



Detection and Analysis of Veterinary Drug Residues in Meat and Chicken from the Valley of Kashmir

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ABSTRACT

Background: Indiscriminate use of drugs (antibiotics and anthelmintics) leads to increased chances of drug residues in animal foods like meat and chicken causing harmful effects on the health and well being of humans, animals in particular and to the overall environment in general.

Methods: A study was conducted in 2021 to assess residues of antibiotics and anthelmintics in meat and chicken procured from the markets of Kashmir Valley. A total of 100 samples (50 samples of mutton and 50 samples of chicken) were randomly taken and analyzed for drug residues using the technique of Reverse Phase High Performance Liquid Chromatography.

Result: The study revealed that 5 samples of chicken were positive for Oxytetracycline, 4% of the overall samples of meat including chicken detected to be positive for enrofloxacin, 7% of samples positive for ceftriaxone and 2% of the samples positive for Fenbendazole. However all of the drug residues were found to be within the limits of International MRLs.

Key words: Chicken, Drug residue, Indiscriminate, Mutton, One health, Reverse phase high performance liquid chromatography.

INTRODUCTION

The irrational use of drugs in veterinary practice increases the possibility of drug residues in animal foods like meat. Over use of antimicrobials and anthelmintics in veterinary medicine, for both food producing and companion animals, leads to the development of antimicrobial and anthelmintic resistance (Geary *et al.*, 2010). In the past, usage of antimicrobials has increased manifold that many feel today's intensive agricultural production will not be possible in their absence (Booth, 1988; Mitchell and Yee, 1995). The spectrum of Veterinary drugs used in food animals is extremely broad, ranging from teat dips to hormones. Approximately 19% of all veterinary pharmaceuticals used worldwide include anti-infectives (*e.g.*, antibacterials, antifungals and antivirals), 13% as parasiticides, 11% are used as biologicals and 15% represent other drugs (Miller, 1993). Antimicrobials are prescribed to animals by injections (*e.g.*, intramuscular, intravenous, subcutaneous), orally in the food and water, topically on the skin or by intramammary and intrauterine infusions. All these drugs may contribute to residues appearing in foods of animal origin such as milk, meat and eggs. These residues may be responsible for various toxic effects such as transfer of antibiotic resistant bacteria to humans, allergy, immuno-pathological effects, carcinogenicity (sulphamethazine, oxytetracycline, furazolidone), mutagenicity, nephropathy (gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (chloramphenicol) and even allergic shock in humans (Nisha, 2008; Darwish *et al.*, 2013). In the developing world, multi-drug resistant (MDR) bacteria are becoming a serious problem in the cure of diseases (Willis, 2000).

Antimicrobials if not used judiciously, may have impact on human, animal and environmental health in a one

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health context, as up to 90% of the antibiotic parent compounds can be directly excreted in milk and meat leading to antibiotic resistance development in the environment (Daniel *et al.*, 2014; Boeckel *et al.*, 2017). Excessive usage of antibiotics has caused xenobiotic residue occurrence in meat and due to this meat adulterated with antibiotics beyond a safe level are deemed to be unfit for human use (Hillerton *et al.*, 1999). Legislation and other restrictions on antibiotic usage in farm animals are now being introduced across the globe (OECD, Paris, 2019). A recent study assessing global use of antibiotics in poultry,

swine and cattle in 2010 revealed that India accounts for 3% of global consumption and is among the top consumers worldwide, along with China, the United States, Brazil and Germany. Estimates for 2030 indicate an overall increase of about two-thirds in animal antibiotic consumption worldwide. Their use in chickens, in particular, is expected to triple in India by 2030 (Yann and Sivaraman, 2018).

MATERIALS AND METHODS

Analysis of drug residues in meat (mutton and chicken meat)

Hundred samples from market meat (50 samples of mutton and 50 samples of chicken meat) were collected randomly in 2021 from markets of Kashmir Valley and taken for study of selected drug residues of the mentioned antibiotics and anthelmintics through use of Reverse Phase HPLC Technique at Department of Food Technology, University of Kashmir. It was also ensured that samples from locally produced mutton and also mutton imported from other states were taken into study.

Preparation of meat samples

10 grams of mutton/chicken sample was pounded and homogenized using Pestle and Mortar and homogenizer. 10 ml HPLC Acetonitrile and 1 ml of 0.1 M EDTA was added followed by rigorous shaking for 20 minutes and Centrifugation at 3200 rpm for 15 minutes. The supernatant was collected and 3 ml HPLC grade n-hexane was added to it and vortexed for 30 seconds. It was again subjected to centrifugation for 15 minutes at 3200 rpm. The upper layer of n-hexane was discarded and the rest was filtered through 0.45 µm syringe filter and concentrated in rotary vacuum evaporator to 0.5ml which was finally diluted by 400µL of mobile phase before injecting for HPLC analysis.

RESULTS AND DISCUSSION

Detection of antibiotic and anthelmintic drug residues in meat and chicken samples from market

The above validated methods proved successful to detect and quantify the given veterinary drug residues thus indicating that the applied method was appropriate for the detection of antibiotic and anthelmintic residues in meat therefore the standardized and validated method was used for the analysis of total of 100 meat samples (50 samples of mutton and 50 of chicken) randomly collected from the market. The results are summarized in Table 1 below and some positive representative chromatograms are shown in Figs 1, 2, 3 and 4.

Representative chromatograms of samples

Drugs are used widely in animal husbandry and the presence of drugs in foods like milk and meat is a health hazard. Food products can potentially be contaminated with hundreds of chemicals used in day to day life including pesticides, antibiotics, anthelmintics, hormones, heavy metals *etc.* (Unnikrishnan *et al.*, 2005; Khaniki, 2007).

Antibiotics are widely used in veterinary practice which may appear as residues in foods like meat for certain period of time (Wassenaar, 2005). The use of drugs in veterinary practice has become a cause of public health concern as these residues may impose a health risk to consumers. The presence of drug residue in foods like milk and meat may be the result of various factors like failure to observe the withdrawal and withholding periods, extra label use of drugs, faulty or inappropriate dosage of drugs. Lack of judicious drug use in veterinary practice may lead to occurrence of drug residues in animal foods like meat (Ivona and Mate, 2002; Paturkar *et al.*, 2005). Milk and meat containing veterinary drugs above certain concentrations are dangerous. The maximum concentrations of drug residues have been set for different drugs so as to prevent harmful effects (Hays, 2003; FDA, 2013).

Detection and analysis of antibiotics and anthelmintics in market meat (mutton and chicken)

(a) Tetracyclines

In the present study 5% of the meat samples (5 samples of chicken meat) were found positive for Oxytetracycline however none of the samples crossed the MRLs as put forth by European Union (2010) and codex commission (2015). The present study was a part of a wider study also involving the detection and analysis of veterinary drug residues in milk in which none of the milk samples was found positive for antibiotic through the technique of Reverse Phase HPLC (Akhoon *et al.*, 2025) Tetracycline residues have also been detected by Waghmare *et al.* (2020) who reported that on HPLC Analysis 9.5% and 5.26% muscle and liver samples respectively were positive for tetracycline residues in chicken farms and processing units located around Mumbai, India. The results are also in agreement with work of Singh *et al.* (2016) who reported 3.3% in muscle and 16.67% in liver samples of chicken, but 21.73% samples were above MRL recommended by EU. Likewise, Salama *et al.* (2011) and Okerman *et al.* (2001) recorded incidence of 12.67% and 7.01% respectively in poultry meat. Salehzadeh *et al.*, (2006) found 27.77% of chicken meat samples in Iran positive for Oxytetracycline residues exceeding the MRLs through HPLC, while Shahid *et al.* (2007) found 4 out of 7 samples of chicken meat in Pakistan positive for Oxytetracycline. Aman *et al.* (2017) performed detection of tetracycline drug residues in Egyptian poultry meat by HPLC and the results showed that residues of OTC were detected in 28.75% and 75% of breast and thigh muscles respectively. The presence of tetracycline residues in chicken meat can occur due to illegal use or a result of production of medicated feed or extra-label administration in farms as therapeutic or growth promoter purposes and slaughter of birds before observance of a proper withdrawal period.

(b) Enrofloxacin

In our study enrofloxacin was detected in 1 sample of locally produced mutton and 3 samples of chicken meat amounting

to 4% of the total meat samples. None of the samples was found to violate MRL values as put forth by EU and Codex commission. Verma *et al.* (2021) showed that 43.58% chicken meat samples were positive for enrofloxacin through HPLC analysis. Buket *et al.* (2013) in Turkey also reported the residues of quinolones in about 40% of samples of chicken. Amro *et al.* (2013) showed that incidence percentage of antibiotics was 40% in chicken meat. The enrofloxacin drug residues have also been

detected by Aslam *et al.* (2016) through HPLC detection in commercial broilers and he revealed that 52% of chicken meat and 78.7% of liver samples were found positive. Ramata *et al.* (2017) have also detected fluoroquinolone drug residues in beef and pork through HPLC method. Chaiba *et al.* (2017) showed that 36.15% of chicken meat samples were positive to antibiotic residues. Arslanbas *et al.* (2018) through HPLC method reported that 3.6% of chicken samples in Turkey were having enrofloxacin residues.

Table 1: Distribution of positive meat samples for each analyte.

Analyte	Number of positive samples			
	Mutton from imported sheep (n = 40)	Mutton produced locally (n = 10)	Chicken meat (n = 50)	Total (n = 100)
Oxytetracycline	Nil	Nil	5 (10%)	5 (5%)
Tetracycline	Nil	Nil	Nil	Nil
Enrofloxacin	Nil	1 (10%)	3(6%)	4(4%)
ceftriaxone	2 (5%)	Nil	5 (10%)	7(7%)
Ivermectin	Nil	Nil	Nil	Nil
Fenbendazole	1(2.5%)	1 (10%)	Nil	2 (2%)

Values in parenthesis indicate percentage.

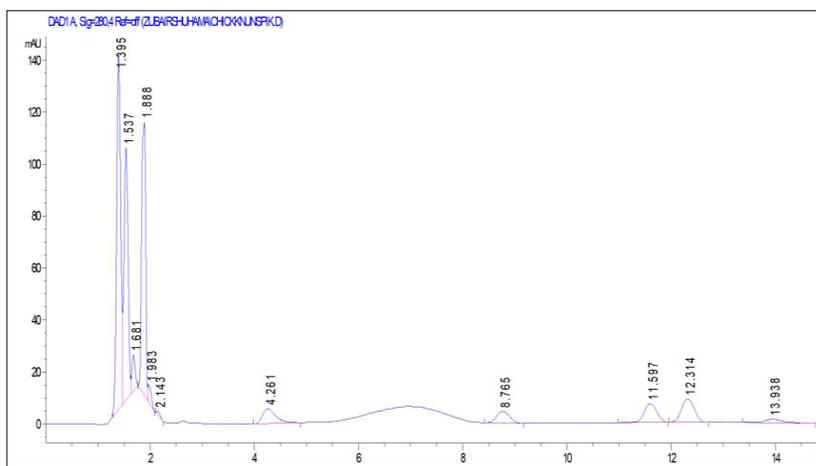


Fig 1: HPLC detection for antibiotic (ceftriaxone) in chicken.

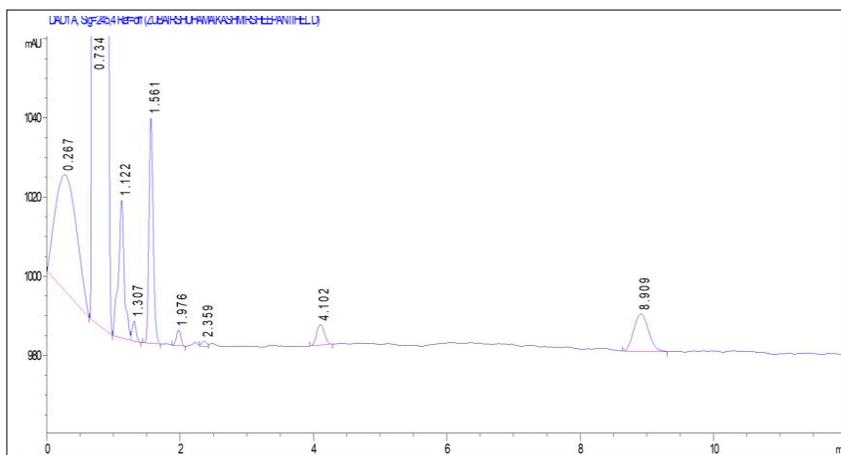


Fig 2: HPLC detection for anthelmintic (Fenbendazole) in meat of local sheep.

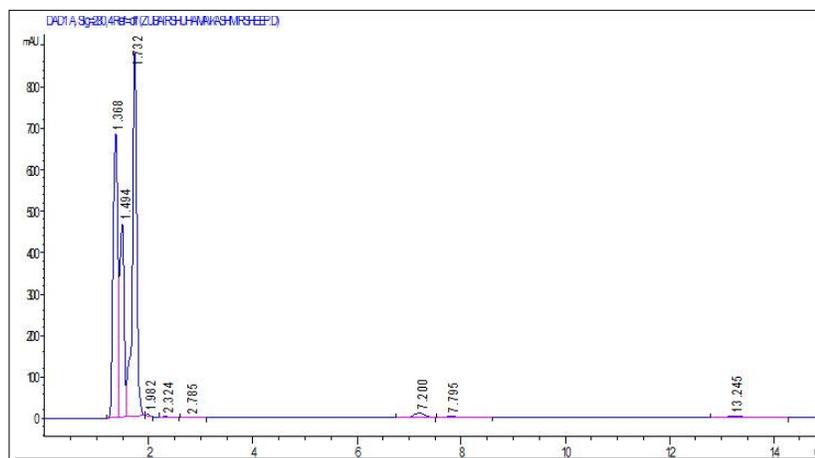


Fig 3: HPLC detection for antibiotic (Enrofloxacin) in meat of local sheep.

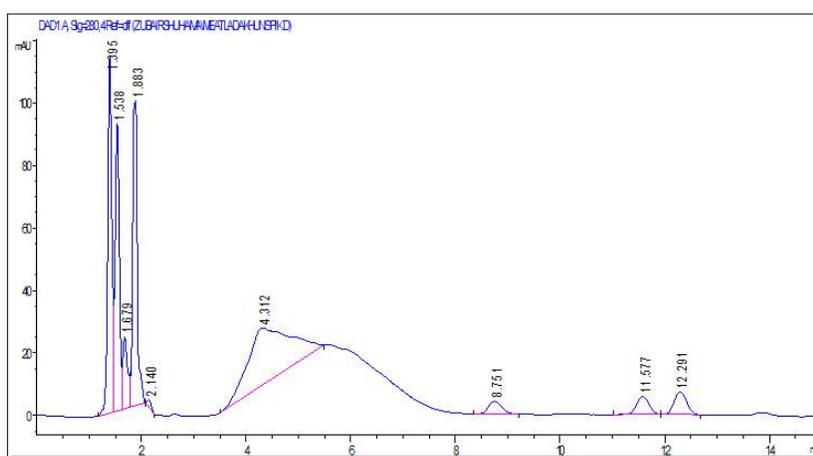


Fig 4: HPLC detection for antibiotic (Ceftriaxone) in mutton.

(c) Ceftriaxone

The study of meat samples revealed that 7% of the overall meat samples including mutton and chicken were found to contain Ceftriaxone residues. This is in agreement with the study of Wilson *et al.* (1986), Novelli *et al.* (2000) Bradley (2002) who reported that uncontrolled use of cephalosporins in animal treatment may bring antibiotic residues in foods of animal origin. A study conducted by Burmanczuk *et al.* (2018) regarding Cefoperazone a related drug in milk of dairy cows reported the time of complete wash-out of 90.90% ranges from 8.0 days to 2.2 days after administration of the drug. However ample literature is not available on the analysis of ceftriaxone in milk and meat due to its recent introduction in veterinary therapeutics.

(d) Fenbendazole

Fenbendazole belonging to benzimidazole group of anthelmintics are commonly used in veterinary industry. None of the meat samples was found to contain Ivermectin. However 2 samples of mutton amounting to 2% of overall meat samples was found to contain Fenbendazole. The determination of Fenbendazole residues in pork after treatment with medicated feed has been already studied

by Capece and Perez, 1999 who reported a recovery of above 75% in muscle tissues. Roy *et al.* (2018) studied Fenbendazole in marketed pork samples of four different north east states of India using Ultra HPLC and found 37 (5.2%) samples out of 720 samples were found to contain residues of fenbendazole. The European Medicines Agency (EMA) in 2011 published a report on the use of Panacur, a liquid suspension of fenbendazole and the study warned that repeated use of panacur may result in anthelmintic resistance. So far no detailed studies are available on the residual harmful effects of fenbendazole on humans but studies have been conducted on rats and rabbits which proved that the drug may cause oral toxicity in rats and some reproductive problems in rabbits (Inchem, 1998).

CONCLUSION

The detection and analysis of commonly used antibiotics and anthelmintics in meat and chicken samples collected randomly from market and analysis done through the use of Reverse Phase High Performance Liquid Chromatography, the selection of drugs to be analyzed was made on the basis of study of their common use under rationality parameter. The antimicrobials and anthelmintics selected

and analyzed were Oxytetracycline, Tetracycline, Enrofloxacin, Ceftriaxone, Ivermectin and Fenbendazole. In the present study 5% of the meat samples (5 samples of chicken meat) were found positive for Oxytetracycline. Enrofloxacin was detected in 1 sample of locally produced mutton and 3 samples of chicken meat amounting to 4% of the total meat samples. 7% of the overall meat samples including mutton and chicken were found to contain ceftriaxone residues. None of the meat samples was found to contain Ivermectin. However, 2 samples of mutton amounting to 2% of overall meat samples were found to contain Fenbendazole residues. None of the samples studied was found to violate MRL values as put forth by EU (2010) and Codex Alimentarius Commission (2015).

The occurrence of the studied antimicrobial and anthelmintic drug residues is comparatively lower than most of the other studies and none of the drug residues crossed the MRL values put forth by the European Union and Codex Alimentarius Commission. This may be due to the sampling errors, processing errors or observing proper withdrawal period. However more and more exhaustive studies are needed in this direction covering other drugs commonly used in veterinary practice.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

Not needed.

Conflict of interest

All authors declare that they have no conflict of interest.

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