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Monitoring of Sulfamethoxazole and Sulfadiazine in Chicken Meat in Lahore City, Pakistan

M. Y. TIPU¹, K. MUHAMMAD², M. ABBAS^{1,*}, A. M. KHAN¹ and S. ALI¹

¹Quality Operations Laboratory, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan

²Department of Microbiology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan

*Corresponding author: E-mail: hafizmateen2002@yahoo.com

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Residues of sulfa drugs (sulfamethoxazole and sulfadiazine) were determined in the samples of chicken meat collected from four towns of city Lahore, Pakistan. Total 116 samples were collected during a period of 5 months (June through October 2009) and were processed for monitoring sulfamethoxazole and sulfadiazine through high performance liquid chromatography (HPLC). Each sample was analyzed separately for monitoring each of the sulfa drugs using UV detector of HPLC. Twenty seven percent meat samples (31/116) contained sulfamethoxazole and 10 % (12/116) contained sulfadiazine levels exceeding the tolerance levels for residues of animal drugs (0.1 ppm). The mean value of sulfamethoxazole was 1.11 ranged 0.01-3.91 $\mu\text{g/mL}$ whereas mean value of sulfadiazine was 0.54 ranged 0.01-1.06 $\mu\text{g/mL}$. Residues of sulfa drugs in the chicken meat samples is an enormous health risk factor for end consumers. There is need to improve regulatory conditions of marketable poultry meat in the city that will mitigate the sulfa drug residues in chicken meat available for general public.

Key Words: Sulfamethoxazole, Sulfadiazine, HPLC, UV detector, Chicken meat.

In food animals, administration of drugs requires not only consideration of effects on the animal but also the effects on humans who ingest food from these animals. In short, after food-producing animals have been exposed to drugs in order to cure or prevent disease or to promote growth, the effects of the residues of such treatment may have on humans should be known. These residues consist of the parent compound or compounds derived from the parent drug (or both) including metabolites and residues bound to macromolecules¹. Concern has been expressed about possible harmful effects on humans through the use of drugs, such as: (1) increased microbial drug resistance, (2) drug residues in food, (3) allergic reactions and sensitization to antimicrobials and (4) drug toxicity². In Pakistan, according to an estimate, poultry industry contributes 707,000 tons of meat³ and 11,258,000 eggs for human consumption⁴. These poultry birds are frequently raised in conditions where there is high level of stress and where different types of antibiotics are used. This might result in drug residues in chicken's meat.

The study was designed to monitor the residues of sulfa drugs *i.e.*, sulfamethoxazole and sulfadiazine in broiler meat available for end consumers in the market.

Sources of meat sample: Broiler chickens are reared in broiler farm houses in suburban and rural areas of Lahore and supplied to various poultry shops of the city for sale. One hundred and sixteen samples of poultry meat (250 g each) were purchased randomly from the market of Gunjbukhsh Town, Ravi Town, Shalamar Town and Gulberg Town during

June 2009 to October 2009. The samples (29 samples from each town) were collected once a year. The meat samples in polythene bags were transported in ice-packed cooler to the laboratory where samples were stored at -20 °C until analyzed for Sulfa drugs.

Phosphate buffer saline (MP Bio), Dichloromethane (MP Bio-HPLC grade) and Acetonitrile (MP Bio-HPLC grade) were used for sulfa drug analysis. Standard sulfadiazine and sulfamethoxazole were purchased from (Sigma Aldrich). Standard solutions of 10 $\mu\text{g/mL}$ were prepared separately for each of sulfadiazine and sulfamethoxazole in distilled water. This solution was stable for up to 1 month when stored at 4 °C. The water used for analysis was double distilled with Millipore water purification system (Bedford, MA, USA). The equipment used in the sample preparation was Centrifuge (Hettich® USA), tissue homogenizer (Ika® USA), filter assembly (Lichroult® USA), HPLC (Agilent® USA), Water purification system (Millipore® Bedford, MA, USA).

Extraction procedure: All the samples were processed for extraction, evaporation, dilution and quantitative detection of sulfa drugs by HPLC using the method as described by Furusawa and Hanabusa⁵. One g of tissue sample was homogenized with 4 mL of phosphate buffered saline (PBS) solution using tissue homogenizer. After homogenization, 8 mL of dichloromethane was added to it and mixed on vortex mixture for 3 min. The content was subjected to centrifugation at 4000 rpm for 20 min and the lower layer was removed with the help

of micropipette kept in a clean test tube. Three mL of dichloromethane was again added to the sample, mixed by vortex for 3 min and again centrifuged at same rpm for same time and lower layer was taken out by micropipette and added to the test tube.

The test tube was placed in a thermostatic bath for evaporation at 50 °C, into a fume gas cabinet. A 500 µL aliquot of mobile phase was added to the tubes to re-suspend the extract. It was filtered through a 0.45 µm nylon membrane and filtrate was aspirated by HPLC automatic sampler at a rate of 1.0 mL per minute for analysis.

Analytical condition: HPLC analysis was carried out on Agilent 1100 system equipped with degasser, quaternary pump, auto-sampler, auto-injector and diode array detector. Separation was achieved on a C₁₈ (250 mm × 4.6 mm, 5 µm, Merck®, Germany). The mobile phase was a mixture of acetonitrile/0.05M NaH₂PO₄, pH 2.5 (33/65 v/v) with a flow rate of 1 mL/min. Both drugs were detected at 254 nm. Flow rate of mobile phase was 1.0 mL/min and column temperature was maintained at 30 °C. An aliquot of 20 µL was injected into HPLC system. The results regarding sulfa drugs levels in chicken meat were statistically analyzed by mean ± SD⁶.

Sulfonamides are very stable molecules, chemically characterized by the *p*-aminobenzene-sulfonamide group. The drugs are antimicrobial compounds commonly used in the veterinary treatment against gram-positive and gram-negative bacterial infections such as coccidiosis, diphtherias, bacterial enteritis, choleric infections, typhoid, bacterial pneumonias, necrotic dermatitis and respiratory infections. The drugs are also effective against some protozoa infections⁷. Since 1990, the European Council has issued regulations establishing maximum residue limits (MRLs) of veterinary medicinal products in foodstuffs of animal origin to limit diet intake of veterinary drug residues. These regulations fix MRL of 100 µg/kg in animal tissues for all sulfonamide drugs and for all animal food⁸. Drug withdrawal time is the time required for drug residue to reach a safe concentration for human or animal consumption, defined as maximum residue limit (MRL)⁹.

Sulfa drugs are routinely used for control of bacterial diseases in chickens. Usage of sulfa drugs (sulfadiazine and sulfamethoxazole) and antibiotics in poultry feed industry is increasing day by day in countries like Pakistan. The antibacterial drugs given in feed to chicken attain a level of more than 1 µg/mL in plasma 24 h after offering medicated feed; levels in tissues are less than that in plasma and occurred 2-3 days later. It is clear that antibacterial agents achieved high tissue penetrating ability⁷. The antibiotics are rapidly absorbed from the gastrointestinal tract of chicken^{10,11}. However, factors such as the physicochemical properties of the drug, presence of bivalent ions in the gut and nutritional sources may affect absorption from the digestive tract of chicken^{12,13}. Some studies reported lower tissue levels¹⁴, comparable levels¹⁵ or higher levels¹⁶ of residues in chicken. These differences may be due to difference in route of drug administration or method of drug detection¹⁷. This parameter is generally based on data derived from healthy animals¹⁸ and established on the basis of drug residue levels in various tissues, *e.g.*, kidney or muscle¹⁹.

In present study, 29 samples were randomly purchased from different poultry meat shops in each of four towns of Lahore city (Tables 1 and 2). Sulfamethoxazole was present in 28, 23, 17 and 22 % of chicken meat samples collected from

Gunjbukhsh Town, Raiv Town, Shalamar Town and Gulberg Town, respectively. Similarly, 8, 10, 7 and 5 % of samples from Gunjbukhsh Town, Raiv Town, Shalamar Town and Gulberg Town, respectively were containing sulfadiazine residues.

TABLE-1
CONCENTRATION (µg/mL) OF REPORTABLE
SULFADIAZINE IN CHICKEN MEAT (n = 29)

Town	Maximum	Minimum	Mean	± SD
Gunjbukhsh	0.67	0.03	0.24	0.37
Ravi	1.06	0.09	0.41	0.57
Shalamar	0.55	0.02	0.20	0.30
Gulberg	0.21	0.01	0.14	0.11

TABLE-2
CONCENTRATION (µg/mL) OF REPORTABLE
SULFAMETHOXAZOLE IN CHICKEN MEAT (n = 29)

Town	Maximum	Minimum	Mean	± SD
Gunjbukhsh	2.54	0.06	0.52	0.86
Ravi	3.13	0.04	1.08	1.26
Shalamar	3.91	0.01	1.53	1.64
Gulberg	0.72	0.02	0.21	0.23

It is concluded from the results that withdrawal time required to reach minimum tissue residual level of the sulfa drugs is not being observed generally in poultry farming. Resultantly, the end consumers are exposed to undesired residues of sulfamethoxazole and sulfadiazine in chicken meat in areas under study of Lahore.

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