Original Article

Serotype Distribution and Antimicrobial Resistance of Salmonella Isolates in Human, Chicken, and Cattle in Iran

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ABSTRACT
Salmonella is a foodborne zoonotic enteric bacterium able to infect both humans and animals. This study aimed to identify the antimicrobial resistance of Salmonella serovars isolated from human, cattle, and poultry. Moreover, we investigated the probable transmission trends of antibiotic-resistant Salmonella isolates from food animals to human. A total of 242 Salmonella isolates collected from various human and animal sources were serotyped. The polymerase chain reaction was performed to detect the *invA* virulence gene. The isolates were subsequently tested against 14 antimicrobials and the resistance rates among the isolates from three sample sources were statistically analyzed by the Chi-Square test. Serotyping revealed the isolates belonged to various serovars with the dominance of Enteritidis (37%), Typhimurium (35.3%), and Infantis (21.1%). A high frequency of resistance to streptomycin was observed followed by tetracycline, trimethoprim, sulfonamides, spectinomycin, chloramphenicol, florfenicol, ampicillin, kanamycin, ceftazidime, and cefepime. In addition, multidrug resistance was observed in more than 40% of the isolates. The results of the statistical analysis showed a significant relationship (P < 0.001) between the rate of antibiotic resistance among the three sources of Salmonella isolates. Furthermore, the antibiotic resistance had a statistical relationship between the different serotypes isolated from different sources. These findings demonstrate the possible transmission of resistance to human from animal sources. The prevalence of the Typhimurium, Enteritidis, and Infantis serovars in both human and animals suggested that Salmonella contamination in chicken and cattle may be the major source of salmonellosis in human. The high incidence of antibiotic resistance in Salmonella isolates along with the close relationship between the antimicrobial resistance of animal and human isolates indicate the role of food animal products as an important source of resistance.
Keywords: Animals, Antibiotic resistance, Human, Salmonella, Serotype

La distribution des Sérotypes et la Résistance aux Antimicrobiens D'isolats de Salmonella chez L'homme, le Poulet et le Bétail en Iran
Résumé: Salmonella est une bactérie entérique zoonotique d'origine alimentaire capable d'infecter aussi bien les les humains que les animaux. Cette étude visait à identifier la résistance aux antimicrobiens des différents sérovars de Salmonella isolés chez l'homme, le bétail et la volaille. De plus, nous avons étudié les tendances probables de transmission d'isolats résistants aux antibiotiques chez des animaux destinés à l'alimentation humaine. Un total de 242 isolats de *Salmonella* provenant de diverses sources humaines et animales ont été sérotypées. Une réaction en chaîne de la polymérase a été réalisée pour détecter le gène de virulence invA. Les isolats ont ensuite été testés contre 14 antimicrobiens et les taux de résistance parmi les isolats de trois échantillons ont été analysés de manière statistique par le test du chi carré. Le sérotypage a révélé que les isolats appartenaient à divers sérovars avec une dominance d'Enteritidis (37%), Typhimurium (35,3%) et Infantis (21,1%). Une fréquence élevée de résistance à la streptomycine a été observée, suivie de la tétracycline, du triméthoprime, des sulfamides, de la spectinomycine, du chloramphénicol, du florfénicol, de l'ampicilline, de la

kanamycine, du ceftazidime et du céfépime. De plus, une multirésistance aux médicaments a été observée dans plus de 40% des isolats. Les résultats de l'analyse statistique ont montré une relation significative (P<0,001) entre le taux de résistance aux antibiotiques parmi les trois sources d'isolats de *Salmonella*. En outre, la résistance aux antibiotiques présentait une relation statistique entre les différents sérotypes isolés de différentes sources. Ces résultats démontrent la transmission possible de la résistance aux antibiotiques à l'homme par des sources animales. La prévalence des sérovars Typhimurium, Enteritidis et Infantis chez l'homme et les animaux suggère que la contamination par Salmonella chez le poulet et le bétail peut être la principale source de salmonellose chez l'homme. L'incidence élevée de la résistance aux antibiotiques dans les isolats de Salmonella ainsi que la relation étroite existant entre la résistance aux antimicrobiens des isolats animaux et humains indiquent que les produits des animaux destinés à l'alimentation sont une source importante de résistance.

Mots-clés: Animaux, Résistance aux antibiotiques, Humain, Salmonella, Sérotype

INTRODUCTION

Salmonellosis is one of the most important bacterial diseases affecting a great number of hosts throughout the world. Two species of Salmonella genus have been recognized, namely *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* has six subspecies and 2500 serovars. The subspecies *enterica* contains the main serovars, which are hazardous to human and warm-blooded animals (Ruby et al., 2012). Salmonella causes typhoid fever and acute gastroenteritis in human beings. Typhoid fever is generally induced by Typhi serovar and results from the invasion of bacteria to the bloodstream. However, gastroenteritis is mainly caused by non-typhoidal serovars from foodborne sources (Mulvey et al., 2006). According to the latest reports, almost 1.3 billion cases of non-typhoidal salmonellosis annually occur all over the world, (Porwollik et al., 2002). Most of the human *Salmonella* outbreaks are related to the consumption of contaminated products with animal origin (Wray and Wray, 2000). In cows, the bacteria are mostly shed to the environment from feces and sometimes from milk, urine, uterine discharge, fetus, and contaminated placenta. Therefore, water, food, and the environment are contaminated endangering the lives of healthy people (Halimi et al., 2014). Poultry and poultry products are the chief reservoirs of many zoonotically important pathogens, among which Salmonella is of

prime importance (Behravesh et al., 2014). Animal-origin foods, such as beef, poultry meat, egg, and milk were indicated as the carriers of this pathogen. As a result, this pathogen not only affects the livestock and poultry industry but also occurs in human (Gillespie et al., 2003). Laboratory diagnosis of Salmonella is difficult, time-consuming, and sometimes with false-negative results due to the limited fecal shedding of the bacteria. This bacterium can be efficiently detected by polymerase chain reaction (PCR) method, in the clinical and subclinical cases (Malorny et al., 2003). *InvA* gene, which is an important virulence gene, has a sequence specific to Salmonella genus making it an appropriate PCR target for the diagnosis of this bacterium (Salehi et al., 2007). Recently, the resistance of different pathogens to antimicrobial agents is rising in both foods and clinical isolates (Van Hoek et al., 2005). Antibiotics are applied for the treatment of humans and animals, as well as the prophylaxis and growth promotion of animals. The inaccurate usage of antimicrobial agents may lead to resistance in various bacteria causing the treatment of bacterial infections to be more difficult (Schwarz and Chaslus-Dancla, 2001). It is assumed that antibiotic resistance in human bacterial isolates could originate from animals through direct contact or food products of animal origins, the latter being of utmost importance. In order to find a mechanism for controlling the rising occurrence of antimicrobial resistance, it is essential to investigate the

epidemiology of Salmonella serovars and antimicrobial resistance levels in humans and animals. Consequently, this study aimed to identify the Salmonella serovars isolated from chicken, cattle, and humans by detecting their *invA* gene and to test the antimicrobial sensitivity of these isolates. In addition, the resistance patterns were compared between the different species and serovars. Therefore, the probable transmission of resistant serovars from food animals to humans could be evaluated.

MATERIAL AND METHODS

Sampling and Serotyping. A total of 242 isolates isolated from diverse origins, including human (N=109), cattle (N=60), and chicken (N=73) during 2008-2015 were used in the present study. It should be noted that all the human samples were from clinical cases. All the isolates were confirmed to be Salmonella based on colony morphology and biochemical tests. The Salmonella strains were stereotyped by commercial antisera (Difco, Detroit, Michigan, USA) and the results were interpreted according to the Kaufmann-White scheme (Guibourdenche et al., 2010).

Detection of *invA* Gene. Specific primers of *Salmonella invA*, S139, and S141 with the nucleotide sequence of 5´- GTG AAA TTA TCG CCA CGT TCG GGC AA - 3´ and 5´- TCA TCG CAC CGT CAA AGG AAC C - 3´ were used (Zahraei Salehi et al., 2007). The DNA extraction was performed by DNA extraction kit (MBST, Iran) based on the instructions of the manufacturer. The PCR was completed in a 25 μL amplification mixture containing 2 mM MgCl2 (Sinaclon, Iran), 10 mM dNTPs (Sinaclon, Iran), 0.5 μM of each primers (Sinaclon, Iran), 2.5 μL 10 X PCR buffer (Sinaclon, Iran), 1 U Taq DNA polymerase (Sinaclon, Iran), and 1.5 μL DNA templates. The amplification process was carried out by thermocycler (Bio-Rad, USA). The amplification program entailed initial denaturation at 94 °C for 10 min, 30 cycles at 94 °C for 1 min, 62°C for 1 min, 72 °C for 1 min and the final amplification at 72 °C for 10 min. The

amplification products were electrophoresed by a 1.2% agarose gel (Sinaclon, Iran), stained with ethidium bromide (Sinaclon, Iran), and finally visualized under ultraviolet light.

Antibiotic Susceptibility Testing. The antibiotic susceptibility of the isolates was determined through the disc diffusion method on Mueller-Hinton agar using antibiotic discs. The examined antimicrobial agents were ampicillin (AMP, 10 μg), ceftriaxone (CRO, 30 μg), ceftazidime (CAZ, 30 μg), cefepime (CPM, 30 μg), chloramphenicol (CHL, 30 μg), florfenicol (FFC, 30 μg), tetracycline (TE, 30 μg), streptomycin (S, 10 μg), spectinomycin (SP, 100 μg), gentamycin (GM, 10 μg), kanamycin (K, 30 μg), tobramycin (TOB, 10 μg), trimethoprim (TM, 5 μg), and sulfonamides (SU, 200 μg) (MAST, UK). The inhibition zones were measured and scored as "sensitive", "intermediate susceptibility", or "resistant" according to the recommendations of the Clinical and Laboratory Standards Institute. For antibiotic disc control, *Escherichia coli* ATCC 25922 was used as a reference strain.

Statistical Analysis. All the data were analyzed using SPSS software version 19. The statistical relationship between the resistance rates of human and chicken samples, as well as human and cattle samples, was analyzed by the Chi-Square test. P-value ≤ 0.05 was considered as statistically significant.

RESULTS

The biochemical profile of Salmonella was indicated in all the 242 study samples. The PCR test target gene, *inv*A, was amplified and the associated bands with the size of 284 bp were observed (Figure 1). The serotyping of the isolates was performed using commercially reliable antisera. Our results demonstrated that out of the 242 positive isolates of *Salmonella* species, 12 different serovars were detected. Moreover, among 109 human isolates, the most predominant serovar was Enteritidis (N=43), followed by Typhimurium (N=41), Infantis (N=11), and Kentucky (N=6). In addition, other serovars,

including Paratyphi B, Virchow, Senftenberg, Paratyphi A, Heidelberg, Senegal, and Emek were detected. it was shown that 40 and 20 of 60 cattle isolates belonged to Typhimurium and Enteritidis serovars, respectively. In chicken samples, Infantis (N=38) was the most prevalent serovar, followed by Enteritidis (N=25) and Typhimurium (N=9) serovars (Table 1).

Table 1. Serotyping Results

Serovar	Human (%)	Cattle (%)	Chicken (%)
Enteritidis	43 (39.4)	20 (33.33)	25 (34)
Typhimurium	41 (37.6)	40 (66.66)	9 (12)
Infantis	11 (10)	-	38 (52)
Kentucky	6 (5.5)	-	-
Paratyphi B	2 (1.8)	-	-
Virchow	1 (0.9)	-	-
Heidelberg	1 (0.9)	-	-
Senftenberg	1 (0.9)	-	-
Paratyphi A	1 (0.9)	-	-
Emek	1 (0.9)	-	-
Senegal	1 (0.9)	-	-
Havana	-	-	1 (1.3)

According to the results of this study, the highest resistance was to streptomycin (43.5%). The resistance status regarding the other antibiotics was as follow: tetracycline (35.7%), trimethoprim and sulfonamides (33.6%), spectinomycin (33.1%), chloramphenicol (31.8%), florfenicol (25.4%), ampicillin (21.9%), kanamycin (15.9%), ceftazidime (12.9%), and cefepime (4.7%). The highest susceptibility was revealed for gentamycin (100%). The rates of antibiotic resistance for distinct serovars in human, chicken, and cattle isolates are shown in tables 2 and 3. A significant relationship was found between human, cattle, and chicken isolates by the statistical analysis of drug resistance rates (P<0.005). Furthermore, the antibiotic resistance rates in human were revealed to have a significant relationship with both cattle and chicken isolates (P<0.001). Regarding Enteritidis and Typhimurium serovars, significant relationships were

reported between antibiotic resistance rates of human, cattle, and chicken (P<0.001). Moreover, Infantis serovar, which was observed just in human and chicken was found to have correlated resistance (P<0.001).

Table 2. Results of antibiotic susceptibility test (%)

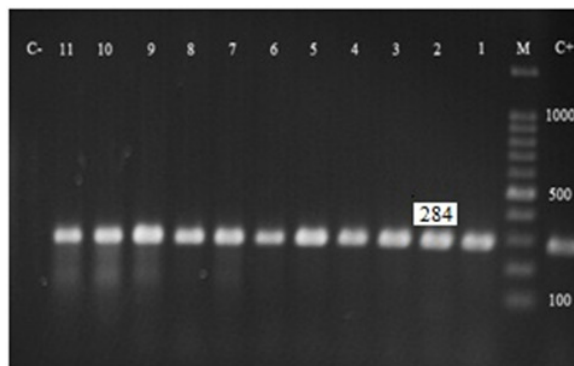| Antibiotic	Human	Cattle	Chicken
Ampicillin	26.6	25	8.21
Chloramphenicol	26.6	20	47.94
Florfenicol	20.18	10	45.2
Tetracycline	31.19	15	57.53
Streptomycin	42.2	26.66	56.16
Spectinomycin	24.77	22.11	53.42
Trimethoprim	23.85	22.11	56.16
Sulfonamides	24.77	20	56.16
Kanamycin	3.66	20	31.5
Tobramycin	0	0	0
Gentamycin	0	0	0
Cefepime	4.58	0	8.21
Ceftazidime	11	10	20.54
Ceftriaxon	0.91	0	1.36

Figure 1. Results of *invA* gene PCR; A 284 bp band was considered as definitively representative of *Salmonella* isolates. Lane 1: positive control (*S.* Typhimurium ATCC 14028), Lane 2: marker 100 bp, lane 3-11: understudy isolates, Lane 12: negative control.

DISCUSSION

Salmonella exists in nature extensively and can colonize or infect a variety of domestic and wild animals from mammals to birds or reptiles. Non-typhoidal salmonellosis generally results from the

consumption of contaminated food products. Although the infection is typically self-limited, it can be life-threatening in immunocompromised patients, children, and the elderly (Rayamajhi et al., 2008). *InvA* gene encodes a protein in the inner membrane of bacteria, which is responsible for the invasion of the host epithelial cells. The presence of this gene in Salmonella isolates proves the virulence of the organisms and the capability of the bacteria for penetrating the host epithelial cells leading to infection (Oliveira et al., 2003). Serotyping and antibiogram typing as the phenotypic methods (R-typing) are useful markers for epidemiological studies that demonstrate the epidemic strain (Threlfall, 2010). The results of this study revealed that the most prevalent serovars in human cases were Enteritidis, Typhimurium, and Infantis. Enteritidis and Typhimurium serovars are the main serotypes in human isolates. In addition, Infantis is categorized among the top ten prevalent serovars in many countries (Threlfall, 2010). In Iran, *Salmonella Enteritidis*, *Typhimurium*, and *Infantis* are the most common serovars detected in human. Ranjbar et al. (2011) reported these three serovars as the most frequent ones isolated from human cases of enteritis in Tehran, Iran. A wide range of *Salmonella* serotypes is capable of colonizing the poultry throughout the world. These serotypes include *S. Typhimurium*, *S. Enteritidis*, *S. Hadar*, *S. Virchow*, *S. Infantis*, and recently, *S. Paratyphi B*, all of which regularly being isolated from poultry in several countries (Kagambega et al., 2013). In Iran, there are several reports concerning the presence of various Salmonella serovars in poultry with the dominance of Enteritidis and Infantis (Bozorgmehri Fard et al., 2009; Rahmani et al., 2013; Ghaderi et al., 2016). However, it appears that *S.* Infantis is presently the most important serovar in the poultry industry of Iran, particularly due to its multiple drug resistance. Nonetheless, the prevalence of serovars may change over time and one may be replaced by another (Rahmani et al., 2013). Typhimurium (66.6%) and Enteritidis (33.3%) serovars have been detected in

samples from cattle. Our study is consistent with the investigation performed by Halimi et al. (2014), in which the prevalence of *S. Typhimurium*, as the most common serovar in dairy cattle isolates, was 54%. In the study completed by Salehi et al. (2007), this serovar accounted for 66.7% of all *Salmonella* isolates detected in bovine diarrheal samples. The comparison of *Salmonella* serotypes common in human infections and animal sources revealed many similarities showing the likelihood of *Salmonella* spread by the food chain. In terms of *S. Enteritidis*, which was the most prevalent Salmonella serovar isolated from human, both poultry and cattle can be considered as remarkable reservoirs of this serovar. *S. Enteritidis* was observed in chicken (34%) and bovine isolates (33.3%) and could also be detected in human samples as a result of food poisoning. Although *S. Typhimurium* was the second prevalent serovar identified in human isolates (41%), it was observed in a few chicken isolates. Furthermore, this serovar was of high prevalence in bovine isolates (66.6%). It could be concluded that human *S. Typhimurium* infections might originate from cattle. Vo et al. (2006) investigated Salmonella serotypes in livestock, meat, and human. They found that *S. Typhimurium* was the most predominant serovar in human isolates. Moreover, this serovar was repeatedly isolated from pork and pig feces. Consequently, pigs were also considered as an important reservoir of *S. Typhimurium*. In the study carried out by Angkititrakul et al. (2005), *S. Rissen* was found as the most prevalent serovar in human (20.4%) and pork isolates (61.5%), as well as the second common serovar in chicken meat (16.7%). In addition, these authors suggested that Salmonella contamination in pork and chicken meat may be the leading cause of salmonellosis in human. Using antibiotics is known as the most important factor promoting the emergence, selection, and dissemination of antibiotic-resistant microorganisms in both human and veterinary medicine (Neu, 1992). In the present study, the highest resistance was to streptomycin (43.5%), followed by tetracycline (35.7%),

trimethoprim and sulfonamide (33.6%), spectinomycin (33.1%), chloramphenicol (31.8%), florfenicol (25.4%), ampicillin (21.9%), kanamycin (15.9%), ceftazidime (12.9%), and cefepime (4.7%). On the other hand, the highest susceptibility was found to gentamycin (100%) and tobramycin (95.2%). We also detected a high percentage of multi-drug resistant Salmonella isolates. The antimicrobial resistance rate in chicken samples was higher than the two other groups. This higher rate can be attributed to the overuse of antibiotics for growth enhancement and treatment in poultry flocks leading to the maintenance of resistance genes in these bacteria (Table 2). Rahmani et al. (2013) stated that the resistance of Infantis serovar was higher, compared to the other serovars. As indicated in this study, the multiple antibiotic resistance of *Salmonella* serovars isolated from human was significantly correlated with cattle and chicken isolates in a specific

resistance pattern and a closely related pattern in pulsed-field gel electrophoresis. In the present study, *S.* Enteritidis isolates showed the lowest rate of antibiotic resistance. This may be due to mild clinical symptoms in most of the affected flocks or animals resulting in not treating with antibiotics. In contrast, the serovar Typhimurium was demonstrated to have the highest resistance. For a long time, ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol were used to treat Salmonella infections. However, due to the increasing rate of resistance to these antibiotics, the efficacy of these agents significantly reduced. Despite recommending the extended-spectrum cephalosporins for the treatment of invasive Salmonella infections, resistance to these antibiotics was soon observed. Thereafter, additional isolates were recognized (Winokur et al., 2000). In this research, cephalosporin resistance was found in Salmonella isolates among

Table 3. Antibiotic resistance rates for the most common serovars of human, chicken, and cattle

Species	Human			Chicken			Cattle	
Serovar	Enteritidis	Typhimurium	Infantis	Enteritidis	Typhimurium	Infantis	Enteritidis	Typhimurium
Antibiotic								
Ampicillin	16.27	48.78	9.09	0	0	15.78	0	37.5
Chloramphenicol	13.95	46.34	27.27	20	22.22	73.68	0	30
Florfenicol	18.6	29.26	18.18	20	22.22	68.42	0	22.5
Tetracycline	16.27	31.7	72.72	20	22.22	92.1	0	22.5
Streptomycin	13.95	68.29	63.63	16	22.22	92.1	0	40
Spectinomycin	2.32	46.34	45.45	8	44.44	86.84	0	32.5
Trimethoprim	13.95	26.82	63.63	12	44.44	86.84	15	25
Sulfonamides	11.62	29.26	63.63	12	44.44	86.84	15	25
Kanamycin	0	0	36.36	8	22.22	50	0	30
Tobramycin	0	0	0	0	0	0	0	0
Gentamycin	0	0	0	0	0	0	0	0
Cefepime	6.97	2.43	9.09	16	0	5.26	0	0
Ceftazidime	11.62	2.43	18.18	12	11.11	31.57	20	5
Ceftriaxon	0	0	9.09	4	0	0	0	0

period of time (P<0.05). Consequently, it can be concluded that resistant isolates can be transmitted to human through animal food products. Even if these isolates are not human pathogens, they can transfer the resistance genes to human pathogens. This transmission creates problems in treating human clinical cases of salmonellosis and other bacterial diseases. Kagambega et al. (2013) indicated that the *S. Typhimurium* isolates detected from poultry and human had the same

which poultry isolates showed the highest resistance, especially to ceftazidime. In addition, intermediate susceptibility to extended-spectrum cephalosporins was observed. As a result, the transmission of these resistant strains to human through the food chain will be a serious public health issue. In conclusion, the present study demonstrated that Salmonella serovars, including Typhimurium, Enteritidis, and Infantis were the most prevalent serovars in both human and animals. This

high prevalence suggested that Salmonella contamination in chicken and cattle may be regarded as the main salmonellosis source in human. Furthermore, we observed a high incidence of antibiotic resistance in Salmonella isolates. A close relationship was found between the antimicrobial resistance of animal and human isolates highlighting the role of food animal products as an important source of microbial resistance. Therefore, food animals must be taken into consideration and monitored in terms of Salmonella resistance to antimicrobial agents. Moreover, it should be noted that the administration of growth promoters and other medications must be restricted.

Ethics

The authors of this study hereby declare that all the ethical standards were followed in the procedure of preparing the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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