



Detection of Antimicrobial Drug Residues in Poultry Products by Four-Plate Test Method in Chaharmahal and Bakhtiari Province

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ABSTRACT

Objectives: Despite the benefits of antibiotics, their residues in foodstuffs prepared for human consumption could be cancerous and mutagenic and cause allergic reactions with toxic side effects, disorders in intestinal wall, adverse effects on microbial intestinal flora, and the emergence of resistant strains of bacteria. Therefore, the present study aimed to investigate the residual antibiotics in liver and muscle tissues of poultry samples using the four-plate test (FPT) method in Chaharmahal and Bakhtiari Province.

Materials & Methods: A total of 6406 samples were randomly collected from liver and muscle tissues of 3203 poultry samples from June 2016 to March 2017. Antibiotic residues were detected in liver and muscle samples using the four-plate test (FPT) method.

Findings: During a two-year study period, from a total of 6406 samples collected, 3203 (50%) samples were collected from liver tissues, and 3203 (50%) samples were collected from muscle tissues of poultry samples, respectively. Among the liver and muscle samples, 12 (384/3203) and 6.4% (206/3203) were positive for the presence of antibiotic residues, respectively. According to the seasonal distribution of muscle samples, the highest rate of antibiotic residues was detected in the autumn with 3 % (95/3203), while the lowest rate was observed in the winter with 0.25% (8/3203) for both bacteria under study (*Bacillus subtilis* and *Micrococcus luteus*). According to the seasonal distribution of liver samples, the highest rate of antibiotic residues was detected in the autumn with 5% (159/3203), while the lowest rate was observed in the spring with 1.1% (35/3203).

Conclusion: In conclusion, a large number of liver tissues collected from poultry samples were contaminated with antibiotic residues, especially at pH=6 and in the autumn season. Therefore, it is recommended to implement appropriate strategies for managing and controlling the use of antibacterial agents in the veterinary industry. Also, it is necessary to monitor the withdrawal time of antibiotics and screen the maximum residue limits (MRLs) in poultry products including liver, egg, and meat.

Keywords: Antibiotic residues, Four plate test, Poultry, Liver, Muscle

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Introduction

In today's world, the use of antibiotics for livestock is inevitable for various reasons such as treatment, prevention of different diseases, and more recently, "enhancing the food efficiency"^[1-2]. Antibiotics are used differently in terms of type and dose for treatment or prevention. The amount of antibiotics used to increase the growth and efficiency of livestock feed is much less than the amount used for treatment^[3]. However, antibiotics used to increase the growth are somewhat different from therapeutic antibiotics^[4-5].

Unfortunately, different types of antibiotics are used by veterinary technicians and even non-experts working in this profession, without considering their side effects and excretion period, leading to the residue of antibiotics and other chemicals, resulting from their metabolism, in livestock products^[6-7]. Despite the benefits of antibiotics, their residues in foodstuffs could be cancerous and mutagenic for human and cause allergic reactions, disorders in intestinal wall, adverse effects on microbial intestinal flora, and the emergence of resistant strains of bacteria^[8-10].

Adding antibiotics as supplement to animals feed is a public health risk. The residual antibiotics in animal products are not a major concern, but the major problem is the development of antibiotic-resistant bacteria that could be transmitted to humans^[10]. Taking a small amount of an antibiotic over a long period of time through food could cause antibiotic-resistant pathogenic or nonpathogenic bacteria to develop in the body^[3, 11]. In people who are more sensitive to small amounts of antibiotics, their possible allergy increases by consuming foods containing antibiotics. The presence of antibiotics in some foodstuffs and products poses potential risks to consumers and may interfere with the production of animal

products, like milk and its products^[12-13]. On the other hand, since poultry meat usually receives boiling heat, heat has a diminishing effect on carcass antibiotic residues and is a way to reduce the side effects of antibiotics on consumers^[14-15].

Objectives: In this regard, since there was no comprehensive study investigating the status of antibiotic contamination of poultry samples in our region; therefore, the present study aimed to investigate the residual antibiotics in the liver and muscle tissues of poultry samples by four-plate test (FPT) method.

Materials and Methods

Sample collection: A total of 6406 samples were randomly collected from liver and muscle tissues of 3203 poultry samples from June 2016 to December 2017. The collected samples were labeled and under refrigeration transferred to the laboratory and kept at -20°C before being tested. These samples were then tested for the presence of antibiotic residues.

Preparation of test plates: Mueller-Hinton agar was prepared according to the manufacturer's instructions. After sterilizing the media, the media pH was adjusted at 6, 7.2, and 8 for *Bacillus subtilis* and at 8 for *Micrococcus luteus* bacteria using a digital pH meter. After cooling to approximately 45°C, 0.1 mL of bacterial suspension was inoculated to each 100 mL of agar before solidification, bacterial suspensions equivalent to 0.5 McFarland turbidity were prepared from typical colonies of *B. subtilis* and *M. luteus*. The plates were incubated at 30 °C for *B. subtilis* and at 37 °C for *M. luteus* for 24 hours^[16].

Detection of antibiotic residues in samples: Antibiotic residues in liver and muscle samples were detected using four-plate test as described previously by Almashhadany et al. (2019)^[16]. Briefly, a disc-

shaped chicken sample of 2 mm in thickness and 8 mm in diameter was arranged and transferred on the surface of Mueller-Hinton agar inoculated by the sensitivity-test organisms (*B. subtilis* and *M. luteus*). The plates were incubated aerobically at 30°C for *B. subtilis* and at 37°C for *M. luteus* for 24 hrs. The inhibition zone diameter equal to or more than 2 mm was considered as positive, a zone of 1 to 2 mm was considered as suspicious, and a zone less than 1 mm was considered as negative [17].

Findings

During a two-year study period, from a total of 6406 samples collected, 3203 (50%) samples were collected from liver tissues, and 3203 (50%) samples were collected from muscle tissues of poultry samples, respectively. Among liver and muscle samples, 12 (384/3203) and 6.4% (206/3203) were positive for the presence of antibiotic residues (Inhibition zone diameters ranged from 2 mm to 8 mm), respectively (Table 1). The details of poultry samples frequency and seasonal distribution are presented in Table 1, 2, and 3.

Antibiotic residues in liver: According to the susceptibility testing of *B. subtilis* at different pH values, out of 2535 liver samples, the most liver samples (303/3203) harboring antibiotic residues against *B. subtilis* were observed at pH=6, and the least cases (206/3203) were observed at pH =8. In addition, 5.6% (179/3203) of liver samples harbored antibiotic residues against *M. luteus* at pH=8.

According to the seasonal distribution of liver sample, the highest rate of antibiotic residues was detected in the autumn with 5% (159/3203), while the lowest rate was observed in the spring with 1.1% (35/3203) (Table 2 and 3).

Antibiotic residues in Muscles: According to the susceptibility testing of *B. subtilis* at

different pH values, the most muscle samples (4.7%) (149/3203) harboring antibiotic residues against *B. subtilis* were observed at pH =6 and 7.2, and the least samples (3.06%) (98/3203) were observed at pH=8. Also, 1.8% (46/2535) of muscle samples harbored antibiotic residues against *M. luteus* at pH=8.

According to the seasonal distribution of muscles samples, the highest rate of antibiotic residues was detected in the autumn with 3 % (95/3203), while the lowest rate was observed in the winter with 0.25% (8/3203) for both bacteria (Table 2 and 3).

Discussion

Antibiotic residues in animal-derived products could pose adverse health effects on humans. For use of pharmacologically active substances, MRLs regulation has been assigned by European Union (EU) [17-18]. However, previous studies have reported that antibiotic residues with high concentrations are remarkably common in the poultry and livestock industries [16, 19-20].

Despite the variety of poultry products, including eggs, chicken, etc, only a few studies have focused on investigating antibiotic residues. Therefore, this study aimed to investigate the antibiotic residues in liver and muscle tissues of poultry samples based on FPT during two years [18].

In the present study, the incidence rate of antibiotic residues in all poultry samples was 9.2% (590/6406). The highest rate (12%) was found in liver samples, while the lowest (6.4%) rate was observed in muscle samples. According to the obtained results (Table 2), the highest detection rate of antibiotic residues in liver and muscle samples was in the autumn.

In agreement with this study results, Tajik et al. (2010) reported that 17.5% of liver, kidney, and muscle samples of chicken carcasses were positive for antibiotic

Table 1) Total distribution of antibiotic residues based on the poultry samples

Organ	Liver (N=3203)			Muscle (N=3203)		
	2016	2017	Total	2016	2017	Total
Spring	0	35 (15)	35 (9.1)	0	18 (15)	18
Summer	3 (2)	150 (64.4)	153 (39.9)	0	85 (70.8)	85
Autumn	111 (73.5)	48 (20.6)	159 (41.4)	78 (90.7)	17 (14.2)	95
Winter	37 (24.5)	0	37 (9.6)	8 (9.3)	0	8
Total	(39.3) 151	233 (60.7)	384 (100)	86 (41.7)	120 (58.3)	206 (100)

Table 2) Relationship between season and incidence of antibiotic residues in poultry samples in 2016.

Organ	Liver No. (%)					Muscle No. (%)				
	Total samples	<i>B. subtilis</i> pH=6	<i>B. subtilis</i> pH=7.2	<i>B. subtilis</i> pH=8	<i>M. luteus</i> pH=8	Total samples	<i>B. subtilis</i> pH=6	<i>B. subtilis</i> pH=7.2	<i>B. subtilis</i> pH=8	<i>M. luteus</i> pH=8
Spring	0	0	0	0	0	0	0	0	0	0
Summer	85	0	0	0	3 (3.5)	85	0	0	0	0
Autumn	321	74 (23)	57(17.8)	46(14.3)	56(17.4)	321	60(18.7)	51(15.9)	36(11.2)	16(5)
Winter	169	3(1.8)	7(4.1)	4(2.4)	29(17.2)	169	0	6(3.6)	2(1.2)	0
Total	575	77	64	50	88	575	60	57	38	16

residues using FPT method. Similar to this study, they did not report the percentage and concentration of each antibiotic residue [21]. Moreover, Khan Nazer et al. (1999) investigated antibiotic residues in poultry samples. Their results indicated that 12.4% of samples contained residual antibiotics at one or more side, 11.2% in the breast muscle and 8% in the liver samples, consistent with this study findings [22].

Ghasemi et al. (2014) also reported that 18.7% of poultry carcasses were positive for

the presence of antibiotic residues [20]. Additionally, another study conducted by Shareef et al. (2009) found that 7% of liver and breast muscle samples were positive for antibiotic residues, which is lower than this study finding [23]. Also, Rahimi et al. (2017) revealed that 6% of chicken meat samples were contaminated with sulfonamide antibiotic residues, which are in contrast with the present study findings [24]. On the other hand, higher rates have been reported previously. Karmi et al. (2014)

Table 3) Relationship between season and incidence of antibiotic residue in poultry samples in 2017

Organ	Liver					Muscle				
		No. (%)					No. (%)			
Season	Total samples	<i>B. subtilis</i> pH=6	<i>B. subtilis</i> pH=7.2	<i>B. subtilis</i> pH=8	<i>M. luteus</i> pH=8	Total samples	<i>B. subtilis</i> pH=6	<i>B. subtilis</i> pH=7.2	<i>B. subtilis</i> pH=8	<i>M. luteus</i> pH=8
Spring	398	33 (8.3)	30 (7.5)	25 (6.3)	16 (4)	398	19 (4.8)	14 (3.5)	14 (3.5)	11 (2.8)
Summer	889	125(14.1)	131(14.7)	103(11.6)	59(6.6)	889	54(6.1)	66(7.4)	32(3.6)	9(1)(1)
Autumn	673	68(10.1)	57(8.5)	28(4.2)	16(2.4)	673	16(2.4)	12(1.8)	14(2.1)	10(1.5)
Winter	668	0	0	0	0	668	0	0	0	0
Total	2628	226	218	156	91	2628	89	92	60	30

reported that 90% of fresh and local-frozen samples were positive for residues of different antibiotics^[25].

Over the past few years, a great number of diagnostic methods have been identified for determining the drug residuals in foodstuff as well as consumable organs of animals. Furthermore, multiple methods have been invented to determine antibiotic residues in foods in the world. Microbiological, chromatographic, ELISA, and physicochemical methods are the most common methods. In the present study, four-plate test (FPT) with high sensitivity for detecting antibiotic residues in foods was used^[26-287].

Furthermore, the existence of antibiotic residues in different food products is an important concern in the world. Accordingly, Al-mashhadany et al. (2016) detected antibiotic residues among raw beef. Their results showed that the occurrence of antibiotic residues was 10.8%^[16]. In another study, antibiotic residues in poultry meat was reported as 5%^[28]. In a study performed by Rahimi et al. (2017), 25, 20, 12, 12, 6, 5, 4, and 2% of sheep meat, cow kidney, cow liver, cow milk, chicken meat, sheep liver, hen egg, and sheep kidney samples were contaminated by

sulfonamides, respectively^[24].

In another study conducted by Ehsani et al. (2015), 12.5% of commercial eggs were positive for antibacterial residues^[19].

Given these findings, it seems that antibiotic residues with high detection rates or high concentrations are common, especially in the livestock and poultry industries.

However, difference in the reported results of antibiotic residues in similar studies in Iran and other countries may be due to the difference in such factors as detection method, sample size, sample preparation, seasonal distribution, and type of food product.

Conclusion

a large number of liver tissues collected from poultry samples were contaminated with antibiotic residues, especially at pH=6 and in the autumn season. Therefore, it is recommended to implement appropriate strategies for managing and controlling the use of antibacterial agents in the veterinary industry. Also, it is necessary to monitor the withdrawal time of antibiotics and screen the maximum residue limits in poultry products, including livers, eggs, and meats.

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