such animals may be contaminated with sulfonamide residues.

The residue violation rates of quinolone and penicillin increased in Korea, and the overall antimicrobial residue violation rate for different animal species was 0.5%. According to the Vietnamese antibiotic residues monitoring program, an average residue violation rate of 11.9% occurred in chicken, pork, and beef. In Malaysia, drug residues in chicken, swine, and cattle were monitored and the average violation rate was observed to be 2.7%. Oxytetracycline was detected in 93.8%, 37.5%, and 82.1% of the meat samples taken from the slaughterhouses in Addis Abeba, DebreZeit, and Nazareth, respectively in Ethiopia [9].

Risk factors for antibiotic residues

The most common risk factors for drug residues may result from human management, such as improper usage, including extra-label or illegal drug applications. The great majority of residues found in edible tissues of animals have their source at the farm of origin [10].

Extra Label Drug Use (ELDU): Extra Label Drug Use (ELDU) is the use of an authorized medicine in a way that is not in conformity with the approved label recommendations. It happens when a medicine that is solely allowed for human use is used in animals, when a drug that is approved for one species of animal is used in another, when a drug is used to treat a disease for which it was not approved, or when pharmaceuticals are used at levels that are higher than suggested doses. In veterinary medicine, for example, the use of enrofloxacin solution as a topical ear treatment (only approved for injection) is a prevalent ELDU.

Withdrawal time: However, the most obvious reason for unacceptable residues might be due to failure to keep to the

withdrawal period including using overdose and long acting drugs. The withdrawal time is the time necessary for the toxicological concern residue to reach a safe concentration as determined by tolerance. It can range from a few hours to days or weeks depending on the medicine, dose type, and mode of administration. It is the time between the removal of an animal from medicine and the permissible time of slaughter for the production of safe foodstuffs [11].

Disease state: An animal's disease status can impact the pharmacokinetics of medications provided, which can affect the possibility for residues. This can happen when the illness impairs the metabolic system (and hence medication metabolism), or when infection and/or inflammation cause the drug to accumulate in afflicted tissues. For example, in cattle with intensely inflamed mastitis quarters, apramycin enters these parts of the body, with concentrations of the medication ten times higher than in cows without mastitis.

Another risk factor: Another risk factor is inadequate good sanitary care during animal or product transportation, including the cross contamination of animal feeding stuffs with inadvertently applied drugs, environmental and animal to animal transfer of drugs may also cause residues [12].

Maximum residue level

MRLs is the maximum allowable level or concentration of a chemical in feed or food at a specified time of slaughter or harvesting, processing, storage and marketing up to the time of consumption by animal or human. The MRL in various foodstuffs (muscle, liver, kidney, fat, milk and eggs) is determined to minimize the risk of consumer exposure, considering dietary intake. Such considerations as food technology, good farming practices and the use of veterinary medicinal products may alsobe considered when setting the MRL. It should be established purely on the basis of safety to the person consuming the product and has no pharmacodynamic reality in the animal to which the drug has been administered [13].
Countries worldwide rely on national regulatory agencies and international committees in evaluating the safety of all drugs used with food animals for potential human health risk as an integral part of the drug registration process. The Codex Alimentarius and Joint FAO/WHO programme have been developing the standards concernings the residues in foods since 1985. These standards are based upon scientific assessments performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) determining the acceptable daily intakes (ADIs) and giving recommendations for (MRLs) (Table 2).

Table 2. Codex MRL and ADI for some important antibiotics in animal food origin CX/MRL 2-2018.

MRL in tissue target (µg/kg)								
Drug	Species	Muscle	Liver	Kidney	Milk	Egg	Fat	ADI
Tetracycline	Cattle	200	600	1200	100	-		0-30 µg/kg bw
	Poultry	200	600	200		400		
	Sheep	200	600	1200	-			
	Pig	200	600	1200				
Erythromycin	Chicken	100	100	100		50	100	
Sulfadimidine	Cattle				25			
Tylosin	Cattle, sheep & Chicken	100	100	100			100	0-30 µg/kg bw

Detection method of antibiotic residue in animal food

Methods are generally divided into screening and confirmatory methods. For drug residue control, some laboratories add a third intermediary level based on post-screening test which gives structural or biological activity information about the residue [14].

Screening methodologies: Screening method can be defined as methods that are used to detect the presence of a substance or class of substances at the level of interest. An ideal screening methodology is easy to use and handle, have low set-up and running costs, high through put, possibility of automatization, ensure reduced time to obtain the result, have high detection capability, and good sensitivity and specificity. Several tests have been described for the screening of drug residues in various food of animal origin. The most frequently used screening methods for antimicrobial are microbiological inhibition assays, immunoassays and biosensor tests [15].

Microbial inhibitions assays: Though its use dates back as early as 1964 and was adopted initially to monitor the dairy industry with a view to preventing problems in the fermentative dairy industry, it has now been extended as a regulatory residue screening method in slaughter animals even till date. The microbiological methods used for detecting antimicrobial residues in foodstuffs are based on inhibiting microbial growth, microbial receptor activity and enzymatic reactions. Microbial inhibition assays involve culturing a microorganism from a standard strain, usually *Bacillus stearothermophilus*, *Bacillussubtilis*, *Bacillus cereus*, *Micrococcus luteus*, *Escherichiacoli*, *Bacillus megatherium*, *Sarcinalutea* and/or *Streptococcus thermophiles*.

There are two main test formats: the tube test and the (multi) plate test. A tube (or vial, or ampoule) test consists of a growth medium inoculated with (spores of) a sensitive test bacterium, supplemented with a pH or redox indicator. At the appropriate temperature, the bacteria start to grow and produce acid, which will cause a color change. The presence of antimicrobial residues will prevent or delay bacterial growth, and thus is indicated by the absence or delay of the color change. This format is commonly applied in routine screening of milk, but it is also increasingly used for analysis of other matrices. A plate test consists of a layer of inoculated nutrient agar, with samples applied on top of the layer, or in wells in the agar. Bacterial growth will turn the agar into an opaque layer, which yields a clear growth-inhibited area around the sample if it contains antimicrobial substances [16].

The advantage of microbiological inhibition assays, compared to immunoassays and instrumental analytical methods, is that microbiological tests can detect any antibiotic residue that shows antibacterial activity. Moreover, these tests have the potential to cover the entire antibiotic spectrum within a single test. The limitations of these techniques are their lack of selectivity, especially the tube microbiological inhibition test, relatively high detection limits and the long bacterial incubation time. Consequently, microbiological inhibition assays are not suitable for detection of banned antibiotic compounds like chloramphenicol [17].

Immunoassays: The immunoassay method is a relatively common way to inspect veterinary drugs in animal origin products through immunological detection techniques. The immunological techniques work on the principle of antigen- antibody interactions and it is usually very specific and helps in detecting residues from in food producing animals. This method is fast and easy to operate, so it is suitable for mass screening of samples. The most usual technique consists the Enzyme Linked Immunosorbent Assay (ELISA) and Fluorescence Immunoassay [18].

Enzyme Linked Immunosorbent Assay (ELISA)-ELISA is an ultramicro-experimental detection technology with high sensitivity

and specificity established by combining modern detection methods with immune technology. ELISA has the advantages of easy operation, convenience, high efficiency, strong specificity, and low detection cost and due to it is still used for the screening of veterinary drug residues in animal-derived foods.

There are different formats for antigen quantification like direct competitive (dc)-ELISA and indirect competitive (ic)-ELISA, of which the ic-ELISA method is more advanced. The basic principle of ELISA is to combine a specific antigen-antibody immunological reaction with an enzymatic catalytic reaction and to display the primary immune response with amplification of the enzymatic reaction and targeted compounds can be qualified with depth of color. Several ELISA methods for the detection of veterinary drugs have been reported to detect different types of veterinary drug residue in edible animal tissue and feed. Those studies were used confirmatory analysis like HPLC-UVD (Table 3).

Table 3. ELISA methods for the detection of antibiotics in animal derived foods.

Veterinary drugs	Animal derived food	Detection method	Recovery (%)	RSD (%)	(µg/kg or LOD µg/L)
Erythromycin	Milk	ic-ELISA	76.9–85.7	5.1–11.3	0.3
Fluoroquinolones and Sulfonamides	Milk	DC-ELISA	67.0–105.0	4.8–16.4	2.4–5.8
Florfenicol and Thiamphenichol	Animal tissues	ic-ELISA	80.6–105.5	3.5–14.1	0.07–0.14
Tetracycline		Competitive ELISA	-	-	1.80 µg/kg

Fluorescence Immunoassay Technology (FIT): Fluorescence immunoassay technology is also one of the commonly used immunoassay methods. It adopts the principle of energy level transition to produce fluorescence by giving electrons energy to complete the transition from the ground state to the excited state. In this method, the antibody antigen is labeled with a fluorescent isotope, and the detection of the compound is achieved by checking its fluorescence intensity.

Time resolved fluoroimmunoassay was used to quantify the residual streptomycin in milk. This method uses a Europium (EU^{3+}) chelate labelled secondary antibody as a tracer, and trichloroacetic acid is used to deproteinize milk samples. The results showed that the detection limit of streptomycin in milk was 1.8 mg/KG, the sample recovery rate was 86.2-96.3%, and the relative standard deviation was less than 11%.

An important advantage of immunoassays is that they are able to detect the presence of antibiotics at very low levels, which makes them even useful for screening of banned substances. The kits allow the analysis of a large number of samples per kit, don't require sophisticated instrumentation, the results are available in a few hours and are quite specific and sensitive. However, the main challenge of immunoassays is the production and supply of antibodies that should be selective about the targeted antibiotic compound or group.

Biosensor: Biosensors have been developed in recent years as an alternative approach to screen veterinary drugs residues in animal derived food. With the continuous development of detection technology, advanced sensor-led detection technology has emerged, providing a fast, efficient, and cost-effective method for detecting veterinary drug residues in animal-derived foods. The instrument is made up of biological recognition element (bio receptor), which recognizes the target antimicrobial residue and a signal transduction element (transducer) which converts the recognition event into a measurable signal. It is usually in close contact and connected to data acquisition and processing systems [19].

The type of bio receptor or transducer used forms the basis for classifying biosensors. A bio receptor can be an organic molecular species (e.g. an antibody, enzyme, protein, or nucleic acid) or a living biological system (e.g. cells, tissues or whole

organisms) using biochemical recognition mechanism. Different types of biosensors methods are used as alternative detection methods to detect veterinary drug residues in animal-derived foods, including methods involving electrochemical biosensors piezoelectric biosensors optical biosensors and MIP biosensors (Figure 1 and Table 4).

Figure 1: Schematic representation of the configuration of a biosensor.

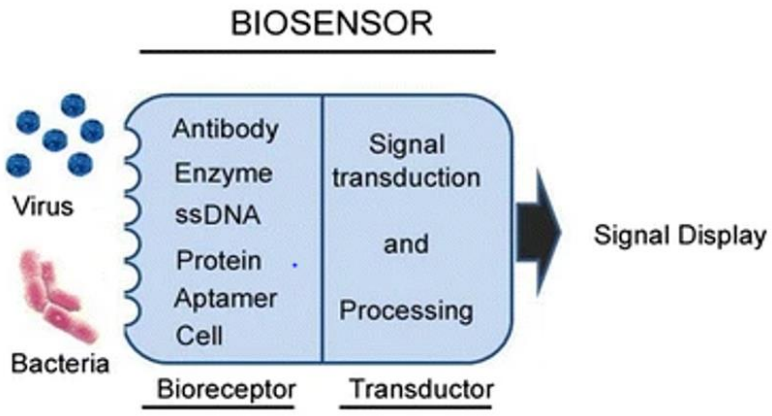


Table 4. Biosensor methods for the detection of antibiotics in animal derived foods.

Veterinary drugs	Animal derived food	Detection method	Recovery (%)	(μg/kg or LOD μg/L)
Ampicillin	Milk	Electrochemical biosensor	95.0–98.1	1.0×10^{-3}
PCN and Tetracycline	Chicken and beef	Electrochemical biosensor	–	10.5–15.2
Chloramphenicol	Porcine muscle, honey, milk and prawn	MIP biosensor	87.0-103.0	7×10^{-5}
Sulfathiazole	Honey	immunosensor piezoelectric	100.0–113.0	0.1 μg/kg

In general, these new technologies are getting good reception in control laboratories due to its advantages of easy to use, availability of results in short time, analysis of multiples residues in one shot, full automatisation, and a high productivity (high throughput technique of up to 120 samples per hour). The primary limitation of biosensors is the uncertainty of the biological sensing element. For instance, the sensing mechanism may be affected by duration of use, type of molecules and/or environmental factors (pH, temperature and ionic strength). Another restriction of biosensors is the transducer size within the biosensors. The technique is also high operative costs and the analysis is restricted to available chips [20].

DISCUSSION

Confirmatory methods

Confirmatory methods are methods that provide full or complementary information enabling the substance to be unequivocally identified and if necessary quantified at the level of interest. They must be instrumental spectrometric techniques and therefore are more expensive and time consuming, but are supposed to be highly selective in order to provide unequivocal identification. Analytical techniques based only on chromatographic analysis without the application of spectrometric detection are not appropriate on their own for use as confirmatory methods. However, if a single technique

lacks sufficient specificity, the desired specificity may be achieved by analytical procedures consisting of suitable combinations of clean-up, chromatographic separations, and spectrometric identification.

Until the last decade of the 20th century, the main instrumental techniques used for veterinary drug residue analysis were Liquid Chromatography (LC) using Ultra Violet detection (UV), Diode Array Detection (DAD) and Fluorescent Detection (FLD), and Gas Chromatography (GC) using flame ionisation detection and electron capture detection. Different analytical techniques are available for such purpose. Some examples of the available confirmatory methodologies are as follows:

Liquid Chromatographic Mass Spectrometry (LS-MS): LC-MS is a very sophisticated technique proving confidence and as compared to other techniques it is used as quantitative as well for confirmation. The LC-MS is the most commonly employed method for determination of veterinary drug residues in animal derived foods products. The LC/MS technique uses LC as the separation system and MS as the detection system. The principle of LC-MS can be divided into two parts: separation and analysis. In detail, the first part is separation of different compounds in sample which have distribution ratio in the liquid solid or two immiscible liquids in chromatography system. After sample is repeatedly adsorbed and decomposed in separation, concentration of the separated compound is transmitted into an electrical signal through the detector and, is recorded and displayed by chromatogram.

The second part is the qualitative and quantitative analysis of the separated compounds by mass spectrometer. The process is to ionize the target compounds and sort them according to the mass to charge ratio of each ion. This ratio is then being converted into an electrical signal by the detector. After the signal is amplified, it is converted into a chromatogram of relationship between separation time and signal intensity by the data processing system, and quantitative analysis is carried out by calculating the chromatographic area. Studies have applied liquid LC-MS at present, the pairing of LC with mass spectrometer detectors (MS and tandem MS) has been widely used in the analysis of veterinary drugs in animal derived foods (Table 5).

Table 5. LC-MS methods for the detection of veterinary drugs in animal derived foods.

Class of veterinary drugs	Animal derived food	Detection method	Recovery (%)	RSD (%)	(µg/kg or LOD µg/L)	(µg/kg or LOQ µg/L)
Macrolides	Meat and milk	LC–MS/MS	70.0–93.0	2.7–11.3	3–10	10–30
Amphenicol	Poultry and porcine tissues	GC–MS	78.5–105.5	6.4–16.8	0.1–0.5	0.25–2
Tylosin	Chicken meat	LC-MS/MS	84.3%–87.05%		0.07 µg/kg	-

Although LC-MS is widely used in the field of veterinary drug residue detection, some researchers believe that the relative molecular mass of the analyse given by liquid chromatography mass spectrometry is low, and structural information is lacking, so multi-stage mass spectrometry technology is needed to improve. Additionally, purity of separated chemicals can lead to inaccurate result.

Gas Chromatography (GS-MS)

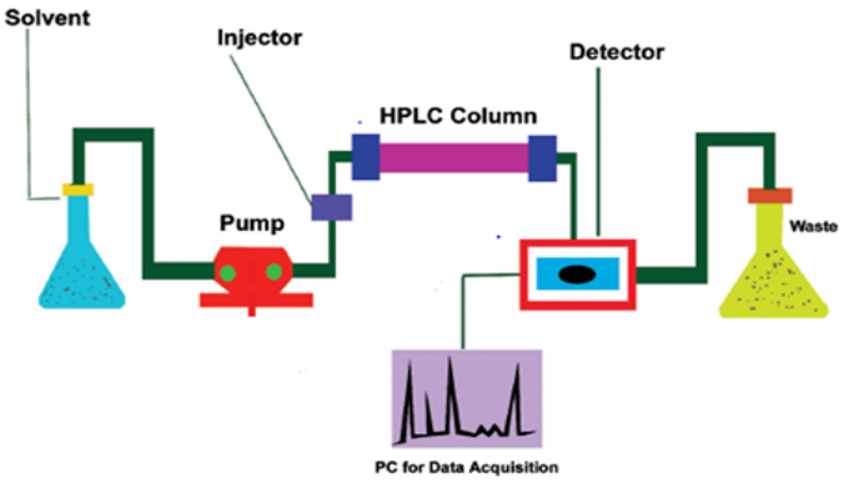
GC is a commonly used chromatographic technique that mainly uses differences in the boiling point, polarity, and adsorption properties of compounds to separate mixtures. For the analysis of veterinary drug residues in animal derived foods, GC instruments are usually connected to classic detectors, mainly including nitrogen phosphorus detectors, electron capture detectors and Mass spectrometry detectors. To date, GC-MS and GC-MS/MS methods are the most commonly used

methods to detect veterinary drugs in animal-derived foods. Compared with nitrogen-phosphorus detectors, electron capture detectors, MS or MS/MS has good recovery, precision, and reproducibility and can confirm false positives. Generally, derivatization reactions are required for the detection of veterinary drugs by GC.

GC usually requires the selection of specific capillary columns to separate the veterinary drugs in the sample, while optimization of the mobile phase, as in the LC method, is not required. GC instruments are relatively expensive, and researchers usually need professional training to operate the instruments. GC is widely used for analysis of pesticides, and GC-MS/MS methods are being gradually developed for research on veterinary drugs. The main reason is that mass spectrum information for some veterinary drugs in the GC mass spectrum library is lacking.

High performance liquid chromatography: It is also called as HPLC technique because solvent push through the pump pressurization. Coupling of HPLC with Mass Spectrometry (MS-MS) has resulted in substantial reduction of analysis time for confirmation in presumed positive samples after initial screening. Such a combination could effectively be used simultaneously for screening and confirmation. Basically, it's divided into five major parts first mobile phase, second detector, third pump (binary and quaternary), fourth column oven, and fifth auto sampler (Figure 2).

Figure 2. Flow diagram of HPLC.



Each and every component of HPLC shows its specific features. In HPLC, previously samples kept in auto sampler where it's come through the injector and carry through the mobile phase flow which it reaches into the column. The High Performance Liquid Chromatography (HPLC) relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column. It has been applied for the detection of antimicrobials in meat, fish and internal organs.

In HPLC, two techniques are involved, first normal phase second reverse phase. In normal phase using mobile phase a form of non-polar work as while stationary phase work as polar. In reverse phase technology, stationary phase and mobile phase work as a non-polar and polar form, respectively. Additionally, High Performance Liquid Chromatography Ultraviolet (HPLC-UV) detection method is an important method for residue detection in food before innovation of LC-MS. Through the separation and purification of the pre-treatment technology, the UV detector, such as a diode array detector, is a method of detecting the residue by detecting the absorption of the solute to the ultraviolet light.

HPLC usage is increasing day by day in the field of veterinary drug residue analysis in biological samples. HPLC have different mobile phases, vast library of column packing and the various modes of operations. HPLC was used for veterinary drug residue determination of ox tetracycline and penicillin G in milk in Ethiopia specially samples collected from Nazareth dairy farms. From the of 400 milk samples, 48 milk samples were found to contain ox tetracycline and penicillin G in the range of 45–192 and 0–28 µg/l, respectively (Table 6).

Table 6. HPLC methods for the detection of veterinary drugs in animal derived foods.

Class of veterinary drugs	Animal derived food	Detection method	Recovery (%)	RSD (%)	(µg/kg or LOD µg L)	(µg/kg or LOQ µg/L)
Tetracyline	Eggs, milk and milk powder	HPLC–UVD	85.3–98.3	1.9–5.3	0.76–1.13	2.53–3.77
Tetracyline and Quinolones	Lamb and chicken tissues, fish, honey, and milk	HPLC–DAD	25.5–82.6	3.4–10.7	0.5–20	1.25–50
Sulfonamides	Cattle meats	HPLC–FLD	44.6–81.0	2.7–4.9	8–15	13–25

The important advantages of HPLC are that, it takes a short time (few min/sample) to have the capacity to analyze multiple residues and to obtain results, has high sensitivity and specificity depending on detector, high automatisation leading to high productivity and the possible receipt of more information from spectra when using diode array detector. The disadvantages include initial investment (equipment), need of expertise, and need of sample preparation (extraction and filtration, addition of internal standard.

Public health impact

In many African countries including Ethiopia, drugs may be used indiscriminately for the treatment of diseases or as feed additives for domestic animals and birds. The ongoing threat of drug residue is one of the biggest challenges to public health that is faced not only by the African people, but also by the human population worldwide. The non-restrictive usage of antimicrobials in animals rearing may lead to problems due to the presence of residues in food and raw materials of animal origin. The drug residue hazards are classified as a direct short term and indirect long term hazards based on the duration of exposure to residues and the time of onset of adverse effects.

Direct short term hazards

The direct short term hazards usually appear immediately following the drug exposure, for example, the occurrence of allergic and hypersensitive reactions in sensitized individual.

Drug hypersensitivity reaction: Drug hypersensitivity is defined as an immune mediated response to a drug agent in a sensitized patient. The drug-mediated hypersensitivity reactions are of two types, one is IgE-mediated and the other Non-IgE mediated and drug allergy is restricted to a reaction mediated by IgE. An allergic or hypersensitive effect following administration of a drug (*i.e.,* drug allergy is quite similar to that typified by allergic response to protein, carbohydrate, and lipid macromolecules. Allergic reactions to drugs may include anaphylaxis, serum sickness, cutaneous reaction, a delayed hypersensitivity response to drugs appear to be more commonly associated with the antibiotics, especially of penicillin.

After exposure even in low amount, the penicillins form reactive neoantigen which produces hypersensitivity mainly the Type I reaction *i.e.,* urticarial. Approximately 10-15% of the human population is considered hypersensitive to the penicillin and the reaction is also seen in the animals. Studies have also shown that damages done to hepatic liver cells can be traced to allergic response to macrolide antibiotics (e.g. erythromycin, clarithromycin).

Indirect and long-term hazards

On contrary, long term exposure to the residues provokes indirect and long-term hazards such as carcinogenicity, teratogenicity and reproductive effects. The presence of dug residue in human food is associated with several adverse public health effects including the followings:

Carcinogenic effect: Carcinogenic effects refer to an effect produced by a drug having carcinogenic or cancer producing activity. Drug whose residues induce cancer in humans or consumers are: Nitrofurans, Nitroimdazoles. Inorganic arsenicis also a known carcinogen and may adversely affect the circulatory and nervous systems. Although, furazolidone had been labeled and approved for anti-protozoal and other uses for a wide variety of conditions in poultry and swine, its metabolites also have been shown to induce cancer in animals and human beings. The synthetic estrogen analogue diethyl stilbesterol, on chronic exposure, leads to vaginal clear cell adenocarcinoma in female offspring and also causes benign structural abnormalities. Similarly, hormonerelated cancer, including cancer of breast, ovary, prostate, testes and colon have been reported following ingestion of milk with hormonal residues. The potential hazard of carcinogenic residues of antibiotics are related to their interaction or covalently binding to various intracellular components such as proteins, Deoxyribonucleic Acid (DNA), Ribonucleic Acid (RNA), glycogen, phospholipids, and glutathione.

Development of antimicrobial resistance: Indiscriminate use of veterinary drugs, mainly antimicrobials, anthelmintics, and acaricides in food animals also play a major role in the development of Antimicrobial Resistance (AMR) which has put the public health at risk. Giving the established fact of an animal to human microbial resistance transfer, resistant micro-organism can gain entrance, directly through contact, into humans or indirectly *via* animal products and by products (e.g. milk, egg, etc.). As the bacteria of animal origin, they may either colonize human endogenous flora or superimpose and additional load to the reservoir of resistance genes already present in man. The consequences of antimicrobial resistance in bacteria causing human infections include increased number of infections, frequency of treatment failures and severity of infection, and finally increased costs to society associated with disease. Increased severity of infection includes prolonged duration of illness and increased frequency of bloodstream infections, hospitalization, and mortality. It has been documented that human develop drug resistant bacteria such as *Salmonella*, *Campylobacter* and *Staphylococcus* from food of animal origin. Examples of drugs that have been shown to cause the growth of resistant bacteria in foods from animals are fluoroquinolones and avaoparin. The resistance of microorganisms, arising from sub therapeutic uses of penicillin, tetracyclines and sulfa drugs; in agriculture is suggested by the WHO to be a high priority issue.

Teratogenicity: Teratogenicity is an ability of a drug or a chemical to produce harmful and toxic effects on developing embryo or foetus during critical phase of pregnancy. Consequently, a congenital malformation that affects the structural and functional integrity of the organism is produced. Sensitivity to teratogen-induced malformation varies during different developmental stages at the time of exposure, where there are critical periods of sensitivity to agents and organ systems. The teratogenic drugs include some chemotherapeutic agents antibiotics like tetracycline and aminoglycosides.

Disruptions of normal intestinal flora: The bacteria that usually in the intestine acts as a barrier to prevent incoming pathogen from being established and causing diseases. However, studies have shown that antimicrobials administered for therapeutic purposes can potentially alter or change the ecological composition of the intestinal flora. Degree of change however, depends on the dosage of the antimicrobial drug, route of administration, its bioavailability, metabolism, exposure length to the drug and distribution in the body including excretion route. The broad-spectrum antimicrobials may adversely affect a wide range of intestinal flora and consequently cause gastrointestinal disturbance. For example, use of drugs like,

flunixin, streptomycin, and tylosin in animals, and also use of vancomycin, nitroimidazole and metronidazole in humans are known for this effect.

Control and prevention

The management of drug residues in foods of animal origin is closely linked to rational pharmacotherapy in livestock animals. Drugs of veterinary use must be registered by government agencies, which are also responsible for establishing MRLs for the chemical agents allowed in food. It should be noted that drug residue monitoring plans in food are crucial to check if the rules are being followed.

The residue control strategy is based on a two-step approach:

- The detection of residues using sensitive tests with a low rate of false negatives;
- Followed by confirmation, requiring quantification against the MRL and identification with a low rate of false positives.

The residue prevention strategy is based on preventing entry of violate residues in meat or milk intended for human consumption by proper drug use guide developed for use by both veterinarians and food animal (dairy and beef) producers. All food animals should be maintained in a clean and healthy environment whenever possible. Antibiotic residues are best avoided by implementing management practice and herd health program that keep animals healthy and producing efficiently. Dairy and beef producers should not use or store un-approved drugs, special mixes, or products within adequate labels as unapproved drugs have no data regarding efficacy, safety, or withholding time.

The use of prescription drug and a veterinary client patient relationship, which is established hence a veterinarian is closely with the owner in health management of the herd. Before administering or dispensing drugs one has to know the drugs approved for all classes of cattle on the farm and be familiar with approved dosage, route of administration, and withholding times. Institute a workable health record for each animal to record all health related events, including administration of medication. Record the identification of all animals in the permanent health record book. These control points address the conditions under which residue testing should be considered the proper selection and interpretation of tests.

CONCLUSSION

Screening and confirmatory analyses of drug residue are needed to ensure the safety of animal derived food. Therefore, many detection techniques have been developed to detect veterinary drug residues in animal derived food. The MIA methods used for detection of residues in foodstuffs has two main test formats: the tube test and the plate test. ELISA is one of the most commonly immunological techniques work on the principle of antigen-antibody interactions. An ELISA kit is fast and easy to operate, so it is suitable for mass screening of samples. However, the main challenge of immunoassays is the production and supply of antibodies. Different types of biosensors have been developed in recent years as an alternative approach to screen veterinary drugs residues in animal derived food. In general, these new technologies are getting good reception in laboratories, but the technique is high operative costs and the analysis is restricted to available chips. Combining HPLC with mass spectrometry could effectively be used simultaneously for screening and confirmation. LC-MS is a very sophisticated technique used as quantitative as well for confirmation of veterinary drug residues in animal derived foods products. But, some researchers believe that the relative molecular mass of the analyse given by LC-MS is low, and structural information is lacking. The presence of drug residues in foods of animal origin, combined with failure to comply

with the instructions for their use (dosage and waiting period) or poor livestock production practices, can have serious consequences for consumer health. Therefore, residue control strategy is very crucial. Based on above conclusions the following recommendations are forwarded:

- ELISA should be used for detection of banned antibiotic compounds like chloramphenicol.
- LC- MS should be used for quantitative as well for confirmation of drug residues in animal-derived foods products.
- Conducting more in-depth research on how to reduce the limitation and cost of antibiotic residue detection technology.
- All the actors related to the livestock production chain assume full responsibility for the rational use of drugs in food-producing animals.
- Strict legislation must be passed for the management of veterinary drugs at all levels.

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