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Determination of Antibiotic Resistance Patterns of *Salmonella* Serotypes Isolated from Broilers in Kermanshah Province

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ABSTRACT

Objectives: The present study aimed to investigate the prevalence of *Salmonella* serotypes in slaughtered broilers in Kermanshah province and investigate the susceptibility of isolated *Salmonella* to antibiotics.

Methods: In this cross-sectional, 10 carcasses were randomly selected from each of 25 slaughtered broiler flocks that were obtained from their cloacae. This study was conducted in 2014 in Kermanshah province. Samples were transferred into the Selenite F enrichment broth and incubated at 43 °C for 12-18 h. Antibiotic susceptibility pattern of all isolates to be performed using the disk diffusion method. Also, the agglutination test on slide was performed on *Salmonella* isolates using the specific antisera to detect *Salmonella* serotypes.

Findings: In the present study, 250 (33%) samples of *Salmonella* were isolated from 750 cloacal samples of broilers. After serotyping, the most frequent serotype was as follow: Enteritidis serotype (55.2%), Infantis serotype (38%) and Typhimurium serotype (25.6%). The results of antibiotic susceptibility pattern indicated that 100% of strains were susceptible to gentamicin, enrofloxacin, imipenem and ceftriaxone; and the highest resistance was observed to nalidixic acid and nitrofurantoin. Furthermore, 202 (80%) out of 250 *Salmonella* isolates had multiple resistance to three or more antibiotics and accounted as MDR isolates.

Conclusion: According to the results of the present study, it is essential to detect pollution sources and pathogenic serotypes, the possibility of transferring *Salmonella* through poultry to humans and increasing the frequency of resistant isolates to antibiotics. It is strongly recommended to avoid the overuse of antibiotics without performing antibiotic susceptibility patterns.

Keywords: *Salmonella*; Serotype; Antibiotic resistance

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Introduction

Foodborne diseases are major health problems and causes of economic losses among industrialized and non-industrialized countries; and Salmonellosis is a common food poisoning in the world [1]. The poultry, especially chicken, is the most widespread reservoir of *Salmonella* [2]. The bacterium is the second most common foodborne disease in the United States with more than 2,500 serotypes [1, 3]. Eating contaminated poultry meat is a way of transmitting *Salmonella* infections to humans and it causes different diseases, including enteric fever, bacteremia and gastroenteritis in consumers [4, 5]. Among different *Salmonella* serotypes, Enteritidis and Typhimurium serotypes are the leading causes of foodborne infection or poisoning [6]. *Salmonella* can cause diseases such as Pullorum, typhoid and paratyphoid in poultry and causes significant losses in poultry and significant damage to the national economy [7]. Also, poultry *Salmonella* is a zoonotic disease that is important in terms of the public health of society [8]. Gartner first reported the prevalence of food poisoning caused by *Salmonella* in Germany in 1888 [9]. In several studies, its prevalence was 2.74% in the US and 8.3% in Iran[10]. It causes about 25 million infections and about 200,000 deaths worldwide [11]. Given the high mortality rate, this is considered as a serious risk. According to researchers' theory and statistical information, chicken, beef, pork, fish, milk, and egg are sources of foodborne salmonellosis [12]. The emergence of antibiotic resistance in this pathogen is due to the increasing use of antibiotics at aviculture and treatment centers and it is a global problem. This is currently considered as a serious risk because of the increasing possibility of transmission of resistant *Salmonella* infections of other transmissible bacteria between humans and animals [13]. According to the examination of results of serotyping tests of the pathogenic *Salmonella* bacterium and its antibiotic susceptibility in different regions of the world, types and frequency of serotypes and bacterial resistance to common antibiotics vary in different regions [14]. Numerous studies of Iran indicate that the rate of chicken *Salmonella* infection ranges from 19.5% to 40% [15]. Antibiotic-resistant Salmonella is one of the most important public health concerns. Recently, studies have revealed that due to long-term antibiotic use during animal breeding, antibiotic resistance has prominently increased. Multidrug-resistant (MDR) Salmonella could pose a serious threat to humans through the food chain [16]. Annually, at least 100,000 infections are due to antibiotic-resistant Salmonella, including those that are resistant to clinically-important drugs such as ceftriaxone and ciprofloxacin. In conducted between 2016 and 2017, 63.6% of cases of salmonellosis were reported from chicken meat in China [16]. According to the EU law, fresh poultry meat products must be free of Salmonella since the beginning of 2011, and the prevalence of *Salmonella* in broilers and laying hens must be less than 1% per country since 2012 [17].

Objectives: Therefore, due to the importance of *Salmonella* contamination in the poultry industry and its effect on food poisoning and public health level and increasing antibiotic resistance of *Salmonella*, the present study was aimed to determine the status of *Salmonella* infection in slaughtered poultry in Kermanshah province. The obtained data will be important in the proper treatment of antibiotics and the prevention of economic and health damage caused by salmonellosis.

Materials and Methods

Sampling and isolation: In the present study, which was conducted in Kermanshah province in 2014, 10 carcasses were randomly selected from each of 25 slaughtered broiler flocks and samples were obtained from their cloacae. Samples were first transferred into the Selenite F enrichment broth (Merck) and incubated at 43 °C for 12-18 h. After this time, the culture was obtained from the fluid MacConkey Agar (MAC) enrichment broth (Merck) and *Salmonella*-Shigella (SS) agar (Merck) and incubated at 37°C for 24 h. The culture was obtained from *Salmonella* suspected colonies in TSI and urea media, and then biochemical tests were performed, including indole, VP, MR and citrate [18].

Antimicrobial Susceptibility testing: Antibiotic susceptibility of all isolates to mentioned antibiotic agents in the Table 1 (Mast Co., UK) was performed on Muller-Hinton agar (Merck, Germany) using the disk diffusion method based on the Clinical and Laboratory Standards Institute (CLSI) [19]. *E. coli* ATCC 25922 was used as a quality control strain for antibacterial susceptibility testing. [20].

Determination of Serotype: The agglutination test on slide was performed on *Salmonella* isolates using the specific antisera to detect *Salmonella* serotypes. To this end, we utilized *Salmonella* specific *Salmonella* antisera (Mast Company) in the microbiology sector of Razi Vaccine and Serum Research Institute. First, a concentrated suspension of the bacterium was prepared in the physiological serum on the slide, and then a drop of mono-valan O serum was added to the mixture; and the formation of agglutination was evaluated in less than 2 min. The sample was then taken adjacent to H antisera (phases 1 and 2) and the serotype of bacterium was determined after observing agglutination according to the Kauffmann–White table 1 [18].

Findings

In general, 250 (33%) out of 750 collected samples from poultry in the present study (25 broiler flocks each of which with 30 samples) were infected with *Salmonella*. The frequency of detected *Salmonella* serotypes in the present study included 138 (55.2%) *Salmonella* serotypes Enteritidis, 98 (38%) *Salmonella* serotypes Infantis, and 64 (25.6%) *Salmonella* serotypes Typhimurium. The antibiotic susceptibility of *Salmonella* isolates to 30 tested antibiotics indicated that 100% of isolates were susceptible to gentamicin, enrofloxacin, imipenem and ceftriaxone. The highest susceptibility was seen to cefotaxime, chloramphenicol, ciprofloxacin, florfenicol, colistin, cefixime, ceftazidime, cefuroxime sodium, kanamycin, cefotaxime, neomycin, piperacillin, cefepime, ofloxacin, and amikacin respectively. The highest antibiotic resistance was also observed for nalidixic acid and nitrofurantoin (Table 1). Furthermore, 202 (80%) out of 250 *Salmonella* isolates had multiple resistance to three or more antibiotics and accounted as MDR isolates.

Discussion

Salmonellosis is gastroenteritis caused by different serovars of *Salmonella* genus and the most common type of food poisoning in the world [5]. It is a major zoonotic and foodborne disease in the world. *Salmonella* is excreted from infected poultry and this infection can remain in the bed and eventually infect even other birds, and it is very important in the epidemiology of disease [21]. Furthermore, the consumption of contaminated eggs, chicken meat and water is important in the transmission of *Salmonella* infections [22].

In a study (2008), Jalali et al. reported 17.9% of raw poultry meat contamination in Isfahan [23]. In the present study, 250 samples (33%) had *Salmonella* infection from 750 poultry cloaca samples, and the *Salmonella* serotypes Enteritidis had the highest number of isolates that it was consistent with results of studies by Carraminana et al and Kasimoghu et al [24]. Kasimoghu et al.

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Table 1) Antibiotic susceptibility pattern of *Salmonella* Serotypes isolated

Antibiotic	Susceptible No. (%)	Intermediate No. (%)	Resistance No. (%)
Cotrimoxazole	160 (64)	4 (1.6)	86 (34.4)
Amoxycillin	65 (26)	85 (34)	100 (40)
Cefixime	195 (78)	45 (18)	4 (10)
Amikacin	238(95.2)	4 (1.6)	8 (3.2)
Ampicillin/Sulbactam	180(72)	60 (24)	10 (4)
Cefotaxime	240 (96)	10 (4)	0
Ceftazidime	246 (98.4)	0	4 (1.6)
Ceftriaxone	250 (100)	0	0
Cefuroxime	244 (97.6)	6 (2.4)	0
Ciprofloxacin	242 (96.8)	8 (3.2)	0
Enrofloxacin	250 (100)	0	0
Gentamicin	250 (100)	0	0
Cephalothin	210 (84)	3	8 (3.2)
Cefotizoxime	246 (98.4)	4 (1.6)	0
Neomycin	190 (76)	60 (24)	0
Fluorophenicol	220(88)	4 (1.6)	16 (6.4)
Piperacillin	235 (94)	9 (3.6)	6 (2.4)
Chloramphenicol	220 (88)	4 (1.6)	16 (6.4)
Tetracycline	70 (28)	60 (24)	120 (48)
Cephalexin	60 (24)	50 (20)	140 (56)
Ampicillin	130 (52)	50 (20)	70 (28)
Cefipime	246 (98.4)	4 (1.6)	0
Aflaxacin	246 (98.4)	4 (1.6)	0
Imipenem	250 (100)	0	0
Kanamycin	236 (94.4)	7 (2.8)	7 (2.8)
Nalidixic acid	27 (10.8)	8 (3.2)	215 (86)
Nitrofurantoin	15 (6)	7 (2.8)	238 (91.2)
Colistin	85 (34)	5 (2)	160 (64)
Furazolidine	36 (90)	44 (17.6)	116 (46.4)
Cefazolin	175 (70)	60 (24)	15 (6)

(2010) isolated and serotyped 32 *Salmonella* from 400 poultry carcasses in Turkey. The detected serotypes consisted of 22 samples (68.7%) of *S.* Enteritidis, 5 samples (15.6%) of *S.* Wirchow, 3 samples (9.3%) of *S.* typhimurium and 2 samples (9.3%) of *S.* Hadar [25]. In another study by Caraminana in Spain (2004) most serotypes isolated from poultry were *S.* Enteritidis, *S.* Hadar, *S.* Newport, *S.* Wirchow, *S.* Typhimurium and *S.* Heidelbege [24]. In another study by Maherjan (2006), isolated serotypes included gallinarum, pullorum, cholorasuis, and typhi [26]. In a research by Mezali et al in Algeria (2012), 61 (19.43%) out of 314 samples were *Salmonella* positive, and 21 different serovars were diagnosed. The most common serovars included *S.* Anatum (14.6%), *S.* Altona (12.5%), *S.* Enteritdis (7.8%) and *S.* Typhimurium (7.81%) [27]. Ammar et al. (2010) isolated *S.* Typhimurium and *S*. livingstone serotypes from poultry [28]. In the present study, *S.* Infantis, *S.* Enteritidis, and *S.* Typhimurium were isolated serotypes; and Enteritidis and Infantis were the most frequent isolates. Based on the above reports, it can be concluded that differences in tested geographical regions may be the result of differences in isolated serotypes in different countries, including Iran. In the evaluation of antibiotic resistance in 18 *Salmonella* isolates in Brazil in 2011, eighteen serotypes were detected. Of these, the most frequently detected were *S*. Enteritidis (48.8%), *S*. Infantis (7.6%), *S*. Typhimurium (7.2%), and *S*. Heidelberg (6.4%). The results of antibiotic resistance pattern of *Salmonella* isolates revealed the highest resistance rate was to streptomycin (78%), florfenicol (62%), sulfonamide (58%) and nalidixic acid (40%). Among 13 antibiotics, the resistance to nalidixic acid was consistent with obtained results in the present study [29]. In a study on the antibiotic resistance by Halawani et al. in Saudi Arabia (2007) on 32 *Salmonella* isolates, two strains, namely Typhimurium (17 cases) and Enteritidis (5 cases) were isolated and all isolates were susceptible to gentamicin, ciprofloxacin, and ceftriaxone; and the result was consistent with the present study [30].

In another study conducted by Kasimoghu et al, out of 32 *Salmonella* strains, 22 (68.75%) displayed multi-drug resistance. Moreover, thirty-two (100.0%) of the isolates were found to be resistant to penicillin G, 20 (62.5%) to nalidixic acid, four (12.5%) to cephalothin, two (6.2%) to streptomycin and two (6.2%) to tetracycline [25]. In a study by Graziani in Italy, there was the resistance of isolated *Salmonella* from poultry to ampicillin (54.3%), gentamicin (3.2%), kanamycin (8.5%), chloramphenicol (24.5%) and ciprofloxacin (1%)[31]. The comparison of these results with results of the present study indicated that isolates of this study were more susceptible to mentioned antibiotics and their resistance was lower. According to antibiotic susceptibility test results in the present study, there was no antibiotic resistance to ceftriaxone, enrofloxacin, gentamicin and imipenem; hence, these antibiotics are probably appropriate treatments for salmonellosis. The rates of resistance to antibiotics such as nalidixic acid and nitrofurantoin were 86% and 91.2% respectively. The highest rates of multi-resistance were reported to antibiotics, namely nalidixic acid, nitrofurantoin, colistin, furazolidone and tetracycline.

The differences in antibiotic resistance and antibiotic susceptibility of *Salmonella* serotypes in the present research and previous studies may be due to the overuse of antibiotics and the genetic transfer of drug resistance among bacteria. The higher and uncontrolled use of antibiotics in medicine and veterinary medicine is a cause of increased antibiotic resistance. Overuse of antibiotics before the antibiogram creates resistant strains. It is important paying attention to the transmission of this resistance among different strains of bacteria and ultimately the human infection with resistant bacteria. As explained, the present research and other studies in Iran and other countries have reported multiple antibiotic resistance of strains. The emergence of multiple antibiotic-resistant isolates has caused problems in the treatment of *Salmonella* infections in humans and animals [32]. It is essential to detect pollution sources and pathogenic serotypes according to the results of the present study and the possibility of transferring *Salmonella* through poultry to humans and increasing the frequency of resistant isolates to antibiotics. It is strongly recommended to avoid the overuse of antibiotics without performing antibiograms to prevent the resistance in different *Salmonella* serotypes because there is a risk of transmission of these resistant strains to humans through the consumption of poultry.

Conclusion

According to the results of the present study and the possibility of transferring *Salmonella* through poultry to humans and increasing the frequency of resistant isolates to antibiotics, the detection of pollution sources and pathogenic serotypes are essential. It is strongly recommended to avoid the overuse of antibiotics without performing antibiograms to prevent the resistance in different *Salmonella* serotypes because there is a risk of transmission of these resistant strains to humans through the consumption of poultry.

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