Review Article

DETECTION OF ANTIBIOTICS RESIDUES IN PROTEIN CONTAINING DIETS (MEAT AND EGGS) OF HUMAN THROUGH DIFFERENT METHODS

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ABSTRACT:

Bacterial and drug contaminants are the major causes of meat and egg borne illness in human. The elaborated methods described in this study, fulfil the criteria for a routine screening method, and should be a useful tool for the detection of antibacterial substances in animal feeding stuffs. Antibiotics are extremely important class of drugs, as they represent a key component in the strategy used in the control of bacterial infections in both humans and animals. It is therefore important that their use in food animals be done with utmost care; antibiotics should be given at recommended doses and with appropriate supervision. Adequate holding period should be observed in all slaughter animals following therapeutic use of antibiotics. Ideally, the use of antibiotics in food animals by non-veterinarians should be discouraged. Regulatory authorities should also ensure proper meat inspection and drug residues surveillance program should be established in the country to ensure food safety.

KEYWORDS: Drugs, Residues, Detection, Toxicity, Tolerance, Withdrawal

INTRODUCTION:

Meat obtained from animals is used as food. The word meat is normally used as the flesh of mammalian species (i.e. cattle, sheep, cow and lamb). Meat is thought to be as the by-product of dairy industry in Pakistan. It is considered as the important part in our diet as it improves nutrition. There are ten essential nutrients present in meat i.e. vitamin A, vitamin B1, vitamin B2, vitamin B6, vitamin B12, iron, zinc, niacin and mainly protein and energy. More than 37 million animals are slaughtered per year in Pakistan. All meat is raised and organized by manufacturers so that humans can consume it

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except that obtained from fish, poultry and certain other animals. Those cattle and buffaloes which are not able to do work or produce milk are usually sold and used for slaughtering purpose. But male calves are generally presented for slaughtering at early age. Animals are mostly slaughtered with unclean knifes and other tools under unhygienic conditions[1,2].

There are many reasons of contamination in meat e.g. concentrations of nutrients, high water activity, food source of mineral, vitamins and other growth factors like pH. These factors make meat favorable for the growth of microorganisms. The growth of microorganisms may lead to many abnormal changes such as off-odor, off-tastes, texture changes**,** slime formation metabolic activity and spoilage[3].

The microbial contamination of meat and poultry can occur at butcher shop during transportation, storage and handling. In developing countries like Pakistan its environment, sanitary situations, transportation and storage condition are not merely the causes of contaminations but these also boost the growth of different microorganisms, spoilage and pathogenic bacteria[4,5].

The extensive use of drugs in food animals because of their secretion in edible animal tissues can affect the human health. In edible tissues these drugs may present in trace amount usually called residues[6]. Consumers can purchase the products like meat and eggs with high quality at a reasonable cost but the edible this meat especially poultry has harmful concentration of antibiotic residues[7].

Livestock is an important sector in the economy of Pakistan. It contributes about 11.9 percent in the gross domestic products (GDP) of Pakistan. During economic year, 2012-2013 cattle production was 38.3% and buffalo, sheep and goat production was 33.7%, 28.8% and 64.9% respectively. Antibiotics/Antimicrobials are commonly used substances which are produced by living organisms and they are able to destroy and inhibit the growth and development of microorganisms. The residues in foodstuff may be due to overuse or misuse of these antimicrobial agents for treatment and prevention of diseases in food producing animals.

DETERMINATION OF ANTIMICROBIAL RESIDUES IN DIETARY MEAT BY DIFFERENT METHODS:

A. Simple Methods

a. Agar Diffusion Method:

Different methods are used to detect the antimicrobial residues in meat including agar diffusion, indirect and automated turbidimetry. To detect the penicilline G (Pen G), enrofloxacin and ciprofloxacin (EF-CF) and oxytetracycline (OTC) residues in meat three plate agar diffusion method using *Bacillus subtilis BGA* as test bacterium was used. Residues in kidney and muscles tissues were compared with special reference to MRLs (Minimal risk levels). Pencillin G residues were detected in both kidney and muscle tissues while enrofloxacin, ciprofloxacin and oxytetracycline residues were detected in only kidney tissue. So the detection through microbiological and group level identification methods are reliable and applicable[8].

b. Four-plate Test:

Antibiotic residues in meat, milk, eggs and other products can cause serious and health hazard effects in human beings. It's presence in food stuff may cause resistance of bacteria, contagiousness, allergic reactions, carcinogenic effects, damaging and changes of natural environment of intestine in humans. To determine the antibiotic residues in poultry eggs four-plate test was applied (in 60 samples of eggs) which was based on the formation of zone of inhibition around the sample in culture media having different pH and test bacteria. The result revealed that 30% cases were having antibiotic residues which may cause diseases in humans, 61.11% cases were relevant to macrolides and 22.22% cases were relevant to aminoglycosides. Due to these reasons the antibiotic residues in chicken eggs has to monitor as routine test due to side effects on human health[9].

c. Swab test:

To identify the different drug residues in food that is used for consumption like meat, milk and eggs various types of microbiological methods are used. These methods are beneficial for the

improvement of the quality of food and to manage different health related issues. One of this microbiological inhibition method is swab test on animal food (STAF) which was used for the identification of different drug residues present in animal meat. For this purpose the pure culture of test organism *Bacillus subtilis* was used. It was selected because this bacterium can detect a very large number of antimicrobial. The zone of inhibition around neomycin control disc was measured as 10-16 mm at 2x107 spores/ml concentration which was used in the formation of STAF plate. Nutrient agar having 0.4% dextrose was used as spore suspension medium. Zones of inhibition were examined. The control disc of neomycin was used and the diameter was measured and observed. The swab samples which were having the zones of 2mm in width or more were taken as positive for the presence of antibiotic residues and those swab samples which showed a zone measuring less than 2 mm or no zone of inhibition were thought to be negative. The concluding results from STAFF test showed the high presence of antimicrobial residues in animal food samples[10].

d. Tube Test:

A tube test was evaluated for the ability of *Bacillus stearothermophilus* var. *Calidolactis-C953* in detecting the residues of anticoccidal drugs. The different concentrations of drugs of sulfamethazine, furazolidone and amprolium were injected into liver and kidney tissues of chicken. To determine the drug detection limits for each drug these tissues were tested. 95% of the test results were positive. The drug concentrations were 0.35µg/ml for furazolidone, 0.70µg/ml for sulfamethazine and 7.8µg/ml for amprolium in liver tissues whereas in kidney tissues the concentrations were 0.54µg/ml for sulfamethazine, 0.30µg/ml for furazolidone and 7.6µg/ml for amprolium[11].

B. Chromatographic Analysis

a. High Performance Liquid Chromatography:

(HPLC) was used with diode array detection to identify the changes by different cooking procedures on tetracycline in chicken meat, and to determine the cooking time necessary to make the cooked sample safer for human health. Chicken meat with tetracycline was boiled, roasted and cooked on microwave for different period of time and it was analyzed by HPLC. The cooking method, food preparation period were prove to cause the loss of tetracycline residues in meat of chicken. Among all cooking procedures microwaving was more effective and among all tetracycline drugs doxycycline was the most heat tolerable, while oxytetracycline was the most heat labile. The time which was needed to destroy 90% of the initial tetracycline level was 23.9min for microwaving, 53.2min for boiling and 101.6min for roasting. It was revealed that adequate cooking and duration of time could have a considerable effect on the losses of tetracycline residues in chicken meat[12].

A research was conducted in Nigeria to detect the residues of antimicrobials in livestock. To check the residues of oxytetracycline HPLC method was used. Hydrochloric acid and acetonitrile was used for the extraction purpose and liquid- liquid partitioning with dichloromethane and petroleum ether was used for cleaning purpose. Lichrosorb RP–18 in HPLC machine was used for recognition and quantification with UV detector. 54.44% of 180 beef samples were having the residues of oxytetracyxline out of these 34.44% had oxytetracycline residues above maximum residue limits (MRLs). 51.8µg/kg, kidney is 372.7µg/kg and liver is 1197.7µg/kg were the mean residues present in muscle and standard deviation of residue was 718.9µg/kg in liver, 366.8µg/kg in kidney, and 90.53µg/kg in muscles. The finding from this study showed that people who are consuming this meat are at high risk. Therefore, regular management of this meat should be required[13]. A same type of study was conducted in Nigeria to check the antimicrobials in poultry samples. For this purpose, samples were collected from three major poultry markets at Enugu Urban area of Enugu State, South Eastern, Nigeria. Premi Test Kit was usedwhich is based on the principle of inhibition and it was able to detect antibiotic residues. Forty-two out of 70 samples from three major poultry markets were positive for having the residues. It was noticed that the concentration of residues were highest in kidney i.e 48.6%, then in Gizzard i.e. 30.1%, in

liver 25.8%, and in muscle 24.3% respectively. There was no any kind of association among the occurrence of antibiotic residues and the location of the poultry markets that were sampled at $P < 0.05$ while there was strong association between antibiotic residues and the type of organ at $P < 0.05$[14].

Cyromazine residues in chicken meat and eggs were analyzed by HPLC method using NH_2 column and 75% acetonitrile. The cleaning of samples was done by C18 Sep-Pak cartridge its treatment was done with NaOH and exraction was done with acetonitrile having20% concentrated NH_4OH. Then separation of peaks of cyromazine and melamine was done within 12 min. It was found that both compounds were having the detection limit of 0.02 ppm. At the level of 0.2-0.7 ppm the recoveries of both compounds were from 92.8-97.3 percent and 91.0-96.1. There was linear standard curve of both compounds which was better than 0.999. Only one beef sample was having cyromazine residues out of forty-six then gas chromatography-mass spectroscopy verified it[15].

In Egypt a research was conducted to determine the residues of sulphonamides. For this purpose 30 chicken samples were collected in 2001 and 34 chickens were collected in 2002. The samples were taken from the breast muscle and liver. These samples were kept in refrigerator until the time of analysis. The samples which were supposed to be positive for having the residues of sulphonamides were further analyzed through the process of High Performance Liquid Chromatography (HPLC). It was observed that 12.5% of the meat samples and 50% of the liver samples were positive for having the residues sulfonamide. Out of these 3.13 % of the meat samples and 12.5% of the liver samples were having the residues of sulphonamide above the maximum residual limit[16].

For identification of residues of sulfamonomethoxine, sulfadimethoxine, and their metabolites of hydroxy/*N*4-acetyl the samples of chicken plasma, muscle, liver and eggs were collected. The selected method to detect the residues was gradient high-performance liquid chromatography (HPLC) with a photo-diode array detector. The samples were extracted with the help of ultrasonic homogenizer by using ethanol. After homogenizing the samples centrifugation was performed. C4 column was used with a gradient elution for the separation purpose. It was observed that the samples which were spiked at 0.1–1.0 g/g or g/ml have more than 90% residues of drug with relative standard deviations within 4%. More than 30 ng/g was the detectable limits of quantitation[17].

For the detection of eight frequently used sulphonamides i.e. sulfaquinoxaline, sulfadiazine, sulfamerazine, sulfathiazole, sulfamethazine, sulfamonomethoxine, sulfadimethoxine and sulfamethoxazole, chicken meat samples were collected from the local market. The liquid mass spectrometry method was used with solid-phase extraction. The sulfonamide residues in these poultry samples were detected from a range of 66g/kg to 157 g/kg. This method of liquid mass spectrometry was greatly successful for detecting the residues sulfonamides in meat samples. There are many confirmations about the occurrence of other antibiotic residues in poultry meat[18].

Another research was conducted to detect the residues of sulphadimidine eggs after orally administration of this drug to chickens for 15 days. The concentration of sulphadimidine was high in 6th day of drug administration and this concentration decreased quickly 10th day and on the 11th day and was 0.1 mg kg-1 which is below the MRL[19]. The residues of sulphadimidine in chicken eggs were observed by the HPLC method. The residues were detected through this technique within 8 days after the last administration[20].

To investigate the residues of sulfadiazine and sulfamethazine a survey was conducted in Uganda. For this purpose 60 egg samples were collected from sixty shops. Reverse phase HPLC method with photodiode array detector was used for the analysis. Through this study it was observed that 98% of the samples were having the detectable level of sulphonamides and 98.3% of the farmers use these drugs either in feed[21].

b. HPLC with Diode Array Detection: Due to the low cost of tetracyclines these are broadly used in livestock and poultry. So it is important to wait until the withdrawal period of these

drugs so it may not be harmful for human health but in many countries these residues remain in poultry and livestock until the time of consumption. To analyze the residues of tetracyclines in pork after different cooking procedures samples of pig muscles were collected. Different cooking procedures were applied for this i.e. boiling, deep-frying and microwaving. To analyze the residues of oxytetracycline (OTC), TC, chlortetracycline (CTC) and doxycycline (DC) HPLC with -diode array detection on aXBridgeTM C18 reverse phase chromatographic column method and mixture of McIlvaine buffer-ethylenediaminetetraacetic acid (EDTA)/methanol (75:25) was used. The results of this HPLC method showed that there was 45.35 to 67.05% reduction of these drug residues in muscle samples after boiling for 9 min, 38.17 to 65.74% reduction after deep-frying for 9 min and 38.17 to 48.47% reduction after microwaving for 1 min. From these results it can be concluded that cooking method can reduce the residues of TC in pig muscle and therefore these cooking methods must be beneficial for reducing the residues in other meat[22].

c. Thin Layer Chromatography:

The method of thin layer chromatography was used for the detection of different antimicrobials oxytetracycline, neomycin, gentamycin, sulfadiazine. For this purpose, 75 samples stored chicken liver, breast and thigh muscle were used. Out of these 39 (52%) samples were having the drug residues. Sulfadiazine and oxytetracycline was present in 28% of liver and breast samples and out of thigh muscles samples 28% were having the residues of oxytetracycline and 16% were having the residues of sulfadiazine. While neomycin and gentamycin were absent in all the samples. About 28% of the samples containing oxytetracycline and 24% were having sulfadiazine. 56% of liver and breast samples were positive and 44% of thigh muscle samples were positive for these antibiotic residues[23].

d. Florisil Column Chromatography:

The total of 519 samples of three categories i.e. eggs, poultry and lamb or beef meat were collected from Jordan to detect the residues of organochlorine pesticide (OCP) including the material of dichlorodiphenyltrichloroethane, aldrin and metabolites (DDTs), dieldrin, endrin, hexachlorocyclohexane isomers (HCHs), endosulfan isomers, heptachlor, heptachlor epoxide and hexachlorobenzene (HCB). The sample cleaning was done by Florisil column chromatography followed by electron capture detector analysis. The 38 (28%) egg samples out of 134, 23 (20%) chicken samples out of 115 and 131(49%) beef and lamb samples out of 270 were organochlorine pesticide residues. Hexachlorocyclohexane isomers and metabolites (DDTs) were highly present in all samples and there were only 7% incident of heptachlor, heptachlor epoxide, HCB, aldrin and endrin compound[24].

C. Spectroscopic Analysis

a. Mass Spectrometry in Combination with Liquid Chromatography:

The technique of mass spectrometry in combination with liquid chromatography was used to check the residues of tetracyclines (TCs) in chicken in Turkey. Tetracycline recoveries were from 56.9% to 101.2%. The residues were detected from 7.9µg/kg to 14.6 µg/kg. Out of 60 samples four were positive for doxycycline which were range from 19.9 to 35.6µg/kg and one sample was positive for tetracycline which was detected at 17.2µg/kg while all samples were negative for chlortetracycline and oxytetracycline. This successful method for the confirmation of tetracyclines indicated that poultry meat in turkey contained tetracyclines residues so there should be regular analysis of residues before human consumption[25].

Antibiotics are extensively used in dairy, poultry, livestock, aquatic animals and for the production of honey in different countries around of the world[26]. To check the effect of cooking on enrofloxacin residues the chicken muscle samples were collected. Five cooking procedures were adopted i.e. microwaving, roasting, boiling, grilling and frying. LC-MS method was used which showed the residues of enrofloxacin and ciprofloxacin and limit quantification (LOQ) of quinolones i.e. 2 and 5 ng g-1 was also found in chicken muscle samples. The detected mean RSD in intra-day

at concentration of 50 ng g-1 was six percent and RSD in inter-day was twelve percent. It was concluded that from tissues sixty five to one hundred percent of the drug and its metabolite could be isolated. In roasted chicken breast at a concentration of 11 ±1,01 ng g- (n=6), the percentage of RSD 9.18%. While during boiling at 100 ºC enrofloxacin residues in chicken muscles remained stable for three hours. From this study, it was easy to estimate the drug can enteredto the people who are consuming these poultry products because heat does not affect the residues of enrofloxacin while the quinolone absorption apparently become decrease in tissues by cooking and from samples it enters in water during boiling. While there was an increase in residue level by other coking processes which have no water[27].

The residues of β-agonists (salbutamol, terbutaline, cimaterol, fenoterol, clorprenaline, ractopamine, tulobuterol, clenbuterol, and penbuterol) were detected in four farm animals muscles by the new methodofLC-MS/MS. Acetonitrile–10% sodium carbonate solution was taken for the extraction of matrix muscles and it was cleaned by acetone synthesized new polymer SPE cartridge packed. Luna C_{18} column with 0.1% fumeric acid in water and acetonitrile was used for chromatographic separation of the compounds. A positive electrospray mode was used for the mass spectrometer. Three levels of high-quality precision and accuracy were studied for most of analysts i.e. 1.0, 10, and 50 µg/kg. These nine β-agonists were detected from 0.04-18 and 0.15-0.69 µg/kg. This method gained high importance to detect nine β-agonists in pork, beef, mutton, and poultry[28].

b. Corona Discharge Ion Mobility Spectrometry:

The detection of furazolidone, chloramphenicol and enrofloxacin in chicken meat was done by using corona discharge ion mobility spectrometry technique. The extraction of drugs followed by the solid phase extraction was done using C18 sorbent. The detected quantity for all samples was less than 20 micro kg. Spiked and real samples were analyzed to check the validity of this method[29].

D. ELISA Method:

A research was conducted in Ankara, Turkey to check the residues of quinolones residues in poultry meat and beef. For this purpose 127 samples of poultry meat and 104 samples of beef meat samples were collected from different shops of city by simple random method. The residues of quinolones were checked by ELISA method. From this method it was found that 51.1% out of 231 of the poultry meat and beef samples were positive for having quinolones antibiotics. Out of which 45.7% samples of poultry meat and 57.7% samples of the beef meat were positive. 30.81 ± 0.45 µg/kg was the mean levels (±SE) of quinolones in chicken and 6.64 ± 1.11 µg/kg was the mean levels of quinolones in beef. So, some of the poultry meat and beef meat used in Ankara city were having the residues of quinolone[30].

The drugs causes economic lose because it affects the food industry. Three basic methods are used for the detection of drug residues in food these includes; microbiological methods, immuno-enzymatic methods and chemical methods. Microbiological methods are quantitative methods which are used before the qualitative methods so that the expense, chemicals and time could be saved[31]. In Bulgaria a research was conducted to check the residues of different antimicrobials in two different abattoir using chicken breast, liver and kidney. Two test organisms *Bacillus subtilis* and *Bacillus mycoides* were used in four-plate agar diffusion method. In first abattoir there were two samples of breast muscles were positive and in 2nd abattoir no sample of breast muscle were having antimicrobial residues while there was high incidence of antibiotics in kidney and liver samples. Therefore the chicken meat must be use after the withdrawal time of antimicrobials so that it could not be harmful for human health[32].

E. Combination of Methods:

To evaluate the harmful concentration of drugs in meat one hundred fresh and frozen broiler fillet samples were collected. Commonly three methods were used to identify the occurrence of antimicrobial residues in meat. These three methods are microbiological inhibition assay, high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). The measurable level of oxytetracycline residues in meat revealed the

extensive use of antibiotic (e.g. oxytetracycline) in farms[33]. Sulfonamides are most frequently used antibiotics in poultry. These antibiotics can be effortlessly taken in and can easily spread through the body of the chicken[34]. So the random collection of 30 samples of poultry eggs and 30 meat samples was done to identify the accumulation level of sulphonamides drugs in poultry. For this purpose liquid-liquid extraction procedure was used with acetonitrile and n-hexane for extraction of sulphonamides and for recognition of sulphonamide residues high performance liquid chromatography (HPLC) was performed. 43% meat and 30% egg samples were having a detectable level of sulfonamide residues that is unfit for human because of high residual levels[33].

Most of the veterinarians use synthetic antibiotics sulfonamides (SAs) for treatment and growth in poultry. The chicken breast and liver samples were used for the detection of four commonly used SAs i.e. Sulfadiazine (SDZ), Sulfamethoxazole (SMX), Sulfamethazine (SMZ) and Sulfaquinoxaline (SQX) in poultry of 11 states of Peninsular Malaysia. The technique of reverse phase HPLC with ultraviolet detector was used. The result indicated that sulfonamides concentrations in chicken breast samples were 0.006 to 0.062 µg/g and in liver samples these concentrations were from 0.08 to 0.193 µg/g. the sulfonamides concentrations were less than MRL in all samples. The people of Malaysia were exposing to sulfonamides in ranged from 0.002-0.088 µg/kg body wt/day. The people of Johor were highly exposed to sulfonamides contains high level of of Sulfamethoxazole (SMX)[36].

Antibiotic residues in food derived from animals may cause adverse health effect for the consumer. Various screening methods were developed for antimicrobial residues in edible animals. An easy and quick screening technique using bioassay analysis for drug residues (penicillins, cephalosporins, macrolides, tetracyclines, quinolones, etc.) in meat was used[37]. The mixture was made by dissolving 5 g sample with 5 mL of methanol. This homogeneous mixture was then centrifuged at 3,000 rpm for 10 min. The pulp disk method was developed for this assay by using test organisms (i.e. *Bacillus subtilis BGA* (Antibiotic Medium 5 (pH 8) and 8 (pH 6)), *Micrococcus luteus* ATCC 9341 and *Geobacillusstearothermophilus*). The drugs like penicillin G, ampicillin, cefapirin, cefalexin, erythromycin, spiramycin, oxytetracycline, chlortetracycline, streptomycin, dihydrostreptomycin, enrofloxacin and oxolinic acid were detected at levels between 0.005 to 2.5 mg/g in meat. Therefore this screening process could be used routinely to determine antibacterial residues in livestock products[38].

Various antimicrobials are being used for the treatment, control and nutritive reasons in veterinary animals. If these antimicrobial remain in meat that is consumed by human being it causes allergies and various health hazardous reactions by causing resistance toward various pathogenic bacteria[39]. Eighty-seven samples of three kinds of meat (meat meals, meat and bone meals) were collected from nine different plants in Germany for the detection of tetracycline residues. For this purpose four different analytical methods i.e. fluorescence test, HPLC using succinate buffer extraction procedure, HPLC using hydrochloric acid extraction procedure and HPLC using hydrochloric acid after sedimentation of bone partials were used. From this study it was found that 100% samples were having the tetracycline residues. The highest concentration of oxytetracycline in meat meals was 2048 µg.kg(-1), the concentration of tetracyclines was 1393 micro.g.kg(-1) and the concentration of chlortetracycline was 608 µg/kg(-1). The oxytetracyclin, tetracycline and chlortetracycline concentrations in meat and bone meals were 2295 µg/kg(-1), 848 µg/kg(-1) and 1274 µg/kg(-1) respectively. From this study it was concluded some concentration of tetracycline are expected to be present in that in field samples (Korner *et al.,* 2001). A research was also conducted in Africa to check the residues of antimicrobial in food. Through this study it was found that tetracyclines are the most abundant antimicrobial use in this country i.e. 41%. The residues of β-lactams were 18%[40].

Three examinations were done i.e. to check the residues of antimicrobials, to check the presence of *Salmonella* and to study the resistance of *Salmonella* raw and ready to

consume poultry and beef meat samples were used in Bangkok and nearby areas. To check the residues of Tetracycline, Penicillin and Sulphonamide groups of antibiotics a qualitative method was applied by using drug residue determining test kit. A culture method ISO 6579 (2002) was adopted to detect the *Salmonella* in samples. Kirby-Bauer antibiotic discs diffusion method was used to check the antibiotic resistance by using different antibiotics. It was found that out of collected 130 samples 51 were positive for having the at least antibiotics residues in each group. The most abundantly found antibiotic among all was tetracycline. It was found in 28% samples. The sulfonamide residues were present in 23% samples and Penicillin residues were present in 20% samples. The *Salmonella* was present in 9% of the collected poultry meat samples and 5% was present in beef samples. Same result was found between raw and ready to eat meat samples. Out of these isolated *Salmonella* 83% were resistant to two antibiotics and 67 % were resistant to multiple antibiotics and most isolated *Salmonella* were resistant to β-lactamase (Ampicillin, Amoxicillin) and were susceptible to Gentamicin and Kanamycin[41].

Very less people have knowledge of the bad impacts of the drug residues present in food. These residues can cause carcinogenicity and mutagenicity[42]. Five hundred samples of beef and mutton were collected to analyze the antibiotic residues. It was found that 22.8% of the beef samples and 14% of the mutton samples were having the residues of antibiotics. So by consuming these highly contaminated meat products peoples are at high risk of having the drug residues. So these meat products must be available in market after the withdrawal time of these drug residues[24].

A research was conducted to check the residues of different drugs in poultry meat. In this purpose 10g of poultry meat was crushed and then it was dipped into 10ml ethanol. Then it was centrifuge for evaporation of solvent. This sample was loaded and run into the silica F256 plates for observing of chromatograms on UV light. Form this method it was observed that more than 50% were positive for antibiotic residues[43].

As antibiotics in chicken meat can enter in consumers body so in order to save the people of U.S. the U.S. federal government employs many qualitative methods to detect the drug residues in edible tissues to make sure that concentrations remain in poultry products do not go beyond the tolerance level. So an experiment was performed to analyze fluoroquinolone residues by collecting different breast samples of poultry meat. For this purpose four different groups of 160 chickens were made. The chickens having age of 33 days were dosed with the fluoroquinolone antibiotic, those which were having age of 3 days were dosed with enrofloxacin (Baytril) at 25 ppm, enrofloxacin at 50 ppm was used for chickens having age of 3 days and enrofloxacin at 50 ppm was used for the chickens having age of 7 days. During dosing and withdrawal period, the breast samples from each chicken (5 birds per day per group) were collected for analysis. These breast samples were divided into 4 segments (upper left, upper right, lower left, and lower right) and each segment was analyzed for fluoroquinolone concentration. It was found that there was not any considerable distinction ($P > 0.05$) during the dosing or withdrawal periods between breast sections in the amount of enrofloxacin residues. So it was concluded that to evaluate the residues of fluoroquinolone samples can be taken from any part of breast[44].

To check the residues of gentamcin in eggs 32 hens were divided into four groups and each group were having 8 hens. They were administrated with 2 or 4 mg/kg gentamicin intramuscularly or subcutaneous continuously for 3 days. The residues of gentamicine were detected one by one from albumen, yolk and whole egg by HPLC. It was found yolk is highly dosed with gentamicin as compared to albumen because of the absorption and storage of drug in pre-ovulatory yolks during the dosing period. In 12-15 days hens were injected with 2 and 4 mg/kg gentamicin for constantly for 3 days and then the whole eggs were analyzed. The amount of gentamicin residues was 0.01µg/g[45].

CONCLUSION:

There are varieties of analytical methods available to determine antibiotic residues in human proteinous diet like eggs and meat. This

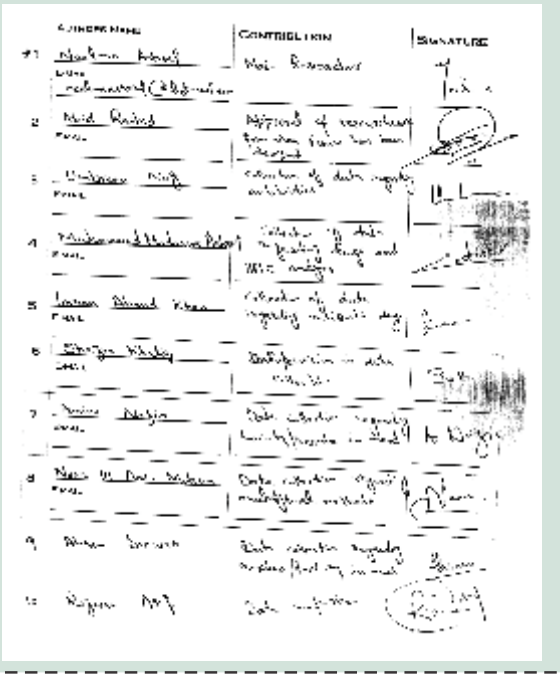
review study showed that antibiotic residues are present in eggs and different meats like beef, mutton and poultry. This food contamination due to antibiotics may result into development of antibiotic resistance and different side effects even toxicities. Although a range of methods are available to detect these antibiotic residues in food stuff but HPLC method is the best and most commonly used technique for the purpose. For healthy and safe human life, meats, eggs and other diets should only be available in market or provided to final consumers after completion of with drawl period of antibiotics administered in animals.

REFERENCES:

1. Aftab M, Rahman A, Qureshi MS, Akhter S, Sadique U, Sajid A and Zaman S. Level of *Salmonella* in beef of slaughtered cattle at Peshawar. J Ani Plant Sci 2012; 22: 24-27.
2. Sun XD and Holley RA. Antimicrobial and antioxidative strategies to reduce pathogens and extend the shelf life of fresh red meats. Comprehensive Rev Food Sci Food Safety 2012; 11: 340-351.
3. Adu-Gyamfi A, Torgby-Tetteh W and Appiah V. Microbiological quality of chicken sold in accra and determination of D10-Value of *E.coli*. Food Nutr Sci 2012; 3: 693-698.
4. Soomro AH,Khaskheli M, Bhutto MB, Shah G, Memon A and Dewani P. Prevalence and antimicrobial resistance of *Salmonella* serovarsisolated from poultry meat in Hyderabad, Pakistan. Turk J Vet Anim Sci 2010; 34: 455-460.
5. Ahmad MUD, Sarwar A, Najeeb MI, Nawaz M, Anjum AA, Ali MA and Mansur N. Assessment of microbial load of raw meat at abattoirs and retail outlets. J Ani & Plant Sci 2013; 23: 745-748.
6. Seri HI. Introduction to veterinary drug residues: Hazards and Risks. J Sci Tech 2013; 1: 1-7.
7. Hussein MA and Khalil S. Screening of Some Antibiotics and anabolic steroids residues in broiler fillet marketed in el-sharkia governorate. Life Sci J 2013; 10: 2111-2118.
8. Myllyniemi AL. Development of microbiological methods for the detection and identification of antimicrobial residues in meat. Acad Dissert 2004; 1-87.
9. Hakimzadegan M, Khosroshahi AK and Nasab SH. Monitoring of antibiotic residue in chicken eggs in Tabriz city by FPT. Int J Adv Biom Res 2014; 2: 132-140.
10. Jabbar A and Rehman SU. Microbiological evaluation of antibiotic residues in meat, milk and eggs. J Micro Biotech Food Sci 2013;2: 2349-2354.
11. Shitandi A, Oketch A and Mahungu S. Evaluation of a Bacillus stearothermophilus tube test as a screening tool for anticoccidial residues in poultry. J Vet Sci 2006; 7: 177-180.
12. Abouraya SH, Shalaby AR, Salama NA, Emam WH and Mehaya FM. Effect of ordinary cooking procedures on tetracycline residues in chicken meat. J Food Drug Analys 2012; 21: 80-86.
13. Olufemi OI and AgboolaEA.Oxytetracycline Residues in edible tissues of cattle slaughtered in Akure, Nigeria. Int J Food Saf 2009; 11: 62-66.
14. Ezenduka EV, Ike OS and Anaelom NJ. Rapid detection of antimicrobial residues in poultry: A consequence of non-prudent use of antimicrobials. Health 2004; 6: 149-152.
15. Chou SS, Hwang DF and Lee HF. High performance liquid chromatographic determination of cyromazine and its derivative melamine in poultry meats and eggs. J Food Drug Anal 2003; 11: 290-295.
16. Salem DA. Monitoring of some antimicrobial residues in chicken from assiut, Egypt. EnvirEncyclopaedia Ass Uni 2004.
17. Kishida K and Furusawa N. Simultaneous determination of sulfamonomethoxine, sulfadimethoxine, and their hydroxy/*N*4-acetyl metabolites with gradient liquid chromatography in chicken plasma, tissues, and eggs. Talanta 2005; 67: 54-58.
18. Lu KH, Chen CY and Lee MR. Trace determination of sulfonamides residues in meat with a combination of solid-phase microextraction and liquid chromatography–mass spectrometry. Talanta 2007; 72: 1082-1087.
19. Ivona, K, Mate D, Hussein K, Katarína R,

Marcincak S and Pavlína J. High performance liquid chromatographic determination of sulfadimdine residues in eggs. Acta Veterinaria 2004; 54: 427-435.
20. Hussein K, Marcincak S, Mate D, Kozarova I, Sokol J and Zdolec N. Use of Premi® test for the detection of sulphonamide residues in chicken eggs. Acta Veterinaria 2005; 55: 493-500.
21. Sasanya JJ, Okeng JWO, Ejobi F and Muganwa M. Use of sulfonamides in layers in Kampala district, Uganda and sulfonamide residues in commercial eggs. Afr Health Sci 2005; 5: 33-39.
22. Nguyen V, Li M, Khan MA, Li C and Zhou GH. Effect of cooking methods on tetracycline residues in pig meat. Afr J Pharma Pharmaco 2013; 7: 1448-1454.
23. Shareef AM, Jamel ZT and Yonis KM. Detection of antibiotic residues in stored poultry products. Iraqi J Vet Sci 2009; 23: 45-48.
24. Ahmad R, Salem NM and Estaitieh H. Occurrence of organochlorine pesticide residues in eggs, chicken and meat in Jordan. Chemosphere 2010; 78: 667-671.
25. Cetinkaya F, Yibar A, Soyutemiz GE, Okutan B, Ozcan A and KaracaMY. Determination of tetracycline residues in chicken meat by liquid chromatography-tandem mass spectrometry. Taylor Francis 2012; 23b :1-5.
26. Babapour A, Azami L and Fartashmehr J. Overview of antibiotic residues in beef and mutton in Ardebil, North West of Iran. World App Sci J 2012; 19: 1417-1422.
27. Lolo M, Pedreira S, Miranda JM, Vazquez BI, Franco CM , Cepeda A and Fente C. The effect of cooking on enrofloxacin residues 1 in chicken tissues. Food AdditivContam 2006; 10: 988-993.
28. WangL, Zeng Z, Wang X, Yang J, Chen Z and He L. Multiresidue analysis of nine β-agonists in animal muscles by LC-MS/MS based on a new polymer cartridge for sample cleanup. J Separa Sci 2013;6: 1843-1852.
29. Jafari MT, Khayamian T, Shaer V and Zarei N. Determination of veterinary drug residues in chicken meat using corona discharge ion mobility spectrometry. Anal Chim Acta 2007; 581: 147-53.
30. ErB, Onurdag FK, Demirhan B, Ozgacar SO, Oktem AB and Abbasoglu U. Screening of quinolone antibiotic residues in chicken meat and beef sold in the markets of Ankara, Turkey. PoulSci 2013; 92: 2212-2215.
31. Babapour A, Azami L and Fartashmehr J. Overview of Antibiotic Residues in Beef and Mutton in Ardebil, North West of Iran. World App Sci J 2012; 19: 1417-1422.
32. Pavlov A, Lashev L, Vachin I and Rusev V. Residues of antimicrobial drugs in chicken meat and offals. Trak J Sci 2008; 6: 23-25.
33. Hussein MA and Khalil S. Screening of some antibiotics and anabolic steroids residues in broiler fillet marketed in El-Sharkia Governorate. Life Sci J 2013; 10: 2111-2118.
34. Weiss C, Conte A, Milandri C, Scortichini G, Semprini P, Usberti R and Migliorati G. Veterinary drugs residue monitoring in Italian poultry: Current strategies and possible developments. Food Control 2007; 18: 1068-1076.
35. Mehtabuddin, Mian AA, Ahmad T, Nadeem S, Tanveer ZI and Arshad J. Sulfonamide residues determination in commercial poultry meat and eggs. J Anim Plant Sci 2012; 22: 473-478.
36. Cheong CK, Hajeb P, Jinap S and Ismail-Fitry MR. Sulfonamides determination in chicken meat products from Malaysia. Int Food Res J 2010; 17: 885-892.
37. Hakem A, Titouche Y, Houali K, Yabrir B, Malki O, Chenouf N, Yahiaoui S, Labiad M, Ghenim H, Bounar SK, Chirila F, Lapusan A and Fit NI. Screening of antibiotics residues in poultry meat by microbiological methods. Bulletin Uni Agri Sci Vet Med 2013; 70: 1843-5270.
38. Horie M, Kobayashi H, Ishii R, Ibe A, Fujita K, TannoKand Nakazawa H. Simple and rapid microbiological method for determination of residual antibacterials in meat. Pubmed 2008; 49: 168-176.
39. BabapourA,Azami L and Fartashmehr J. Overview of antibiotic residues in beef and mutton in Ardebil, North West of Iran. World App Sci J 2012; 19: 1417-1422.
40. Darwish WS, Eladaly EA, Abbasy MTE,

Ikenaka Y, Nakayama S and Ishizuka M. Antibiotic residues in food: the African scenario. Jap J Vet Res 2013; 61: 13-22.
41. Gebre BA. Qualitative screening of antibiotic residues and identification of antibiotic resistant *Salmonella* from raw and ready to eat meat in Thailand. Int J Adv Lif Sci 2012; 5: 51-64.
42. Doyle ME. Veterinary Drug residues in processed meats -potential health risk. FRI Briefings 2006; 5: 1-11.
43. Tajick MA and Shohreh B. Detection of antibiotics residue in chicken meat using TLC. Int J PoulSci 2006; 5: 611-612.
44. Herrera R and Donoghue DJ. Antibiotic residues distribute uniformly in broiler chicken breast muscle tissue. J Food Prot 2008; 71: 223-5.
45. Alm-El-Dein AK and Elhearon ER. Antibiotic residue in eggs of laying hens following injection with Gentamicin. New York Sci J 2010; 3: 135-140.



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After Revision

Failures are often the results of timidity and fears; disappointments are the results of bashfulness; hours of leisure pass away like summer-clouds, therefore, do not waste opportunity of doing good

Hazrat Ali (Karmulha Wajhay)