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Detection and Presumptive Identification of Antibiotic Residues in Poultry Meat by Using FPT

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Abstract: A total of 100 chicken samples (breast and thigh), 50 fresh carcass samples, 25 local frozen samples and 25 imported frozen samples were analyzed and the antibiotic residues were estimated by using Four Plate Test (FPT). Results revealed that 90 %, 88 % and 72 % of fresh, local frozen and imported frozen samples were positive for residues of different antibiotics, respectively. Samples were positive for presence of tetracyclines, sulphonamides, aminoglycosides and quinolones, while were negative for presence of beta-lactam and macrolides. Presence of antibiotic residues in chicken carcasses poses a health risk to consumers such as antibiotic resistance, teratogenicity, carcinogenicity, hepatic and renal failure. Veterinary control of withdrawal times in poultry farms and post-mortem inspection of slaughtered carcasses for antibiotic residues can reduce the incidence of antibiotic residues and improve the quality of chicken meat.

Key words: Antibiotic · Residues · Chicken · Meat · FPT

INTRODUCTION

Antibiotics are used by the poultry industry and poultry veterinarians to enhance growth, feed efficiency and reduce diseases. Antibiotic usage has facilitated the production of poultry, allowing the consumer to purchase, at a reasonable cost, high quality meat and eggs. Antibiotic usage has also enhanced the health and wellbeing of poultry by reducing the incidence of diseases. Although these uses benefit all involved, unfortunately, consumer perception for that edible poultry tissues are contaminated with harmful concentrations of drug residues [1]. Antibiotics used in food animals can affect the public health because of their secretion in edible animal tissues in trace amounts usually called residues such as oxytetracycline and enrofloxacin which have been found above the maximum residue level in chicken tissues [2, 3]. Some drugs have the potential to produce toxic reactions in consumers directly such as clenbutarol. Other types of drugs are able to produce allergic or hypersensitivity reactions such as β-lactam antibiotics can cause cutaneous eruptions, dermatitis, gastro-intestinal symptoms and anaphylaxis at very low doses, such drugs include the penicillin and cephalosporin groups of antibiotics [4]. Indirect and long term hazards include microbiological effects, carcinogenicity, reproductive effects and teratogenicity. Microbiological effects are one of the major health hazards in human beings. Antibiotic residues consumed along with edible tissues like milk, meat and eggs can produce resistance in bacterial populations in the consumers, this is one of the major reasons of therapeutic failures amongst such people. Certain drugs like 3-nitrofurans and nitroimidazoles can cause cancer in human population [5, 6, 7]. Several antibiotic families are used in veterinary medicine such as β-lactam, Tetracyclines, Chloramphenicols, Aminoglycosides, Nitrofuranes, Nitroimidazoles, Macrolides, Quinolones and Macrocyclics [8].

To assure food quality and to protect human health, many regulations have been put which regulates the use of veterinary medicines with establishment of maximum

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residue level (MRL) values for veterinary medicinal products in food stuffs of animal origin and regulates the residue monitoring of pharmacologically active compounds in those products[9, 10]. Many sensitive and more specific methods were optimized and validated for detection and determination of different antibiotic residues in animal meat and poultry such as High Performance Liquid Chromatography (HPLC), Liquid Chromatography (LC), Liquid Chromatography-Mass Spectrometry (LC-MS), Thin Layer Chromatography (TLC), ELISA, Four Plate Test (FPT). These analytical methods were used to determine the contamination of the food samples with the antibiotic residues qualitatively and quantitatively [11- 18].

Microbiological assays are qualitative or semi-qualitative methods, based on a specific reaction between a susceptible organism (generally bacteria) and the antibiotic present in the sample. Some advantages of these assays are their reliability, cost effectiveness and simplicity. Moreover, a large number are commercially available [19, 20]. Another important advantage, compared to the Liquid Chromatography-Mass Spectrometry (LC-MS) system, is that microbiological tests can detect any antibiotic or metabolite with antibacterial activity, whereas LC-MS systems are commonly applied to compound previously selected as targets, so that any other antibiotics present would pass undetected. However, the most important drawbacks of the microbiological tests are their lack of specificity and the long incubation times required in some cases [21].

In this study, we investigate the broiler meat for the presence of antibiotic residues and to compare these residues between different types of broiler meat and between different sites inside the broiler carcass by using microbiological assay method.

MATERIALS AND METHODS

Samples: A total of 100 chicken meat samples (Breast and Thigh); 50 fresh chickens, 25 local frozen chickens and 25 imported frozen chickens were purchased from shops and supermarkets. Samples were collected in sterile polyethylene bags, put in ice tank under low temperature and transported to the laboratory for examination.

Analysis: Samples were analyzed by using microbiological assay method: Four Plate Test (FPT) [22]. FPT was intended to detect residues of β-lactam, tetracyclines, sulphonamides, aminoglycosides and macrolides in chicken meat. A fifth plate, with low detection limits of quinolones and flouroquinolones, was used with three antibiotics belonging to this group [23, 24]. Antibiotic standards for tested antibiotics were purchased from Sigma (Bayer, Germany) and Pharmacia and Upjohn (Puurs, Belgium).

Bacterial Strains and Media: *Bacillus subtlis* spore suspension No. 10649 is a ready-to- use suspension (Merck, Darmstadt, Germany), *Micrococcus luteus* suspension No. ATCC9341, *Escherichia coli* suspension, a freeze-dried strain of *E.coli* ATCC11303 was reconstituted (Merck, Darmstadt, Germany). Media used are Test agar pH 6 (Merck, dehydrated medium 10663), Test agar pH 7.2 (Merck, dehydrated medium 15787), Test agar pH 8 (Merck, dehydrated medium 10664). Five different inoculated media were used for antibiotic detection as follow: medium I, test agar pH 6, seeded with *Bacillus subtlis,* medium II, test agar pH 7.2, seeded with *Bacillus subtlis,* medium III, test agar pH 8, seeded with *Bacillus subtlis,* medium IV, test agar pH 6, seeded with *Micrococcus luteus,* medium V, test agar pH 6, seeded with *Escherichia coli*.

RESULTS

Results revealed that 90%, 88% and 72% of fresh, local frozen and imported frozen chicken samples were positive for the presence of antibiotic residues, respectively (Table 1). It was noticed that breast samples were contaminated with antibiotic residues more than thigh samples and higher percentages of positive samples were found in breast samples (Table 2). Higher percentages of positive samples in fresh chicken were for sulphonamides, tetracyclines and quinolones (52%, 50% and 48%), while lower percentage was for aminoglycosides (16%). In local frozen chicken, higher percentages were for tetracyclines, sulphonamides and quinolones (56%, 44% and 44%), while lower percentage was for aminoglycosides (16%). In imported frozen chicken, higher percentages were for sulphonamides and tetracyclines (64% and 48%) while lower percentages were for quinolones and aminoglycosides (16% and 12%). All samples were negative for β-lactam and macrolides groups (Table 3).

Table 1: Incidence of positive and negative samples of antibiotic residues in broiler meat.

		Positive Samples		Negative Samples	
Samples	No.	No.	%	No.	%
Fresh carcasses	50	45	90	5	10
Local frozen	25	22	88	3	12
Imported frozen	25	18	72	8	28

Table 2: Incidence of positive samples for antibiotic residues in different parts of broiler carcass in different five media.

			Medium 1		Medium 2		Medium 3		Medium 4		Medium 5	
		No.	No.	%	No.	%	No.	%	No.	%	No.	%
Fresh carcasses	Breast	50	25	50	26	52	0	0	8	16	24	48
	Thigh	50	16	32	10	20	0	0	3	6	24	48
Local frozen	Breast	25	14	56	11	44	0	0	4	16	11	44
	Thigh	25	7	28	4	16	0	0	1	4	11	44
Imported frozen	Breast	25	12	48	16	64	0	0	3	12	4	16
	Thigh	25	10	40	14	56	0	0	3	12	3	12

Table 3: Occurrence rate of different antibiotic residues in broiler meat.

		Tetra[1]		BL[2]		Sulph[3]		AG[4]		Macr[5]		Q[6]	
Samples	No.	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Fresh carcasses	50	25	50	0	0	26	52	8	16	0	0	24	48
Local frozen	25	14	56	0	0	11	44	4	16	0	0	11	44
Imported frozen	25	12	48	0	0	16	64	3	12	0	0	4	16

1.Tetracyclines 2. Beta-Lactamase 3. Sulphonamides 4. Aminoglycosides 5.Macrolides 6. Quinolones

DISCUSSION

Chicken meat were tested for the presence of different antibiotic residues; β-lactam (penicillin G and ampicillin), tetracyclines (tetracycline, chlorocycline, oxycycline and doxycycline), sulphonamides (sulphadimidin), aminoglycosides (streptomycin), macrolides (erythromycin and tylosin) and quinolones (flumequin, ciprofloxacin and enrofloxacin) by using four plate test (FPT) method and results revealed that samples were positive for tetracyclines, sulphonamides, aminoglycosides and for quinolones groups, all samples were negative for β-lactam and macrolides groups. Higher percentages of positive samples for antibiotic residues were observed in most samples (88% and 72% of fresh, local frozen and imported frozen chicken samples, respectively) and this can be attributed to failure to observe the pre-slaughter withdrawal period by the farmers [25, 26], antibiotics achieved high tissue penetrating ability [27] and rapidly absorbed from the gastrointestinal tract of chicken [28]. 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 [29, 30]. Presence of tetracyclines, sulphonamides and quinolones residues were more than that of aminoglycosides, these results agreed with other studies which reported that tetracyclines are the most predominantly antibiotic residues (41%) and this is can be attributed to that tetracyclines are highly prescribed antibiotic for treatment and as feed additive [31] and lower than other findings which reported 36% for tetracyclines, 29% for penicillin and 21% for sulphonamide in raw chicken [32]. Other results revealed that 28% of breast muscle was positive for sulphonamides and oxytetracycline while 28% of thigh muscle was positive for oxytetracycline and 16% of samples were positive for sulphonamides. Oxytetracycline was the most predomominant antibiotic detected (28%), among the studied antibiotics and followed by sulphonamides (24%). Breast muscle had the highest percentage of antibiotic detected (56%), followed by for thigh muscle (44%)[33].

It was noted that 43% poultry meat samples had detectable levels of sulphonamide residues whereas 23% poultry meat samples exceeded recommended residual level and were unfit for human consumption [34]. The high percentage of sulphonamides detected may be largely due to their using in poultry sector for reducing mortalities due to fowl typhoid, fowl cholera and coccidiosis[35].

Antibiotic residues of 21% in Ghana [36], 44% in Nigeria [37] and 70% in Tanzania [38] is reported from various meat sources. Our findings were similar those obtained in Iran, who found that more than 50% of poultry meat samples had noticeable antibiotic residues [39]. Higher percentage (69%) was reported through examining 33 broiler farms, with 87% and 100% positivists for oxytetracycline in muscle and liver respectively [40]. Higher percentage (96%) was recorded in Canada during 1991-1992 through examination 961 poultry samples [41]. About 45.7% of chicken meat samples were positive for quinolone antibiotic residue [42]. Direct Quantification of tetracycline-resistant genes in poultry meat revealed that tetA gene was found in 40% of chicken meat, whereas tetB gene was found in 45% of chicken meat and these findings were associated with high counts of tetracycline-resistant bacteria in these samples [43].The residue of tylosin was less frequently found in poultry meat [44].

In some studies which used four plate tests to study antibiotic residues in chicken meat (breast muscle), liver and kidney found that 4% from meat samples were positive for antibiotic residues, while liver (17%) and kidney (33%) samples were higher than those from meat and these results showed that chicken meat producers not always keep in mind withdrawal periods of the veterinary products used in their farms [45]. Administration of ampicillin, oxytetracycline or sulphadimidin antibiotics to chickens in broiler rations resulted in an immediate increase in concentrations of antibiotics in plasma and tissues from day 1 until day 40 of the treatment. Withdrawal of medicated feed resulted in a rapid decline in tissue concentration parallel to that of plasma [46]. Penicillin, chloramphenicol and oxytetracycline were the antibiotics detected in the chicken meat examined. About 35.8% of the birds examined were found to have one or more of the antibiotics in their meat. The establishment of the maximum acceptable level of drugs in tissue is of central importance to consumer safety [47]. Strict regulations for the use of antibiotics in the poultry industry and the monitoring of drug residues in chicken meat prior to marketing are needed [48].

CONCLUSION

High percentage of chicken samples were contains residues of different antibiotics. Human as a non-target organism of this drugs receives different amounts of them as a residue which can pose a great health hazards for consumers such as allergic reactions, bacterial resistance, teratogenicity and carcinogenicity. Control of using veterinary drugs for treatment or as a feed additives and good observation of withdrawal times after antibiotic treatment can decrease the incidence of antibiotic residues in poultry meat. Also, routine monitoring of produced chicken for the presence of such residues was important for consumer safety.

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