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Antibiotics in Chicken Meat

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1. POLLUTION MONITORING LABORATORY OF CSE

The Centre for Science and Environment (CSE), a non-governmental organization based in New Delhi, has set up the Pollution Monitoring Laboratory (PML) to monitor environmental pollution. PML is an ISO 9001:2008 accredited laboratory which conducts pollution monitoring and scientific studies on environmental samples. The lab has highly qualified and experienced staff that exercise Analytical Quality Control (AQC) and meticulously follow what is called Good Laboratory Practices (GLP). It is equipped with most sophisticated state-of-the-art equipments for monitoring and analysis of air, water, soil and food contamination, including Gas Chromatograph with Mass Detector (GC-MS), Gas Chromatograph (GC) with ECD, NPD, FID and other detectors, High Performance Liquid Chromatograph (HPLC), Atomic Absorption Spectrometer (AAS), UV-VIS Spectrophotometer, Mercury Analyzer, Particulate Matter Analyzer, Ozone Monitor, etc. Its main aim is to undertake scientific studies to generate public awareness about food safety, water and air pollution. It provides scientific services at nominal cost to communities that cannot obtain scientific evidence against polluters in their area. The lab and its work are directed to use science to achieve environmentally sound and socially relevant public policy.

2. INTRODUCTION

Chicken is the most common type of poultry in the world. The term broiler is applied to chickens that have especially been bred for meat; they grow rapidly. Broiler strains are based on hybrid crosses between Cornish White, New Hampshire and White Plymouth Rock. In broiler production there are two main production phases – keeping of parent stock and production of day-old-chicken (DOC); and growing and finishing of broilers.[1]

Under intensive farming methods, a broiler chicken lives less than six weeks before slaughter. Free-range chickens are usually slaughtered at 8 weeks and organic at around 12 weeks. Chickens farmed for eggs are called egg-laying hens or layers.

According to the ICRA report of 2014, the Indian poultry sector has been growing at around 8-10 percent annually over the last decade. In 2013, with a growth rate of eight percent over 2012, the total poultry market size including layers (chickens raised for eggs) and broilers (chickens raised for meat) is estimated at Rs. 58,000 crore. Specifically, the domestic poultry meat production (broiler - carcass weight) is estimated at 3.5 million tonnes which is known to be growing at over 10 percent for several years. While the market for processed chicken is growing, over 90 percent

[1] http://www.fao.org/ag/againfo/programmes/en/lead/toolbox/indust/indpprod.htm

of domestic purchases are still through wet market due to traditional consumer preferences for getting meat dressed in front. The processed chicken market is expected to grow over 25 percent in the long-term. [2]

Accidentally, it was found that by-products of antibiotic production (dried *Sreptomyces aureofaciens* broth) which contain a high level of vitamin B_{12}, when fed to poultry animals resulted in higher growth. Eventually, it was discovered that the trace amounts of antibiotics remaining in these byproducts accounted for this growth.[3] Since then the antibiotics have been used on poultry in large quantities to enhance production in poultry.[4] However, the use of antibiotics in food animals poses a major risk for humans due to antibiotic resistance.

Antibiotic use is related to emergence of resistant bacteria in the animal which later transmits to human through food, environment and direct contact with the affected meat. Residues of antimicrobial compounds are also found in foods of animal origin as a result of inappropriate or excessive usage of these compounds. These residues are also known to transfer to humans through food and environment. To prevent any residues of antibiotics in food and food products of animal origin, withdrawal periods are set by regulatory agencies. Withdrawal period is a time between the last dose of antibiotic given to food animals and consumption of food animals or food derived from it. It needs to be mentioned on the antibiotics that are used for animals.

3. ANTIBIOTICS

3.1 What are Antibiotics?

Antibiotics are substances that can destroy or inhibit the growth of microorganisms. They are widely used in the prevention and treatment of infectious diseases. They are therapeutically used to protect the health and welfare of humans and animals. Some antibiotics are produced by micro-organisms but most of them are now manufactured synthetically.

The term antibiotic originally referred to any agent with biological activity against living organisms; however, ‘‘antibiotic” now refers to substances with antibacterial, anti-fungal, or anti-parasitical activity.

[2] http://www.icra.in/Files/ticker/SH-2014-1-ICRA-Poultry.pdf

[3] http://whqlibdoc.who.int/monograph/WHO_MONO_10_%28part2%29.pdf

[4] Chapman, H. D., and Z. B. Johnson; Use of antibiotics and roxarsone in broiler chickens in the USA: analysis for the years 1995 to 2000; *Poultry Science* 2002; 81: 356–364

3.2 Major Classes of Antibiotics

Antibiotics can be grouped by either their chemical structure or mechanism of action. They are often complex molecules which may possess different functionalities within the same molecule. Therefore, under different pH conditions antibiotics can be neutral, cationic, anionic, or zwitterionic. They are divided into different sub-groups such as Fluoroquinolones, tetracyclines, Aminoglycosides, β-lactams, macrolides, amphenicol, etc.

Fluoroquinolones have a fluorine atom attached to the central ring system, typically at the 6-position. Examples include Enrofloxacin, Ciprofloxacin, and Norfloxacin. Fluoroquinolones are a class of important synthetic antibacterials, which are active against both Gram positive and Gram negative bacteria. They also have some activity against mycobacteria, mycoplasmas, and rickettsia.[5] They can enter cells easily and therefore are often used to treat intracellular pathogens.[6] Fluoroquinolone antibiotics such as Ciprofloxacin is used to treat infectious diseases in humans such as infectious diarrhea, typhoid fever (enteric fever), lower respiratory tract infections, skin and skin structure infections, bone and joint infections, etc.[7]

Tetracyclines are a group of antibiotics with four ("tetra-") hydrocarbon rings ("-cycl-") derivation ("-ine"). They are defined as "a subclass of polyketides having an octahydrotetracene-2-carboxamide skeleton". They are collectively known as "derivatives of polycyclic naphthacene carboxamide" e.g. Oxytetracycline, Chlortetracycline, Doxycycline, and tetracycline. Doxycycline a Tetracycline antibiotic is used to treat a wide variety of bacterial infections such as respiratory tract infections due to Hemophilus influenzae, Streptococcus pneumoniae, or Mycoplasma pneumonia. It is also used for the treatment of nongonococcal urethritis. Doxycycline is used for many different types of infections, including (due to Ureaplasma), typhus cholera syphilis and acne.[8]

Aminoglycosides consist of an aminocyclitol ring connected to two or more amino sugars linked via a glycoside link. Aminoglycosides are derived from bacteria of the genus *Streptomyces* and are named with the suffix -mycin, whereas those that are derived from *Micromonospora* are named with the suffix -micin e.g. Neomycin, Kanamycin, Gentamicin, Netilmicin, etc. Neomycin

[5] Ashwini Kumar, Ashok Kumar Malik, Dhananjay Kumar Tewary, Baldev Singh; Gradient HPLC of antibiotics in urine, ground water, chicken muscle, hospital wastewater, and pharmaceutical samples using C-18 and RP-amide columns; J. Sep. Sci. 2008, 31, 294–300

[6] Lyczak, J. B., Cannon, C. L., Pier, G. B., Clin. Microbiol. Rev. 2002, 15, 194 – 222

[7] http://www.rxlist.com/cipro-drug/indications-dosage.htm

[8] http://www.rxlist.com/script/main/art.asp?articlekey=38190

an Aminoglycoside antibiotic is used to treat conjunctivitis.[9] Aminoglycosides are used in the treatment of severe infections of the abdomen and urinary tract, as well as bacteremia and endocarditis.[10]

β-Lactam antibiotics are a broad class of antibiotics that contain a β-lactam ring nucleus with a heteroatomic ring structure, consisting of three carbon atoms and one nitrogen atom e.g. penicillin, ampicillin, cloxacillin, amoxicillin. At first, β-lactam antibiotics were mainly active only against Gram-positive bacteria but the recent development of broad-spectrum β-lactam antibiotics which are active against various Gram-negative bacteria has increased their usefulness.

Antibiotics are critical in the treatment of bacterial infections. The discovery of penicillin was followed by an extraordinary progress in research related to antibiotics and their extensive use. Drastic reduction in mortality and morbidity due to infectious diseases during 1980s led to great euphoria and complacence amongst medical fraternity.[11] The result of this was misuse or inappropriate use of antibiotics with emphasis of curative medicine at the cost of disease preventive measures. Excessive use of antibiotics resulted in the emergence of bacterial resistance.[12]

3.3 Use of Antibiotics in Poultry Farming: Antibiotics are used in poultry farming as:

1. **Therapeutic Agents: For treatment of disease**. The infected animals receive a course of antibiotics, which usually involves high doses for a relatively short period of time.
2. **Prophylactic Agents: For prevention of disease.** This involves sub-therapeutic doses of antibiotics to animals via feed or drinking water, when signs and symptoms of infection are absent but suspected. Antibiotics are given periodically for several days during the life cycle of the broiler chicken.
3. **Growth Promoters: To increase growth-rate and productivity.** The use of growth promoters is characterized by administration of very low-dose of antibiotics on a regular basis, mostly over a lifetime of the food-producing animal and given through feed. This is distinguished from therapeutic and prophylactic antibiotic use, which is delivered at higher doses and generally administered through water. Antibiotic growth promoters are known to suppress the gut bacteria leaving more nutrients for chicken to be absorbed for greater

[9] http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=53c0710d-08e5-42ac-afb8-4faa13ae5833

[10] http://www.aafp.org/afp/1998/1115/p1811.html

[11] Aarti Kapil; The challenge of Antibiotics Resistance: need to Contemplate; *Indian Journal of Medical Research* 2005, 121(2): 83-91

[12] Mitchell L. Cohen; Changing patterns of infectious disease; *Nature* 2000; 406: 762-767

weight gain. Research also shows that 'benefits' from the use of growth promoters are more noticeable in sick animals or those 'housed in cramped, unhygienic conditions'.

4. ANTIMICROBIAL RESISTANCE

Antimicrobial resistance (AMR) is resistance of a microorganism to an antimicrobial medicine to which it was originally sensitive. Resistant organisms (bacteria, fungi, viruses and some parasites) are able to withstand the attack by antimicrobial medicines, such as antibiotics, antifungals, antivirals, and antimalarials. Hence, the standard treatments become ineffective, infections persist increasing risk of spread to others. The evolution of resistant strains is a natural phenomenon that happens when micro-organisms are exposed to antimicrobial drugs, and resistant traits can be exchanged between certain types of bacteria. Over use and misuse of antimicrobial medicines accelerates this natural phenomenon.[13] Poor infection control practices encourage the spread of AMR.

A vast majority of drug-resistant organisms have emerged as a result of genetic changes, acquired through mutation or transfer of genetic material during the life of the micro-organisms, and subsequent selection processes. Mutational resistance develops as a result of spontaneous mutation in a locus on the microbial chromosome that controls susceptibility to a given antimicrobial. Resistance can also develop as a result of transfer of genetic material between bacteria. The method of resistance transfer varies for specific drug/bacteria combinations.

Resistance depends on different mechanisms and more than one mechanism may operate for the same antimicrobial. Microorganisms resistant to a certain antimicrobial may also be resistant to other antimicrobials that share a mechanism of action. Such relationships, known as cross-resistance, exist mainly between agents that are closely related chemically (e.g. Neomycin-kanamycin (both Aminoglycoside), but may also exist between unrelated chemicals e.g. erythromycin (macrolide)-lincomycin (lincosamide). Micro-organisms may be resistant to several unrelated antimicrobials.

As a result of animal use of antibiotics, food borne microbes may become resistant to the antibiotics used to treat human diseases. When an animal is treated with an antimicrobial drug, a selective pressure is applied to all bacteria exposed to the drug. Bacteria that are sensitive to the antimicrobial are killed or put at a competitive disadvantage, while bacteria that have the ability to resist the antimicrobial have an advantage and are able to grow more rapidly than more

[13] http://www.who.int/mediacentre/factsheets/fs194/en/

susceptible bacteria. In addition, bacteria can become resistant when resistance genes are passed from a resistant bacterium to a sensitive one. Thus, antimicrobial agents may increase the prevalence of resistant bacteria among both target pathogens and normal bacterial flora.[14]

Foods of animal origin can act as source of food borne disease in humans and therefore, also as vehicles of resistant food borne pathogens and resistant genetic material. Resistant bacteria proliferate and can make resistant other species of bacteria that are present in animals[15]. Resistant bacteria can also transfer to humans through several routes such as direct contact with live animals and carcass at poultry farms and slaughterhouses; human consumption of meat and food with resistant bacteria; and environmental contamination of soil, water and air through animal excreta.

Based on select studies[16] conducted between 2002-13, across private and government medical colleges/hospitals in India, high levels of resistance against common antibiotics was found in several bacteria known to cause common and severe infections, e. g.:

- Resistance to Ciprofloxacin was highly prevalent in several types of pathogenic bacteria such as *E. coli, Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp., *Citrobacter* spp., *Acinetobacter* spp., *Enterococcus* spp., Methicillin resistant *S. aureus* (MRSA).
- High resistance against Doxycycline was found in *Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp., *Citrobacter* spp., and *Acinetobacter* spp.
- *Enterococcus* spp. and Methicillin resistant *S. aureus* (MRSA) were found to be resistant against Tetracycline antibiotic of class Tetracycline

[14] http://www.who.int/foodborne_disease/resistance/publications/en/index.html;

[15] http://www.thelancet.com/commissions/antibiotic-resistance-the-need-for-global-solutions

[16] biomedscidirect; NCBI; J Infect Dev Ctries; saspublisher; jpbms; scirp; japi.org; ncbi; ncbi.nlm; ijpmonline; scopemed; ijpmonline.org

5. REGULATIONS FOR ANTIBIOTICS IN CHICKEN MEAT

European Union:

Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004, lays down specific rules for the organization of official controls on products of animal origin intended for human consumption.

The use of veterinary drugs within the European Union is regulated by means of the Council Regulation (EEC) No. 2377/90 describing a procedure for the establishment of maximum residue levels (MRLs) for veterinary medicinal products in foodstuff of animal origin including meat, fish, eggs and honey. Its annexes present substances, for which MRLs have been established (Annex I); substances for which it is not considered necessary to establish MRLs (Annex II); substances with provisional, temporary MRLs (Annex III); and substances, which are not allowed to be used for food producing species (Annex IV).

While Council Directive No. 96/23/EC defines measures to monitor certain substances and residues thereof in live animals and animal products it divides veterinary drugs into two groups: group A covering prohibited substances in compliance with the Annex IV of the Council Regulation (EEC) No. 2377/90 and group B containing agents, in compliance with Annexes I and III of the Council Regulation (EEC) No. 2377/90.

Since January, 1st 2006 according to Regulation (EC) No. 1831/2003 the antibiotics cannot be used as feed additives.[17]

USA:

The Center for Veterinary Medicine's (CVM) Division of Compliance is responsible for reviewing violative residues reported to the Agency by the USDA's Food Safety and Inspection Service. The Drug Residue Compliance Team provides regulatory support and outreach to prevent illegal drug residues by reviewing inspectional evidence sent to CVM by the FDA District Offices. The evidence is reviewed for compliance with the Federal Food, Drug, and Cosmetic Act and its implementing regulations. USFDA has given specific tolerances for animal drugs in CFR 21, Part 556.[18]

In 1977, the USFDA proposed banning tetracyclines and penicillins as additives in the livestock feed which is yet to be implemented. USFDA imposed a ban on use or distribution of

[17] S. Andrée; S. Stirtzel; F. Schwägele; Federal Research Centre for Nutrition and Food; Location Kulmbach; Institute for Chemistry and Physics; E. - C. - Baumannstr. 20; 95 326 Kulmbach; Germany

[18] http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=556

Enrofloxacin for the purpose of treating bacterial infections in poultry with effect from 12th September, 2005.[19]

In 2012 and 2013, the CVM issued two policy documents known as guidance for industry (GFI) – i) GFI#209 - The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals; ii) GFI #213 - New Animal Drugs and New Animal Drug Combination Products Administered in or on Medicated Feed or Drinking Water of Food-Producing Animals: Recommendations for Drug Sponsors for Voluntarily Aligning Product Use Conditions with GFI #209. The aim was to phase-out the use of medically important antimicrobials in food animals for production purposes and to bring the therapeutic uses of such drugs under the oversight of licensed veterinarians.[20]

Health Canada:

Health Canada's Veterinary Drugs Directorate (VDD) is responsible for ensuring the safety of foods produced in Canada from food-producing animals that have been treated with veterinary drugs. To accomplish this, VDD conducts comprehensive scientific reviews of veterinary drugs before they are approved for sale in this country and also sets standards, e.g., maximum residue limits (MRLs) in the tissues and food products derived from such food producing animals[21].

Human Safety Division of VDD evaluates data on new drugs to assess any potential hazards to human health resulting from the use of veterinary pharmaceuticals in animals used for food, and conducts health risk assessments at the request of the Canadian Food Inspection Agency (CFIA). The Division establishes mandatory withdrawal periods and sets MRLs for residues of veterinary drugs in food derived from animals and develops warning statements for veterinary drug labels.

In addition, it develops policies relating to the human safety of veterinary drugs, including antimicrobial resistance, and maintains a close working relationship with CFIA on matters of food safety.[22]

[19] http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2005/ucm108467.htm

[20] http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm042450.htm

[21] http://www.hc-sc.gc.ca/dhp-mps/vet/mrl-lmr/mrl-lmr_levels-niveaux-eng.php

[22] http://www.hc-sc.gc.ca/ahc-asc/branch-dirgen/hpfb-dgpsa/vdd-dmv/divisions-eng.php

Table 1: Maximum Residue Limits of Antibiotics for Poultry Meat Set by Different Regulatory Agencies

Antibiotics	EU (ppm)	Health Canada (ppm)	Australia (ppm)	USFDA (ppm)
Class Fluoroquinolone				
Enrofloxacin + Ciprofloxacin	0.1 in muscle 0.2 in liver 0.3 in kidney			
Class Tetracycline				
Oxytetracycline	0.1 in muscle 0.3 in liver	0.2 in chicken muscle 0.6 in chicken liver 1.2 in chicken kidney	0.1 in poultry meat	2 in muscle 6 in liver 12 in kidney
Chlortetracycline	0.1 in muscle 0.3 in liver 0.6 in kidney	0.2 in chicken muscle 0.6 in chicken liver 1.2 in chicken kidney	0.1 in poultry meat	2 in muscle 6 in liver 12 in kidney
Doxycycline	0.1 in muscle 0.3 in liver 0.6 in kidney			
Class Aminoglycoside				
Neomycin	0.5 in muscle 0.5 in liver 5 in kidney	0.5 in chicken muscle, liver 10 in chicken kidney	0.5 in poultry meat 0.5 in poultry liver 10 in poultry kidney	

India:

Food Safety and Standards Authority of India (FSSAI) has been established under Food Safety and Standards Act (FSS Act), 2006 to lay down science based standards for articles of food and to regulate their manufacture, storage, distribution, sale and import to ensure availability of safe and wholesome food for human consumption. FSS Act, 2006, among others has repealed various central acts such as Prevention of Food Adulteration Act, 1954, Meat Food Products Order, 1973 to establish a single reference point for all matters relating to food safety and standards, by moving from multi level, multi departmental control to a single line of command.

FSSAI has set the tolerance limit for antibiotics and other pharmacologically active substances only for sea foods including shrimps, prawns or any other variety of fish and fishery products under the Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011.[23]

No tolerance limit has been set for antibiotics and other pharmacologically active substances in poultry meat and meat products by FSSAI.

[23] http://www.fssai.gov.in/Portals/0/Pdf/Food%20safety%20and%20standards%20%28contaminats,%20toxins%20and%20residues%29%20regulation,%202011.pdf

The ministry of Health and Family Welfare India amended the Drugs and Cosmetics Rules, 1945 in sub rule 3 of rule 97 in 2013. As per the amendment, the container of the medicine for treatment of food-producing animals shall be labelled with the withdrawal period of the drug for the species on which it is intended to be used. If the specific withdrawal period is not mentioned, it should not be less than 28 days for meat from poultry. [24]

Export Inspection Council of India (EIC), a statutory body, set up by the Indian government under section (3) of the Export (Quality Control and Inspection) Act, 1963, for implementation of the act. The Act was enacted for the sound development of the export trade of India through quality control and inspection and for matters connected therewith.

EIC has a Residue Monitoring Plan (RMP) for export to EU for fresh poultry meat and poultry meat products to ensure food safety and quality of the products for the export purpose.[25] EIC has adopted the EU council directive regulations and MRLs for different antibiotics.

There are no regulations for domestic consumption of chicken, while for exports EU standard are followed by the EIC.

6. REVIEW OF LITERATURE

Hussein and Khalil (2013) randomly collected 50 samples each of fresh and frozen broiler fillet to evaluate the antibiotic residue levels qualitatively by microbiological inhibition assay followed by quantitative estimation of Oxytetracycline and Enrofloxacin by high performance liquid chromatography (HPLC). The sum of positive samples for antibiotic residues in both fresh and frozen fillet was 21% of the total samples examined. The positive samples resulted from the microbiological inhibition assay were analyzed by HPLC for quantification of Oxytetracycline and Enrofloxacin residues. Oxytetracycline residues were found in 31.5% of fresh samples.[26] The results confirm widespread misuse of antibiotics especially Oxytetracycline in farms and lack of application of recommended withdrawal period. Enrofloxacin residues were found in two samples of fresh fillet and one sample of frozen fillet.

Gebre (2012), collected 130 samples of raw and ready to eat chicken and beef from markets in Bangkok and nearby areas to screen antibiotic residues qualitatively and to assess *Salmonella*

[24] http://www.egazette.nic.in/WriteReadData/2012/E_16_2012_176.pdf

[25] http://www.eicindia.gov.in/services/Pre-Compliance/Residue-Monitoring-Plans.aspx

[26] Hussein MA and Khalil S; Screening of Some Antibiotics and Anabolic Steroids Residues in Broiler Fillet Marketed in El-Sharkia Governorate; *Life Science Journal* 2013; 10(1): 2111-2118

contamination rate and study resistance profiles of *Salmonella* isolates from the same samples. Screening of Tetracycline, Penicillin and Sulphonamide groups of antibiotics was conducted using drug residue determining test kits. Fifty one out of 130 samples (39%) were antibiotic residue positive for at least one of the tested antibiotic groups. Tetracycline (28%) was the leading group of antibiotics found followed by Sulfonamide (23%) and Penicillin (20%).[27]

Cetinkaya et al. (2012) analyzed chicken meat samples available in Bursa and Turkey for the antibiotics of class tetracycline (Oxytetracycline, Chlortetracycline, Doxycycline and tetracycline) using LC-MS/MS technique. Doxycycline was found in four of the 60 samples in the range of 19.9 to 35.6 $\mu g\, kg^{-1}$. Tetracycline was detected in only one sample (17.2 $\mu g\, kg^{-1}$). Chlortetracycline and Oxytetracycline were not detected in any of the samples tested.[28]

Buket et al. (2013) randomly collected 127 chicken and 104 beef meat samples from markets of Ankara (Turkey) and determined quinolones using ELISA technique. Out of 231 chicken and beef samples, 118 (51.1%) were positive for quinolone residues. Of the 127 chicken meat samples tested 58 samples (45.7%) and 60 samples (57.7%) of 104 beef meat samples were positive for quinolones, respectively. The mean levels (±SE) of quinolones were found to be 30.81 ± 0.45 and 6.64 ± 1.11 $\mu g\, kg^{-1}$ in chicken and beef samples respectively.[29]

Mehtabuddin et al. (2012) randomly collected 30 samples each of breast chicken meat and egg from sale points at different locations and poultry farms of Rawalpindi/Islamabad and analyzed for sulfonamide residues. The study revealed the presence of sulfonamide residues in poultry meat because of indiscriminate use of sulfonamides in commercial broilers & layers without observing withdrawal period of this drug. Forty three percent meat samples were found to contain sulfonamide residues in the range of 0.02 to 0.8 $\mu g\, g^{-1}$ and 30% egg samples had sulfonamide residues in the range of 0.02 to 0.8 $\mu g\, mL^{-1}$.[30]

Cheong et al. (2010) estimated four common Sulfonamides (SAs), Sulfadiazine, Sulfamethazine, Sulfamethoxazole and Sulfaquinoxaline in chicken breast and liver samples using reverse phase HPLC. The concentration of SAs detected in samples from 11 states in Peninsular Malaysia

[27] Gebre BA; Qualitative screening of antibiotic residues and identification of antibiotic resistant salmonella from raw and ready to eat meat in Thailand; *International Journal of Advanced Life Sciences* 2012; 5(1): 51-64

[28] Cetinkayaa F, Yibara A, Soyutemiza GE, Okutanb B, Ozcanc A, Karacac MY; Determination of tetracycline residues in chicken meat by liquid chromatography-tandem mass spectrometry; *Food Additives & Contaminants: Part B: Surveillance* 2012; 5(1): 45-49

[29] Buket Er, Onurdağ FK, Demirhan B, Özgacar SO, Öktem AB, Abbasoğlu U; Screening of quinolone antibiotic residues in chicken meat and beef sold in the markets of Ankara, Turkey; *Poultry Science* 2013; 92: 2212-2215

[30] Mehtabuddin AA, Mian T, Ahmad S, Nadeem ZI, Tanveer, Arshad J; Sulfonamide Residues Determination in Commercial Poultry Meat And Eggs; *The Journal of Animal & Plant Sciences* 2012; 22(2): 473-478

ranged from 0.006 to 0.062 μg g^{-1} in breast meat samples and 0.08 to 0.193 μg g^{-1} in liver samples. Concentration of SAs in all the samples was lower than MRLs established by Malaysia (0.1 μg g^{-1}).[31]

7. OBJECTIVES OF THE STUDY

The main objective of this study was to analyze the chicken meats available in Delhi and National Capital Region (NCR) for antibiotic residues to highlight the need for suitable regulations.

8. MATERIALS AND METHODS

8.1 Sampling

A total of 70 chicken samples were tested in two phases from different markets of Delhi NCR region (Delhi, Noida, Ghaziabad, Gurgaon, and Faridabad). Thirty six chicken samples were from Delhi, 12 from Noida, 8 from Gurgaon and 7 each from Ghaziabad and Faridabad.

In the phase-I, a total of 50 samples of chicken were tested during September-October 2013**.** In 4 samples, muscles, kidney and liver were tested separately; for the remaining 46 only muscles were tested.

In phase-II, 20 samples of chicken were tested during May-June 2014**.** In 10 samples, muscles and liver were tested; for the remaining 10 only muscles were tested.

Samples were purchased and packed in good quality polybags, sealed and kept in dry ice immediately after purchase. Samples were transported to the laboratory under frozen condition using dry ice. Details of the samples are given in ***Table 2***.

Each chicken sample was analyzed in triplicate for 6 antibiotics of 3 major classes (Tetracycline, Fluoroquinolone and Aminoglycoside) using High Performance Liquid Chromatograph (HPLC) with Diode Array detector (DAD) and Fluorescence Detector (FLD). Methods used for the analysis were based on published methods and were validated at PML.

8.2 Equipments

- HPLC of Agilent Technologies (1260 Infinity LC System) with auto-sampler and DAD and FLD Detectors

[31] Cheong CK, Hajeb P, Jinap S, Ismail-Fitry MR; Sulfonamides determination in chicken meat products from Malaysia; *International Food Research Journal* 2010; 17: 885-892

- Post Column Derivatizer of Pickering Laboratories
- Tissue Homogenizer
- Ultra Sonicator
- Vortex Mixer
- Centrifuge of Remi Equipments
- Nitrogen Evaporator
- Water Bath
- Solid Phase Extraction Unit

8.3 Chemicals

All the solvents used (acetonitrile, methanol and hexane) were of HPLC grade. Other chemicals used for the analysis were of analytical grade and purchased from E. Merck. Water used was of HPLC grade obtained from Elga USF Maxima Ultra Pure Analytical Grade DI System.

8.4 Glassware

All the glassware was soaked overnight in 10% nitric acid and cleaned with detergent and rinsed thoroughly with distilled water and finally with solvent before use.

8.5 Standards

Standards for all the antibiotics were purchased from Sigma-Aldrich: Supelco.

- Oxytetracycline hydrochloride
- Chlortetracycline hydrochloride
- Doxycycline hyclate
- Ciprofloxacin
- Enrofloxacin
- Neomycin trisulfate hydrate

8.6 Sample Preparation and Analysis

8.6.1 Class Tetracycline - Oxytetracycline, Chlortetracycline and Doxycycline

Standard Solutions

Stock standard solutions (100 µg/mL) of Oxytetracycline, Chlortetracycline and Doxycycline were prepared in methanol and kept in the freezer (-4^0C).

Working solutions were prepared by diluting the stock solutions with a mixture of methanol: 10mmol/L TFA (1:19). The working solutions were prepared daily.

Reagents

McIlvaine Buffer: 1000 mL of 0.1M citric acid and 625 mL of 0.2M disodium hydrogen phosphate were mixed and pH adjusted to 4.0± 0.05 with NaOH or HCl as needed.

Na_2EDTA-McIlvaine Buffer (0.1M): 60.5g $Na_2EDTA.2H_2O$ was added to 1625 mL McIlvaine buffer.

Sample Extraction

Application note from Agilent technologies entitled "Determination of Tetracyclines in Chicken by Solid-Phase Extraction and High-Performance Liquid Chromatography" was used for the sample preparation. Equal weight of breast muscle and leg muscle was mixed and minced and used for analysis. In case of liver more than 5 g of liver tissue was taken and minced and the same was done in case of kidney. About 5 g of the minced sample was placed into a centrifuge tube with 20 mL 0.1 mol/L Na_2EDTA-McIlvaine buffer solution and vortexed for 2 minutes followed by a 10-minute ultrasonic extraction in an ice bath. The sample was then centrifuged at 3000 rpm for 5 minutes. The supernatant was removed and kept in a clean tube. The extraction was repeated twice with 10 mL. The combined supernatant was made up to 40 mL with buffer, mixed well, centrifuged at 4000 rpm for 10 min, and filtered.

SPE Cleanup

Agilent SampliQ OPT 3 mL, 60 mg cartridges preconditioned with 5 mL of methanol and then 5 mL of a 10 mmol/L TFA solution were used for sample cleanup. A 10 mL extract was passed through the SampliQ OPT cartridge at a speed of 1 mL/min. After the sample effused completely, the cartridge was washed with 3 mL of water (pH adjusted to 4.5 with TFA). The entire effluent was discarded. The cartridge was dried under vacuum for 3 minutes. Finally, the cartridge was eluted with 10 mL of 10 mmol/L oxalic acid in methanol. The eluent was collected and dried under nitrogen below 40^0C. The resulting residue was dissolved and made to a constant volume

of 0.5 mL using the methanol/10 mmol/L TFA solution (1/19) and filtered through a 0.45 µm filter membrane and used for analysis.

Analysis

The analysis was performed on an Agilent 1200 HPLC with DAD using the analytical column ZORBAX SB-C8 5 µm, 150 mm × 4.6 mm id from Agilent. The HPLC conditions were as follows:

HPLC Conditions

Flow rate: 1.0 mL/min

Column temperature: 30^0C

Injection volume: 100 µL

Detector wavelength: 360 nm

Mobile phase: Methanol: acetonitrile: 10 mmol/L TFA solution

Run Time: 8.0 minutes

Gradient elution:

Time (minutes)	Methanol (%)	Acetonitrile (%)	10 mmol TFA (%)
0	3	21	76
2.5	4	25	71
8.0	7	35	58

Method Performance:

Parameters	Oxytetracycline	Chlortetracycline	Doxycycline
Retention Time (minutes)	3.1	5.9	6.8
LOD (µg/kg)	2.5	5.0	5.0
LOQ (µg/kg)	5.0	10	10
Average Recovery (%)	70.4	76.3	83.7

8.6.2 Class Fluoroquinolone - Enrofloxacin and Ciprofloxacin

Standard Solutions

Stock standard solutions (100 µg/mL) of Enrofloxacin and Ciprofloxacin were prepared in methanol containing 1% acetic acid and stored at 4^0C.

Working standard solutions were prepared by diluting the stock solutions with 0.1% aqueous formic acid.

Sample Extraction

Equal weight of breast muscle and leg muscle was mixed and minced and used for analysis. In case of liver and kidney more than 2 g of respective tissue was taken separately and minced and used for extraction. About 2 g of minced sample was placed in a tissue homogenizer tube and 10 mL of 0.1% formic acid in acetonitrile was added to it, which was then homogenized in tissue homogenizer for 3 minutes. Then 1.0 g of anhydrous sodium sulfate was added to it and homogenized again for 2 minutes. The supernatant liquid was transferred to a clean centrifuge tube. The tissues were extracted once again by repeating the above steps. Then all the contents in the homogenizer tube were transferred to the same centrifuge tube in which supernatant was collected, which was then mixed well by shaking and centrifuged for 10 minutes at a speed of 5000 rpm. The acetonitrile extract was evaporated in nitrogen evaporator at 40^0C. The residue was resuspended in 2.0 mL of 10% acetonitrile containing 0.1% formic acid and defatted by extraction with 4.0 mL hexane. The mixture was centrifuged for 5 minutes at 4000 rpm. Finally the hexane layer was discarded and aqueous layer was filtered through 0.45 µm filter membrane and used for analysis.[32]

Analysis

The analysis was performed on an Agilent 1200 HPLC with FLD using the analytical column ZORBAX SB-C8 5 µm, 150 mm × 4.6 mm id from Agilent.[33] The HPLC conditions were as follows:

HPLC Conditions

Flow rate: 0.36 mL/min

Column temperature: 30^0C

Injection volume: 50 µL

Detector wavelength: Excitation – 280 nm, Emission – 450 nm

Mobile phase: 0.1% aqueous formic acid: 0.1% formic acid in ACN

Run Time: 10 minutes

[32] http://www.fssai.gov.in/Portals/0/Pdf/15Manuals/ANTIBIOTICS%20AND%20RESIDUES.pdf

[33] https://www.chem.agilent.com/Library/applications/5989-0596EN.pdf

Gradient elution:

Time (minutes)	0.1% Aqueous Formic Acid (%)	0.1% Formic Acid in ACN (%)
0	80	20
1.0	80	20
10.0	20	80

Method Performance:

Parameters	Ciprofloxacin	Enrofloxacin
Retention Time (minutes)	6.0	7.0
LOD (µg/kg)	1.0	1.0
LOQ (µg/kg)	3.0	3.0
Average Recovery (%)	97.3	86.7

8.6.3 Neomycin (Class Aminoglycoside)

Standard Solutions

Stock standard solution of 100 µg/mL as free base was prepared with water. To prepare the working solutions an appropriate volume of stock solution was transferred to 10 mL volumetric flask and then 1.0 mL of ion-pair concentrate was added to it and diluted to the mark with distilled water. All solutions were stored at 4^0C.

Reagents

Mobile Phase: The mobile phase contained 0.01M 1-pentanesulfonic acid sodium salt, 0.056M anhydrous sodium sulfate, 0.1% acetic acid, 11.5% methanol. It was sonicated in ultrasonic bath and filtered through 0.45 µm filter before use.

Post Column Derivatization Reagent: To prepare this reagent, 5.3 g boric acid was weighed and transferred to a 250 mL volumetric flask. Then about 200 mL of degassed water and 7.5 mL of 30% NaOH solution were added to it and mixed. 0.2 g *ortho*-phthalaldehyde (OPA) was dissolved in 10 mL ethanol and added to the flask. Then 0.5 mL 2-mercaptoethanol and 1.0 mL Brij-35 solution were added to the flask and mixed. Finally the volume was made up to the mark with ultra pure degassed water. This solution was filtered through 0.45 µm filter before use.

Potassium Phosphate Buffer: Potassium phosphate buffer (0.2M, pH 8.0) was prepared by dissolving 33.46 g dibasic potassium phosphate and 1.046 g monobasic potassium phosphate in water and diluting to 1000 mL with water.

Ion-pair Concentrate: A 10-fold solution of ion-pair reagent used in mobile phase was prepared to contain 0.1M 1-sodiumpentanesulfonate and 1% acetic acid and filtered through 0.45 μm filter and refrigerated until used.

Sample Extraction

Equal weight of breast muscle and leg muscle was mixed and minced and used for analysis. In case of liver more than 3 g of liver tissue was taken and minced and used for extraction, same procedure was adopted for kidney. About 3 g of minced and mixed sample was transferred to a tissue homogenizer tube containing 8 mL of phosphate buffer. The sample was homogenized for 1 minute at low speed and the contents of the tube were transferred to a centrifuge tube which was then vortexed for 3 minutes and sonicated for 10 minutes and centrifuged for 10 minutes at 4000 rpm. The supernatant was transferred to a clean tube. Eight mL of phosphate buffer was added to the tissue pellet and again vortexed for 3 minutes and sonicated for 10 minutes and centrifuged for 10 minutes at 4000 rpm. The supernatant was combined with previous supernatant and the tissue pellet was washed with 2 mL phosphate buffer and the supernatant was combined with previous supernatants.[34]

Deproteination

The tube containing tissue extracts was immersed in boiling water for 5 minutes with occasional mixing. After cooling it was centrifuged for 10 minutes at 4000 rpm and the deproteinated extract was decanted into another tube. The tissue pellet was washed with 2 mL of phosphate buffer. The wash supernatant was combined with the above deproteinated extract. The pooled sample extract was acidified to pH 3.5 – 4 with H_2SO_4 followed by centrifugation for 10 minutes at 4000 rpm. Ion-pair concentrate 100 μL was added to the filtered 1 mL aliquot of the above extract and used for analysis.

Analysis

The analysis was performed on an Agilent 1200 HPLC with FLD using the analytical column ZORBAX C18 5 μm, 250 mm × 4.6 mm id from Agilent. The HPLC conditions were as follows:

HPLC Conditions

Flow rate: 1.0 mL/min

Column temperature: 35^0C

[34] Shaikh et. al., Journal of AOAC, 1985, Vol 68(1): 29-36

Injection volume: 100 µL

Detector wavelength: Excitation – 280 nm, Emission – 450 nm

Mobile phase: 0.01M 1-pentanesulfonic acid sodium salt, 0.056M anhydrous sodium sulfate, 0.1% acetic acid, 11.5% methanol

Run Time: 25 minutes (Isocratic Elution)

Method Performance:

Parameters	Neomycin
Retention Time (minutes)	17.6
LOD (mg/kg)	0.5
LOQ (mg/kg)	1.0
Average Recovery (%)	90.3

9. RESULTS AND DISCUSSION

A total of 70 chicken samples were analyzed for the presence of antibiotics in two phases. In 14 samples, both muscles and liver were tested. In four samples, muscles, liver and kidney were tested. In the remaining 52 samples only muscles were tested. Each sample was analyzed in triplicate.

In phase-I, analysis for 6 antibiotics of 3 different classes - Oxytetracycline, Chlortetracycline and Doxycycline (class Tetracycline), Enrofloxacin and Ciprofloxacin (class Fluoroquinolones) and Neomycin (class Aminoglycoside) were carried out in HPLC-DAD/FLD. In the phase-II, samples were analyzed for only 5 antibiotics of two different classes - Oxytetracycline, Chlortetracycline and Doxycycline (class Tetracycline) and Enrofloxacin and Ciprofloxacin (class Fluoroquinolones). Samples were not analyzed for Neomycin in this phase.

The antibiotics residues detected in chicken samples were identified on the basis of comparison of the retention time with that of the reference standard peaks and the identity was confirmed by spiking the sample extract with the standard solution of known concentration. A perfect overlap of the sample peak indicated correct identification.

Out of 70 samples tested in two phases 28 samples (40%) were found to contain residues of one or more antibiotics. About 23% (16/70) chickens had residue of one antibiotic while about 17%

(12/70) had residues of more than one antibiotic. Two of the seventy chickens tested had antibiotics from two groups (Tetracyclines and Fluoroquinolones). Highest level of antibiotics 131.75 and 64.59 µg/kg of Enrofloxacin and Ciprofloxacin respectively was found in the liver of sample number 55.

Out of the total 50 samples tested in phase-I, 22 (44%) were found to contain residues of either only one antibiotic or a mix of two or three antibiotics (*Table 3*). Antibiotics of class Tetracycline were found in 9 (18%) samples while that of class Fluoroquinolone were detected in 14 (28%) samples. Oxytetracycline was detected in 8 (16%) samples while Doxycycline was found in 9 (18%) samples. Chlortetracycline was detected in only one sample. Enrofloxacin was detected in 9 (18%) samples and Ciprofloxacin was detected in 7 (14%) samples.

Four samples in which muscles, liver and kidney were tested (sample numbers 2, 16, 27 and 47), 3 samples were found positive for antibiotics. In sample number 16 both Oxytetracycline and Doxycycline were detected in muscle, liver and kidney tissues, and also Enrofloxacin was found in the liver of this sample. In sample number 27 and 47 Tetracycline antibiotics were not detected but the liver of both the samples were found to contain Fluoroquinolone antibiotics. Liver of sample number 27 had both Enrofloxacin and Ciprofloxacin while that of sample number 47 had only Enrofloxacin. None of the antibiotics tested were found in sample number 2.

Similarly in the phase-II, a total of 20 chicken samples were analyzed of which 6 (30%) were found to contain residues of either only one antibiotic or a mix of two or three antibiotics (*Table 3*). Antibiotics of class Tetracycline were found in only 1 (5%) samples while that of class Fluoroquinolone were detected in 6 (30%) samples. Enrofloxacin was detected in 5 (25%) samples while Ciprofloxacin was detected in 3 (15%) samples. Doxycycline was found in only 1 (5%) sample. Oxytetracycline and Chlortetracycline were not found in this phase.

Ten samples in which muscles and kidney were tested, 4 samples (sample numbers 53, 55, 59, and 70) were found positive for antibiotics of class Fluoroquinolones. Of these 4 samples, both liver and kidney of 3 samples (sample numbers 55, 59, and 70) were found to contain Fluoroquinolone antibiotics. Both muscle and liver of sample number 55 had Enrofloxacin and Ciprofloxacin while that of sample numbers 59 and 70 had only Enrofloxacin. Liver of sample number 53 was found to contain Enrofloxacin.

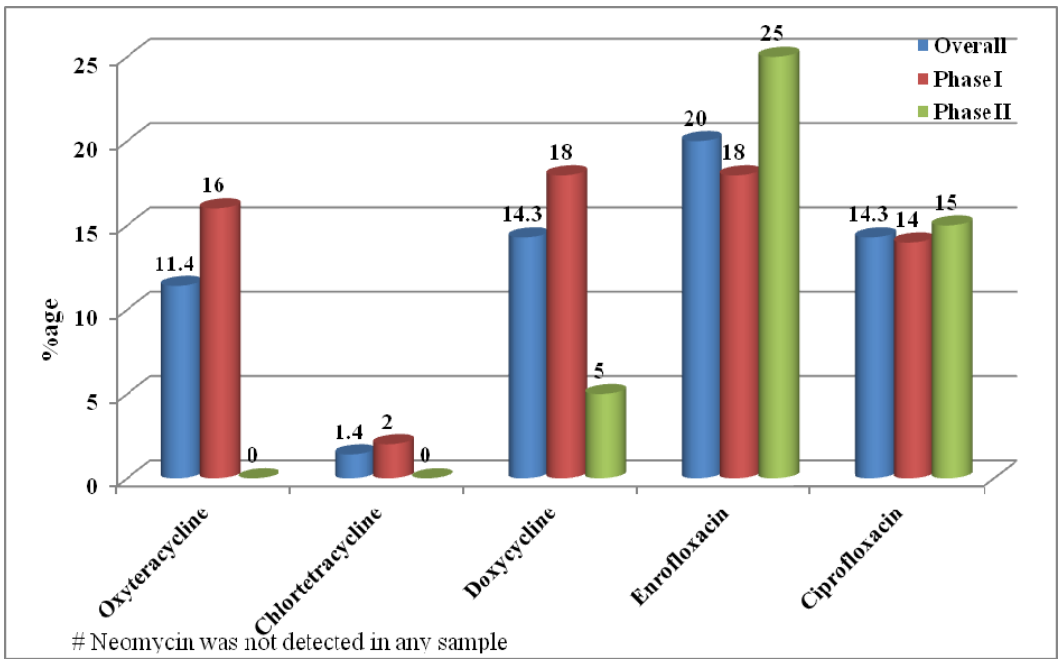

Figure 1: Percentage of Chicken Samples Detected Positive for Antibiotics Residues

Oxytetracycline:

Oxytetracycline was detected in 11.4% of the chicken samples (8/70) in the range of 8.25 to 15.16 µg/kg (*Table 3*). The highest concentration (15.16 µg/kg) was found in a sample from Gurgaon. Oxytetracycline was not detected in any of the samples tested in phase-II.

Chlortetracycline:

Chlortetracycline was found only in one sample (10.2 µg/kg) out of 70 samples tested for Chlortetracycline (*Table 3*). This sample was from Gurgaon. Chlortetracycline was not detected in any of the samples tested in phase-II.

Doxycycline:

Doxycycline was detected in 14.3% of the chicken samples (10/70) in the range of 11.94 to 20.66 µg/kg (*Table 3*). The highest concentration (20.66 µg/kg) was found in a sample from Gurgaon. Doxycycline was found in only one sample in phase-II.

Total Tetracycline:

A total of 14.3% samples (10/70) were detected with one or two or all three antibiotics of class Tetracycline tested. Seven samples had both Oxytetracycline and Doxycycline while one sample was found to contain all the three members of class Tetracycline tested.

The sum of concentrations of Oxytetracycline, Chlortetracycline and Doxycycline is referred here as total Tetracycline. Total Tetracycline was found in the range of 16.01 to 46.02 µg/kg. The highest concentration of total Tetracycline (46.02 µg/kg) was found in the sample from Gurgaon. This sample was found to contain all the three members (Oxytetracycline, Chlortetracycline and Doxycycline) of the class Tetracycline. Toxicological profile of Doxycycline is comparable to that of Oxytetracycline, Chlortetracycline and Tetracycline and has a comparable or slightly higher susceptibility to human enteric microorganisms[35].

Enrofloxacin:

Enrofloxacin, a synthetic antibacterial drug belongs to the class Fluoroquinolone. It was detected in 20% samples (14/70) in the range of 3.37 to 131.75 µg/kg (*Table 3*). The highest concentration (131.75 µg/kg) was found in the liver of chicken from Shahdara, Delhi and muscle tissue of the same chicken had 58.06 µg/kg of Enrofloxacin. In phase-II, 25% samples (5/20) were detected positive for Enrofloxacin.

Ciprofloxacin:

Ciprofloxacin, a metabolite of Enrofloxacin was detected in 14.3% samples (10/70) in the range of 3.55 to 64.59 µg/kg (*Table 3*). The highest concentration (64.59 µg/kg) was found in the liver of chicken sample from Old Delhi while its muscle tissue had 6.03 µg/kg of Ciprofloxacin. In phase-II, 15% (3/20) samples were detected positive for Ciprofloxacin.

Total Fluoroquinolone:

Residues of antibiotics of the class Fluoroquinolone were detected in 28.6% of the samples (20/70) - either one member or both the members tested. While only four samples out of 70 samples (~6%) were found to contain residues of both Enrofloxacin and Ciprofloxacin, 16 samples (~23%) contained only one, either Enrofloxacin or Ciprofloxacin (*Table 3*).

A sum of concentrations of Enrofloxacin and Ciprofloxacin is referred here as total Fluoroquinolone. Total Fluoroquinolone was found in the range of 3.37 to 196.34 µg/kg. The highest concentration of total Fluoroquinolone in muscle (64.09 µg/kg) was found in the chicken from Shahdara, Delhi while its liver had 196.34 µg/kg of total Fluoroquinolone. This sample contained both Enrofloxacin and Ciprofloxacin.

[35] http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500013941.pdf

Kidney of 4 chicken samples and liver of 14 chicken samples were also tested for Enrofloxacin and Ciprofloxacin. Of these 14 liver samples, 7 liver samples were found to contain either Enrofloxacin or both Enrofloxacin and Ciprofloxacin. None of these antibiotics residues were detected in any of the kidney samples analyzed.

Neomycin:

It was not detected in any of the samples.

10. CONCLUSIONS

Antibiotics are known to be used in poultry farming for treatment of diseases, as prophylactic agents for prevention of diseases, and as growth promoters. Residues of antibiotics have been detected in chicken meat by various investigators in several countries as also by PML.

The residues of six antibiotics – Oxytetracycline, Chlortetracycline, Doxycycline, Enrofloxacin, Ciprofloxacin and Neomycin – from three classes of antibiotic i.e. Tetracycline, Fluoroquinolone and Aminoglycoside were determined in chicken samples from Delhi NCR by HPLC.

The antibiotics were indentified on the basis of their retention times (RTs) as compared to the standards and by adding known quantities of the standards to the sample and re-chromatography which showed complete overlap of analyte peaks.

Twenty eight samples of chickens out of 70 (40%) showed the presence of antibiotic residues. Tetracyclines (Oxytetracycline, Chlortetracycline and Doxycycline) were detected in 10 samples (14.3%). Total Tetracycline (i.e. the sum of concentration of Oxytetracycline, Chlortetracycline and Doxycycline) was found in the range of 16.01 – 46.02 µg/kg. In one sample from Gurgaon all the three members of class Tetracycline tested were found to the extent of 46.02 µg/kg.

Fluoroquinolones (Enrofloxacin and Ciprofloxacin) were detected in 20 samples (28.6%) in the range 3.37 – 131.75 µg/kg. Total Fluoroquinolone (i.e. the sum of concentration of Enrofloxacin and Ciprofloxacin) was found in the range of 3.37 to 196.34 µg/kg. Three samples contained both Enrofloxacin and Ciprofloxacin. The rest of the samples contained either of them.

Misuse/overuse of antibiotics in chickens leads to development of antibiotic resistant bacteria in the chicken itself and in the farms. Further a long-term exposure of human pathogenic bacteria in chickens to even low levels of antibiotic residues may lead to development of resistance to antibiotics. No MRLs have been set for these antibiotics in India.

11. RECOMMENDATIONS

1. Reduce antibiotics use; many countries have banned its use as growth promoters, India should also do this
2. Forty percent of the chicken meat samples investigated contained one or more antibiotics which are obviously used in poultry farming. Appropriate MRLs need to be set by the regulatory body in the country and enforced.
3. Withdrawal periods should be strictly followed and enforced to make the meat safer for human consumption.
4. Poultry farmers need to be made aware to best poultry practices to prevent infection and avoid the use of antibiotics.
5. Alternatives to antibiotics in poultry feed need to be developed and used where ever possible.
6. Organic poultry farming may be encouraged by providing appropriate incentives to the farmers in form of subsidy.

Table 2: Details of Chicken Samples Purchased from Different Markets of Delhi NCR

Sample Number	Name of Shop with Address	Date & Time
01	Chicken Hut, In front of CSP Flats, East of Kailash, New Delhi - 110065	30/08/13, 11.30AM
02	Kwality Chicken (Halal), Shop No-179, INA Market, New Delhi 110023	02/09/13, 10.30AM
03	New Laziz, Hasinuddin & Saleem, Shop No-71-A, Khan Market, New Delhi 110003	02/09/13, 11.00AM
04	Green Chick, S. No-4, B-10, CSC Vasant Kunj, New Delhi 110070	02/09/13, 5.30PM
05	Mutton Mahal, M-31, M-Block Market, G. K., New Delhi 110048	02/09/13, 4.30PM
06	Venky's Xpress, F-32 GF, Sector 18, Gautam Budh Nagar, Noida (U.P.)	03/09/13, 4.30PM
07	M K Meat Shop, Shop No-46, Shakumbery Market, Near Sab Mall, Sec 27, Noida, UP	03/09/13, 3.30PM
08	Chicken Place MD1, DDA Market, Pitampura, New Delhi 110088	04/09/13, 5:15PM
09	Indian Mutton Chicken Shop 1/A, LSC-10, DDA Market, Sector-16, Rohini, New Delhi 110085	04/09/13, 3:40PM
10	Mukesh Chiken Corner, G-70, GF Vardhman Plaza-II, Rajouri Garden, New Delhi	05/09/13, 5:00PM
11	Babu Jhatka Mutton & Chiken Shop -35-36, Super Market, Ashok Nagar, New Delhi 110018	05/09/13, 3:55PM
12	Fozy Halal Mutton & Chicken Shop 43, Patparganj, Mayur Vihar, Phase - I, New Delhi - 110091	08/09/13, 5:00PM
13	Sama Chicken Shop, 62/8, Shahdera, New Delhi	08/09/13, 3:10PM
14	New Modern Bazaar Departmental Store, The Meat Shop at Spencers Retail Ltd., MGF Mega City Mall, Gurgaon Haryana	09/09/13, 3:58PM
15	AI Chicken Shop, Krishna Market, Chakarpur, Gurgaon Haryana	09/09/13, 4:30PM
16	Fancy Chicken Shop, Khushrali Gaon, Gurgaon, Haryana	09/09/13, 5:30PM
17	D. K. Chicken Shop, Sector 5, Horla Market, Noida (U.P.)	10/09/13, 2:30PM
18	Mohammad Shakir Chicken & Mutton Supplier, Y-64, Sector-12, Sabji Mandi, Noida (U.P.)	10/09/13, 2:30PM
19	Sahi Keemat, Solus Wholesale Bazaar Pvt Ltd, C-3, C-4, FFCC, Main IGNOU Road, Neb Sarai, New Delhi	11/09/13, 3:50PM
20	Delight Proteins Limited GF, 29/1, Savitri Nagar, Delhi - 110052	11/09/13, 5:01PM
21	Venky's Xpress, F-32 GF, Sector 18, Noida, Gautam Budh Nagar Noida (U.P.)	12/09/13, 2:45PM
22	Vakeel Qureshi Halali Chicken and Motton Shop-236, Ward No-8, Sarai Kherati Meat Market, Old Faridabad, Haryana	15/09/13, 4:20PM
23	Munna Kureshi Chicken Shop, Fance Colony, Railway Road, Faridabad Haryana	15/09/13, 5:00PM

24	Sahi Keemat, Solus Wholesale Bazaar Pvt Ltd, C-3, C-4, FFCC, Main IGNOU Road, Neb Sarai, New Delhi	16/09/13, 5:12PM
25	Haryana Chicken Shop, Sector 37 Faridabad Haryana	15/09/13, 3:30PM
26	Fresh Khana, Lal kuan, Chandni Chowk, Old Delhi 110006	17/9/13, 7:00PM
27	Shop No. 4081,Urdu Bazaar, Zama Maszid Old Delhi 110006	17/9/13, 6:00PM
28	Easy Day, Bharti Retail LTD. 12A, Jaipuria's Sunrise Plaza Ahinsa Khand, Ghaziabad.	18/9/13 4:00PM
29	Md. Ashraf Qureshi, Standard Halal Meat Shop, R.T.G. 128, Royal Tower Shopping Complex, Shipra Suncity, Indirapuram Ghaziabad.	18/9/13 3:00PM
30	Sartaj, Asia Chicken and Mutton Shop, Shop No. 3/150, Takia Maszid, G.T. Road, Sahibabad, Ghaziabad.	18/9/13 4:45PM
31	Delight Proteins Limited GF, 29/1, Savitri Nagar, Delhi - 110052	19/09/13, 4:15PM
32	Babu Meat Shop, No. 3 Sec-37, Devi Shai Market, Faridabad, Haryana	29/9/13,4:00PM
33	Saleem Kureshi, Fatak Road, Old Faridabad, Haryana	29/9/13,5:30PM
34	Jaipur Meat Shop, By pass Road, Sec-29, Faridabad, Haryana	29/9/13,4:40PM
35	Standard Halal Meat & Fresh Chicken Shop, H.S.-24, G.K.I, Kailash Colony Main Market, New Delhi 110048	30/9/13,2:15PM
36	New Janta Meat Shop, Sec-8, Bash Mandi, Noida (U.P.)	2/10/13,4:00PM
37	Kureshi Meat Shop, Shop No.I-25, Sec-12, Noida (U.P.)	2/10/13,4:40PM
38	Pakiza Chicken and Mutton Shop, Sec-12, 22 Sabji Mandi, Noida (U.P.)	2/10/13,5:30PM
39	Standard Chicken, Mutton and Fish, Shop No.76-77, Najafgarh Road, Rajouri Garden, New Delhi	3/10/13,5:20PM
40	Opposite MD Market, Near TV Tower Pitampura, New Delhi	3/10/13,7:00PM
41	Fish Mandi, Sikandarpur, Gurgaon, Haryana	6/10/13,4:00PM
42	U.P. Meat Shop, Rajiv Nagar, Shiv Mandir Chowk, Gurgaon Haryana	6/10/13,4:30PM
43	Chintoo Chicken Corner, Sec-12, Near Telephone Exchange, Gurgaon Haryana	6/10/13,3:00PM
44	Punjab Meat Shop, Shop-1, Samachar Market, Mayur Vihar Phase-I, New Delhi	7/10/13,4:00PM
45	Avon Meat Shop, Bholanath Marg, Shahdara, New Delhi	7/10/13,5:30PM
46	Sahil Halal Meat Shop, RZ 73/9, Kishangarh, Vasant Kunj, New Delhi	8/10/13,3:30PM
47	Mohammad Fayaz Chicken Shop, Shop No. 1, Jagat Cinema, Zama Masjid, Old Delhi	8/10/13,5:30PM
48	Chand Mohammad Chicken Shop, B-1165, Rajiv Nagar, Mohan Nagar, Ghaziabad	13/10/13,1:30PM
49	Jameel Chicken Shop, Macchi (Fish) Market, Ghantaghar, Ghaziabad	13/10/13,12:15PM
50	Meenu Meat Shop, Shop No. 211, Railway Road, Ghaziabad	13/10/13,1:15PM
51	M.K. Halal Mutton Shop, 87/1, Jamrudpur, G.K I, NewDelhi	25/05/14,11:00AM

52	Fresh N Frozen, HS-26, Kailash Colony MKT, New Delhi	25/05/14,3:00PM
53	Halal Meat Shop, Sakarpur I Block, Pitampura, New Delhi	26/05/14,2:25PM
54	New Puppu Chicken and Fish Shop, Shop No. 82, Najafgarh Road, Rajouri Garden (Subji Market), New Delhi	26/05/14,4:00PM
55	Chotu Chhatka Meat Shop, Bholanath Nagar, Tejab Mill Chowk Shahdara, New Delhi	28/05/14,2:30PM
56	Fancy Halal Meat & Chicken Shop, 270/A, Patparganj, Opp. Anand Lok Society, Mayur Vihar Phase-1, New Delhi	28/05/14,5:00PM
57	Javed Enterprises, 184 Urdu Bazaar, Zama Maszid, Old Delhi	01/06/14,3:00PM
58	Bharat Petroleum Corporation Ltd., In & Out Autocentre, D-Block, Defence Colony, New Delhi - 110024	02/06/14,2:30PM
59	Akbar Chicken and Mutton Shop, Sector-31, Noida, G.B. Nagar (U.P.)	03/06/14,2:00PM
60	Punjab Meat Shop, Shop No. 207, Jaipuria Plaza, Sector-26, Noida (U.P.) 201301	03/06/14,4:00PM
61	Qureshi's Kabab Corner, Shopping Mall, DLF-I, Gurgaon Haryana	04/06/14,3:00PM
62	Shahid Qureshi, Ambedkar Marg Colony, Chakkarpur, Gurgaon, Haryana-122002	04/06/14,5:00PM
63	Baba Meat Shop, Sector-31, Faridabad, Haryana	08/06/14, 4:00PM
64	Venky's Xpress, F-32 GF, Sector 18, Noida, Gautam Budh Nagar Noida	10/06/14,3:30PM
65	Bharat Petroleum Corporation Ltd., In & Out Autocentre, D-Block, Defence Colony, New Delhi - 110024	10/06/14,5:45PM
66	Sahi Keemat, Solus Wholesale Bazaar Pvt Ltd, C-3, C-4, FF Enclave, Main IGNOU Road, Neb Sarai, New Delhi	11/06/14,2:45PM
67	Gulzar Chicken & Mutton Shop, Shop No.21, Sector-D Pocket 4, Vasant Kunj, New Delhi-70	11/06/14,4:15PM
68	Venky's Xpress, F-32 GF, Sector 18, Noida, Gautam Budh Nagar Noida	16/06/14,4:00PM
69	Sahi Keemat, Solus Wholesale Bazaar Pvt Ltd, C-3, C-4, FF Enclave, Main IGNOU Road, Neb Sarai, New Delhi	16/06/14,7:00PM
70	Easy Day, Bharti Retail LTD, Makanpur More Mahagun Metro Mall Ghaziabad U.P.	17/06/14,7:30PM

Table 3: Antibiotics in Chicken Samples from Delhi NCR (µg/kg)

S. No.	Sample No.	Location	Oxytetracycline	Chlortetracycline	Doxycycline	Enrofloxacin	Ciprofloxacin
Phase – I (50 Muscle Tissue and 4 Liver and 4 Kidney)							
1	1	East of Kailash	ND	ND	ND	ND	**3.55**
2	2	INA	ND	ND	ND	ND	ND
3	2 (Liver)	INA (Liver)	ND	ND	ND	ND	ND
4	2 (Kidney)	INA (Kidney)	ND	ND	ND	ND	ND
5	3	Khan Market	ND	ND	ND	ND	ND
6	4	Vasant Kunj	ND	ND	ND	ND	**7.79**
7	5	GK 1	ND	ND	**20.05**	ND	ND
8	6	Noida	ND	ND	ND	ND	ND
9	7	Noida	ND	ND	ND	ND	ND
10	8	Pitampura	ND	ND	ND	**11.47**	ND
11	9	Rohini	ND	ND	ND	ND	ND
12	10	Rajauri Garden	ND	ND	ND	ND	**6.12**
13	11	Tilak Nagar	ND	ND	ND	ND	ND
14	12	Mayur Vihar	**10.64**	ND	**14.61**	ND	ND
15	13	Shahdara	**12.47**	ND	**19.69**	ND	ND
16	14	Gurgaon	**15.16**	**10.20**	**20.66**	ND	ND
17	15	Gurgaon	**13.44**	ND	**18.11**	ND	ND
18	16	Gurgaon	**11.85**	ND	**17.67**	ND	ND
19	16 (Liver)	Gurgaon (Liver)	**9.13**	ND	**11.94**	**3.37**	ND
20	16 (Kidney)	Gurgaon (Kidney)	**8.25**	ND	**15.73**	ND	ND
21	17	Noida	**12.39**	ND	**17.71**	ND	ND
22	18	Noida	**8.45**	ND	**16.00**	ND	ND
23	19	Neb Sarai	ND	ND	ND	**4.82**	ND
24	20	Savitri Nagar	ND	ND	ND	ND	ND
25	21	Noida	ND	ND	ND	ND	ND
26	22	Faridabad	ND	ND	ND	ND	ND

27	23	Faridabad	ND	ND	ND	**3.84**	ND
28	24	Neb Sarai	ND	ND	ND	ND	ND
29	25	Faridabad	ND	ND	ND	ND	ND
30	26	Old Delhi	ND	ND	ND	ND	ND
31	27	Old Delhi	ND	ND	ND	ND	ND
32	27 (Kidney)	Old Delhi	ND	ND	ND	ND	ND
33	27 (Liver)	Old Delhi	ND	ND	ND	**15.27**	**7.55**
34	28	Ghaziabad	ND	ND	ND	**13.62**	ND
35	29	Ghaziabad	ND	ND	ND	ND	ND
36	30	Ghaziabad	ND	ND	ND	**6.38**	ND
37	31	Savitri Nagar	ND	ND	ND	ND	ND
38	32	Faridabad	ND	ND	ND	ND	ND
39	33	Faridabad	ND	ND	ND	ND	ND
40	34	Faridabad	ND	ND	ND	ND	ND
41	35	GK (Kailash Colony)	ND	ND	ND	ND	ND
42	36	Noida	ND	ND	ND	ND	**3.92**
43	37	Noida	ND	ND	ND	ND	ND
44	38	Noida	ND	ND	ND	ND	**5.55**
45	39	Rajauri Garden	ND	ND	ND	ND	ND
46	40	Pitampura	ND	ND	ND	ND	ND
47	41	Gurgaon	ND	ND	ND	ND	ND
48	42	Gurgaon	ND	ND	ND	ND	ND
49	43	Gurgaon	**13.60**	ND	**15.96**	ND	ND
50	44	Mayur Vihar	ND	ND	ND	**4.44**	**3.97**
51	45	Shahdara	ND	ND	ND	ND	ND
52	46	Vasant Kunj	ND	ND	ND	ND	ND
53	47	Old Delhi	ND	ND	ND	ND	ND
54	47 (Kidney)	Old Delhi	ND	ND	ND	ND	ND
55	47 (Liver)	Old Delhi	ND	ND	ND	**4.94**	ND
56	48	Ghaziabad	ND	ND	ND	ND	ND

57	49	Ghaziabad	ND	ND	ND	ND	ND
58	50	Ghaziabad	ND	ND	ND	ND	ND
	Statistics of Phase - I		**16% (8/50)**	**2% (1/50)**	**18% (9/50)**	**18% (9/50)**	**14% (7/50)**
			18% (9/50)			**28% (14/50)**	
			44% (22/50)				
Phase – II (20 Muscle Tissue and 10 Liver)							
59	51	GK	ND	ND	ND	ND	ND
60	52	East of Kailash	ND	ND	ND	ND	**26.27**
61	53	Pitampura	ND	ND	ND	ND	ND
62	53 (Liver)	Pitampura	ND	ND	ND	**14.05**	ND
63	54	Rajauri Garden	ND	ND	ND	ND	ND
64	54 (Liver)	Rajauri Garden	ND	ND	ND	ND	ND
65	55	Shahdara	ND	ND	ND	**58.06**	**6.03**
66	55 (Liver)	Shahdara	ND	ND	ND	**131.75**	**64.59**
67	56	Mayur Vihar	ND	ND	ND	ND	ND
68	56 (Liver)	Mayur Vihar	ND	ND	ND	ND	ND
69	57	Old Delhi	ND	ND	**16.01**	**8.05**	**17.35**
70	58	Defence Colony	ND	ND	ND	ND	ND
71	59	Noida	ND	ND	ND	**5.15**	ND
72	59 (Liver)	Noida	ND	ND	ND	**9.62**	ND
73	60	Noida	ND	ND	ND	ND	ND
74	60 (Liver)	Noida	ND	ND	ND	ND	ND
75	61	Gurgaon	ND	ND	ND	ND	ND
76	61 (Liver)	Gurgaon	ND	ND	ND	ND	ND
77	62	Gurgaon	ND	ND	ND	ND	ND
78	62 (Liver)	Gurgaon	ND	ND	ND	ND	ND
79	63	Faridabad	ND	ND	ND	ND	ND
80	63 (Liver)	Faridabad	ND	ND	ND	ND	ND
81	64	Noida	ND	ND	ND	ND	ND
82	65	Defence Colony	ND	ND	ND	ND	ND

83	66	Neb Sarai	ND	ND	ND	ND	ND
84	67	Vasant Kunj	ND	ND	ND	ND	ND
85	68	Noida	ND	ND	ND	ND	ND
86	69	Neb Sarai	ND	ND	ND	ND	ND
87	70	Ghaziabad	ND	ND	ND	**4.42**	ND
88	70 (Liver)	Ghaziabad	ND	ND	ND	**11.06**	ND
	Statistics of Phase - II		**0% (0/20)**	**0% (0/20)**	**5% (1/20)**	**25% (5/20)**	**15% (3/20)**
			5% (1/20)			**30% (6/20)**	
			30% (6/20)				
	Overall Statistics		**11.4% (8/70)**	**1.4% (1/70)**	**14.3% (10/70)**	**20% (14/70)**	**14.3% (10/70)**
			14.3% (10/70)			**28.6% (20/70)**	
			40% (28/70)				

Note: Neomycin was not detected in any sample and was not analyzed in phase – II, ND – Not detected.

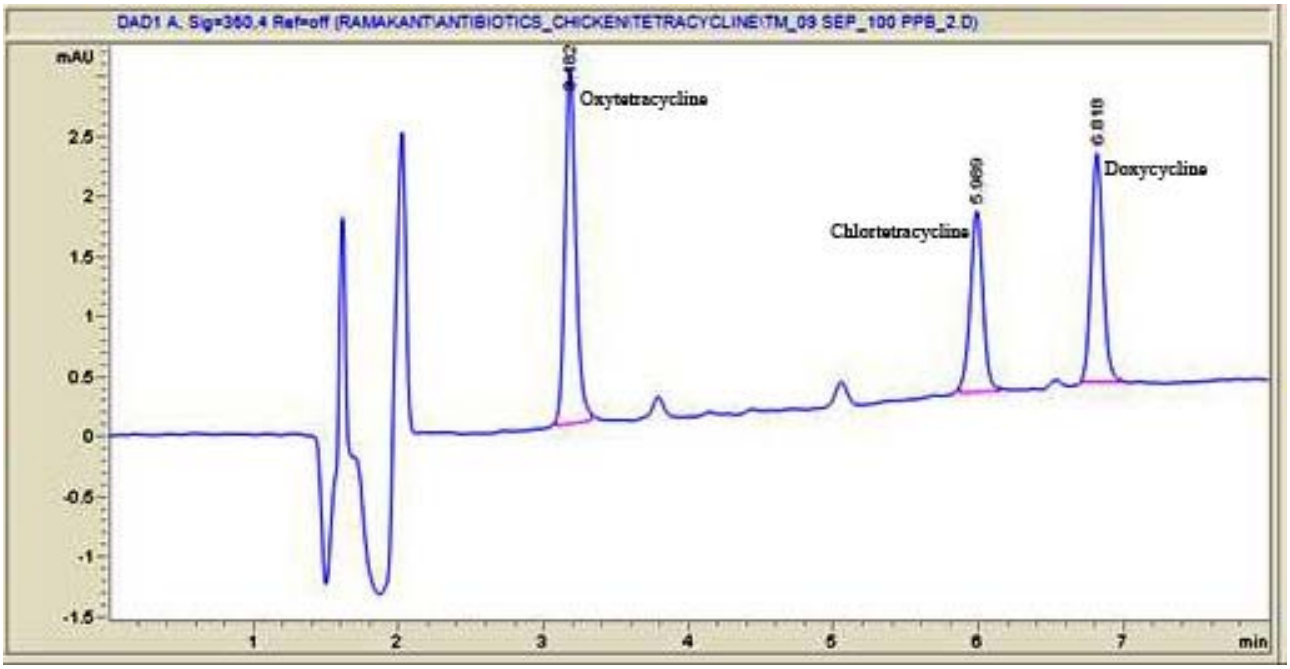

Figure 3: HPLC Chromatogram of Standards of Oxytetracycline, Chlortetracycline and Doxycycline (100 ng/mL)

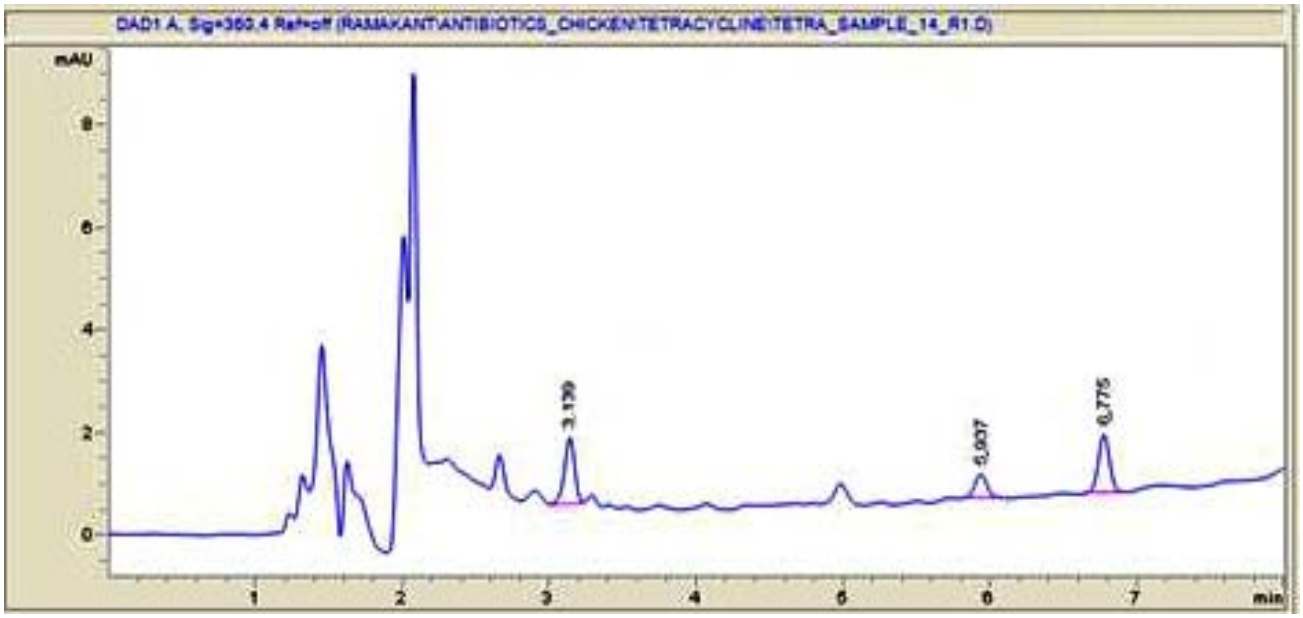

Figure 4: HPLC Chromatogram of Sample Number 14 Detected with Oxytetracycline, Chlortetracycline and Doxycycline

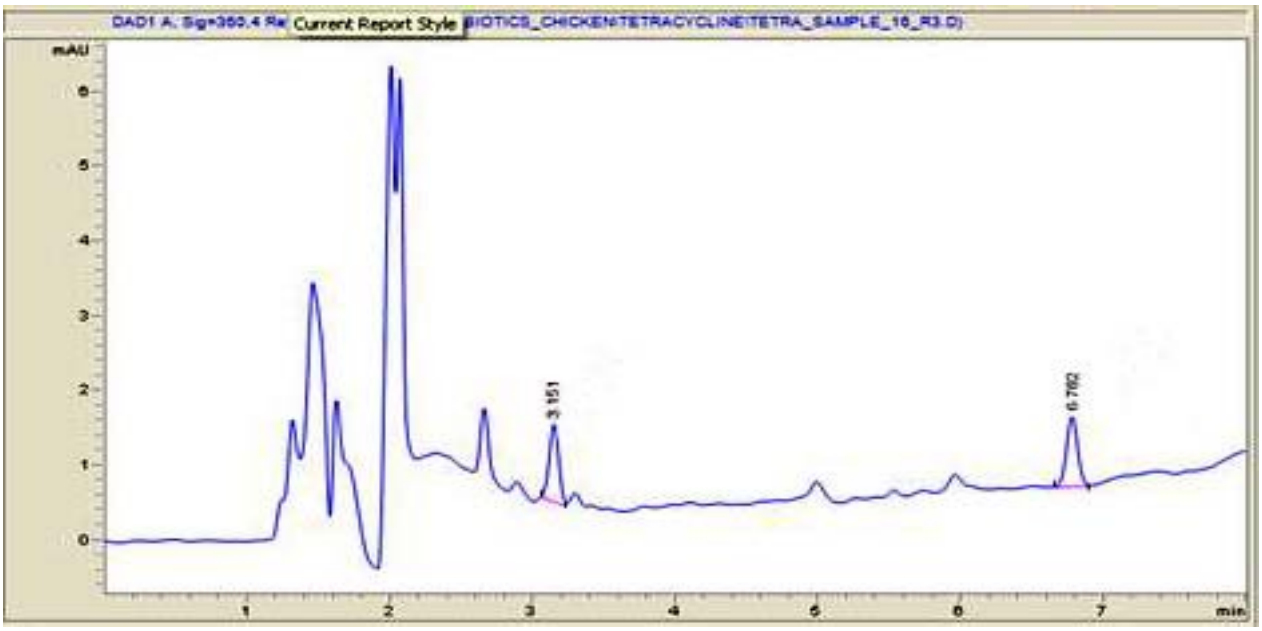

Figure 5: HPLC Chromatogram of Sample Number 10 Detected with Oxytetracycline and Doxycycline

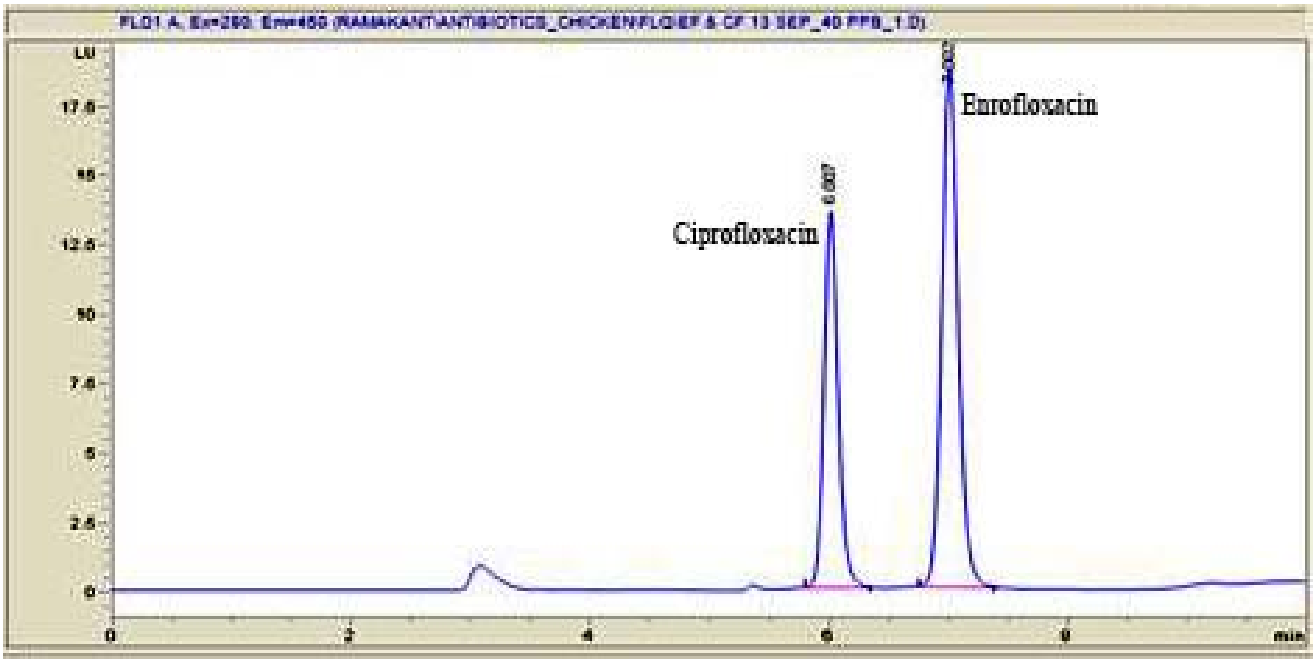

Figure 6: HPLC Chromatogram of Standards of Ciprofloxacin and Enrofloxacin (40 ng/mL)

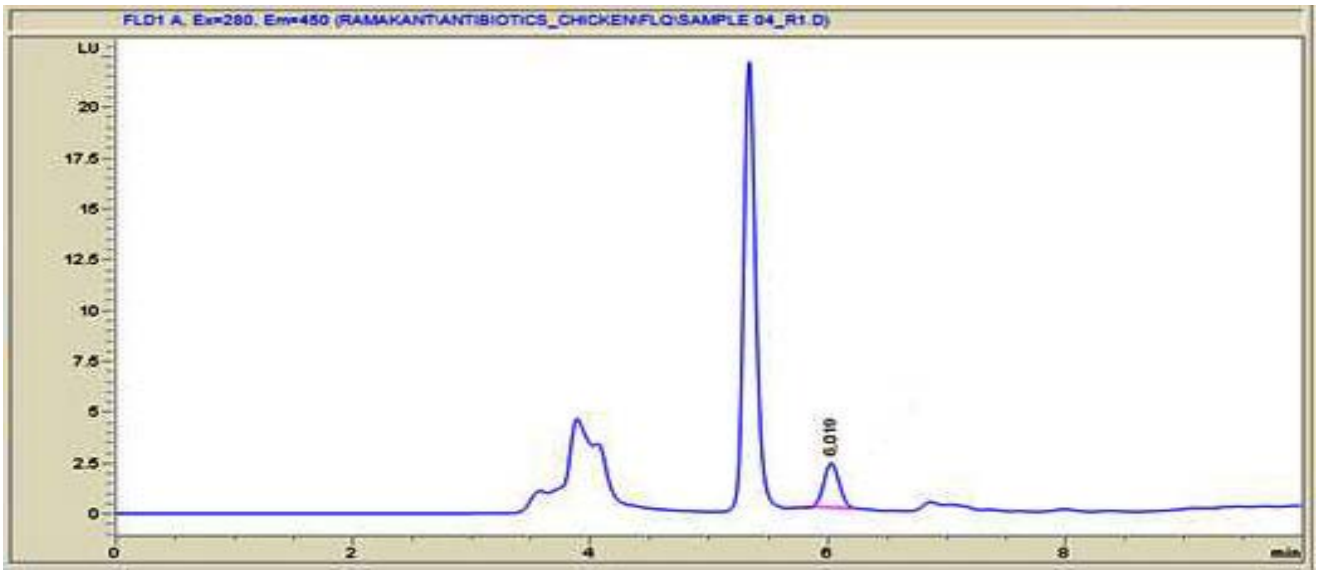

Figure 7: HPLC Chromatogram of Sample Number 4 Detected with Ciprofloxacin

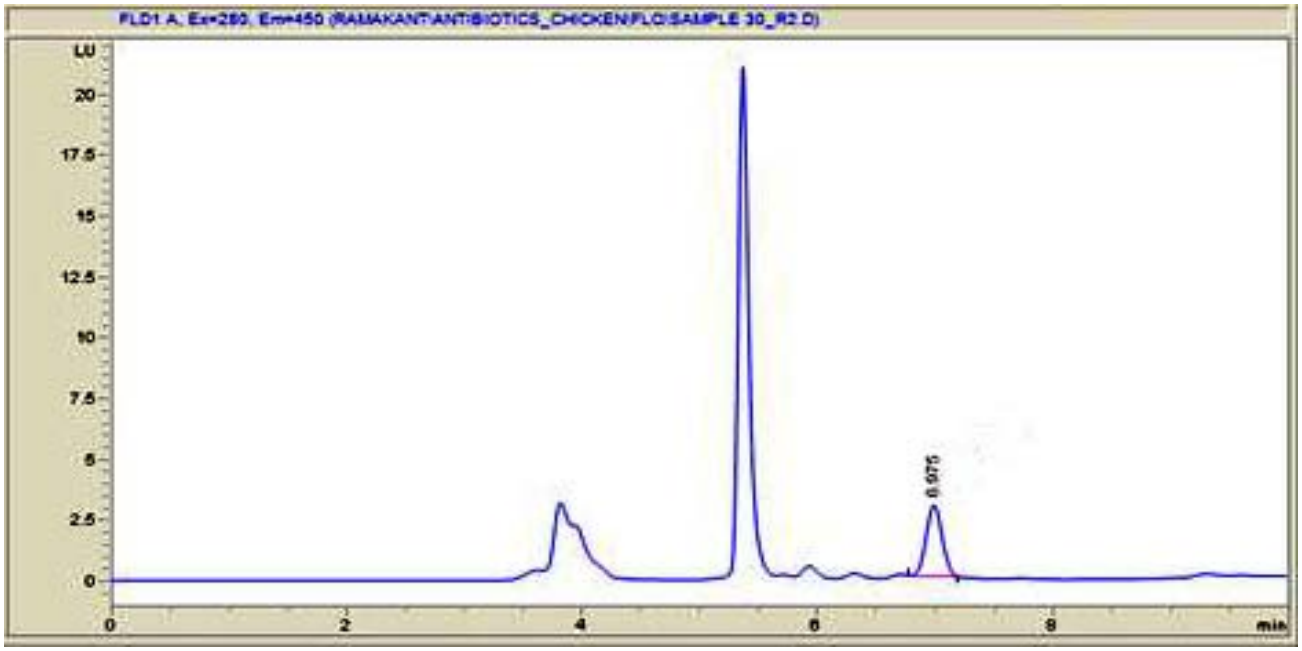

Figure 8: HPLC Chromatogram of Sample Number 30 Detected with Enrofloxacin