Original Article

Occurrence of multidrug-resistant Salmonella enterica serovar Enteritidis isolates from poultry in Iran

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ABSTRACT

Salmonella enterica is recognized as one of the major food-borne pathogens with more than 2,500 serotypes worldwide. The present study addresses antimicrobial resistance of *Salmonella enterica* serovar Enteritidis isolates in Iran. A collection of 151 *Salmonella spp*. isolates collected from poultry were serotyped to identify *Salmonella* Enteritidis. Sixty-one *Salmonella* Enteritidis were subsequently tested against 30 antimicrobials. A high frequency of antimicrobial resistance was observed against nitrofurantoin (n=55, 90.2%) followed by nalidixic acid (n=41, 67.2%), and cephalexin (n=23, 37.7%). Multi-drug resistance were observed in 35 (57.4%) out of 61 isolates. Twenty-six antimicrobial resistance patterns were observed among the 61 *Salmonella* Enteritidis. All isolates were susceptible to ofloxacin, imipenem, enrofloxacin, chloramphenicol, gentamicin, and 3rd and 4th generation cephalosporins. In conclusion, our results revealed that implementing new policies toward overuse of antimicrobial drugs in Iranian poultry industry are of great importance.

Keywords: antimicrobial resistance, resistance profile, multi-drug resistance, Salmonella enterica

INTRODUCTION

Salmonella enterica is a major foodborne pathogen with more than 2,500 serotypes (Grimont and Weill, 2007). Although typhoidal Salmonella serovars are host restricted (Uzzau et al., 2000), non-typhoidal Salmonella (NTS) are zoonotic agents, that are found in a wide range of animal reservoir (Hald et al., 2007) such as poultry, pigs, and cattle that are considered as the common vehicle of human salmonellosis (Braden, 2006). Globally, it is estimated that 93.8 million cases of gastroenteritis due to Salmonella species with 155,000 deaths occurs each year (Majowicz et al., 2010). Salmonellosis is mostly self-limiting and treatment with antimicrobials are not recommended, but this is not the case for very young, the elderly and immunocompromised patients (Martin et al., 2004; Varma et al., 2005). It has been proved that salmonellosis driven by drug-resistant strains has led to 30-50% longer duration of illness period, three times higher risk of hospitalization, and three times higher rate of death compared to pan-susceptible *Salmonella* (Molbak, 2004). *Salmonella* Enteritidis is among the most frequently reported serotypes in Iran (Rad et al.,

2008; Emaddi Chashni et al., 2009; Abdollahi et al., 2011). In a resistance trend study between 1996 and 2006 on clinical Salmonella Enteritidis isolates an increased level of resistance was observed for nalidixic acid (9.0% to 43.0%) and ceftazidime (3% to 23%)(Ashtiani et al., 2009). In a cross-sectional study including 1,950 fecal specimens from children with diarrhea, 26 Salmonella isolates were collected out of which 14 (54.0%) were identified as Salmonella Enteritidis. All the isolates were resistant to nitrofuratoin and ten (71.4%) to nalidixic acid. The resistance against streptomycin, co-trimoxazole, and tetracycline as the most common drugs prescribed for salmonellosis were 7.1%, 21.4%, and 28.6%, respectively (Eshraghi et al., 2010). In 2008, Ranjbar et al., detected 60 serogroup D isolates in a collection of 136 Salmonella spp.. Further research on resistance against ampicillin, chloramphenicol, co-trimoxazole, and tetracycline showed 6.7%, 3.3%, 8.3%, and 11.7% resistance rates, respectively. This collection demonstrated the highest level of resistance against doxycycline up to 51.7% (Ranjbar et al., 2008). In other continents, a study from New Zealand on antibiotic susceptibility of 1,560 human and 1,505 non-human Salmonella isolates between 2002-2009 revealed that almost all isolates were susceptible to ciprofloxacin and gentamicin (Broughton et al., 2010). Of non-human isolates in the United States of America between 1999 and 2003, there was increased sulfisoxazole, decreased tetracycline and fluctuating streptomycin resistance (Kiessling et al., 2007). In Morocco,150 Salmonella Enteritidis isolates obtained between 2000 to 2008 were subjected to seven antibiotics, resulted in 42% of resistance to at least one class of antimicrobial agent with the largest numbers of resistance to nalidixic acid (36%) (Ohmani et al., 2010). Nowadays, progressive increase in resistance rates of microorganisms against antimicrobial agents has complicated control programs of infectious diseases (Hendriksen, 2003a; Okeke et al., 2005). Among the main reasons in emergence of antimicrobial resistance, the role of misuse and overuse of antimicrobial drugs cannot be neglected. In the condition that, countries face growing trend of nonresponsive microorganisms against prescribed treatments, surveillance programs on drug resistance phenomenon will help by implement proper antimicrobial consumption strategies to prevent disastrous consequences of antimicrobial resistance phenomena (Anonymous, 2012a). This study was conducted to determine the level of antimicrobial resistance found in *Salmonella* Enteritidis among Iranian poultry population.

MATERIALS AND METHODS

Bacterial Isolates. During 2011-2012, a total of 585 fecal samples were collected and transferred to the Microbiology Dep. Laboratory of Razi Vaccine and Serum Research Institute Karaj, Iran. All the samples were collected from three different poultry abattoirs in Alborz (No. 35), Marakazi (No. 240), and Fars Provinces (No.310). Isolation and identification of samples were carried out according to standard procedures (Davies et al., 2001; Hendriksen, 2003b). Gram negative short-motile rods with characteristic red slope/yellow butt reaction on TSI (Merck, Darmstadt, Germany) with the production of H₂S were interpreted presumptively as *Salmonella*. To enable comparative analysis of the results a *Salmonella* Enteritidis type strain (ATCC 13076) was added to our collection.

Serotyping. All the biochemically confirmed isolates were serotyped using slide agglutination with standard antisera (Mast, Bootle, England) for somatic and flagellar antigen identification according to the Kauffman-White classification scheme (Grimont and Weill, 2007).

Antimicrobial Susceptibility Testing. The Kirby-Bauer disc-diffusion test, was used according to CLSI standards (Anonymous, The following 2012b). antibiotic discs were used: amikacin, amoxicillin, ampicillin, ampicillin-sulbactam, cefazolin, cefepime, cefixime. cefotaxime. ceftizoxim. ceftazidime. ceftriaxone, cefuroximesodium, cephalexin, cephalothin, chloramphenicol, ciprofloxacin, cotrimoxazole, enrofloxacin, florfenicol, furazolidone, gentamicin, imipenem, kanamycin, nalidixic acid, neomycin, nitrofurantoin, ofloxacin, piperacillin, streptomycin, and tetracycline (Padtan Teb Co. Tehran, Iran). The ATCC reference strain *Escherichia coli* ATCC 25922, was used for quality control purposes.

RESULTS

One-hundred-fifty-one (25.8%) Salmonella isolates out of 585 were analyzed of which, 61 (40.4%) were positive for Salmonella Enteritidis. All the isolates originated from three poultry abattoirs comprised of different urban regions; Arak (n = 29, 47.5%), Shiraz (n = 15, 24.6%), Qazvin (n = 10, 16.4%), Takestan (n = 10, 16.4%)6, 9.8%) and Abhar (n = 1, 1.6%). Antimicrobial susceptibility testing of 30 antimicrobial agents against the entire collection of isolates indicated that most resistance were observed towards nitrofurantoin (90.1%), nalidixic acid (67.2%), and cephalexin (37.7%) followed by furazolidone (18%), cephalothin (14.7%) and tetracycline (11.5%), respectively (Figure 1). On the other hand, the top eleven most susceptible drugs reported against the all isolates includes floxacin, imipenem, gentamicin, ceftazidime, cefotaxime, cefepime, ceftizoxime, chloramphenicol, ceftriaxone, and enrofloxacin (Table 1). Multi-drug resistance against three or more (≥ 3) antibiotics was a common phenomenon which were observed with 34 (55.7%) of 61 isolates. Twenty-five antibiotic resistance patterns were detected (Table 2). Overall, this rate of resistance against at least 2 (2 \leq), 3 (3 \leq), and 4 (4 \leq) drugs was 90.1%, 55.7%, and 22%, respectively. The most frequent resistance profile no.7 (Table 2) CN, FM, NA (cephalexin, nitrofurantoin, nalidixic acid) were obtained from 4 (6.5%) isolates. The largest panresistant pattern was a panel of 12 drugs consisting NA, SXT, TE, CN, AMX, FM, CZ, FR, AM, XM, CFM, SAM as only one isolate from Arak exhibited this pattern. It is noteworthy, that five (8.1%) of the 61 isolates of Salmonella Enteritidis were resistant to seven or more antibiotics, among which two were isolated from Arak and three from Qazvin.

DISCUSSION

Nowadays, food-borne diseases caused by nontyphoid Salmonellae represent an important public health problem. Intestinal salmonellosis will usually resolves spontaneously in five to seven days without the need to exert antibiotic therapy. However, this is not the case for those in which infection spreads beyond the intestinal tract. Hence appropriate and right choice of antimicrobial drugs are of great concern and in rare cases lifesaving (Hohmann, 2001). In this study, Salmonella Enteritidis was the predominant serotype isolated, comprising 40.4% of all isolates from five different cities of Arak, Oazvin, Takestan, Shiraz and Abhar. This prevalence rate is in agreement with the recent epidemiologic investigations in Iran (Eshraghi et al., 2010; Zahraei Salehi et al., 2005). Our findings demonstrated that, all isolates were resistant to at least one antimicrobial drug. Multi-drug resistance (MDR) was a remarkable observation in this study. The (CN, FM, NA) was the most prevalent pattern (6.5%) (Table 2) which corroborates our findings that these mentioned antimicrobial agents posses the three most resistance rate (Table 1) recorded against Salmonella Enteritidis isolates. Nalidixic acid is the prototype quinolone. It has been available in many countries since the mid 1960, but it's use which once has been restricted for a while (Crump et al., 2003), currently is widely prescribed against invasive and systematic infections caused by Salmonella spp. (Malorny et al., 1999; Giraud et al., 2006). Some of the researchers believe that use of fluoroquinolone in Veterinary Medicine has an indicative role in emergence and dissemination of nalidixic acid resistance in Salmonella in food animals, with high probability of transmission to humans (Angulo et al., 2004). Regarding the importance of this antibiotic from human health point of view and its potential risk of damage to growing cartilage (Bennish and Salam, 1992) we gained the 2nd rate of resistance among our poultry study group. Likewise In another study conducted on a human group, this drug has been reported as the 2nd most resistant antibiotic (74.1%) after doxycycline

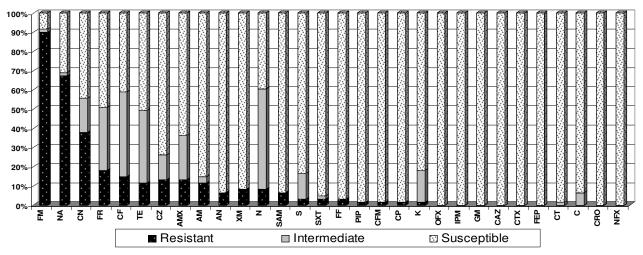


Figure 1. Antimicrobial Resistance aginst 30 drugs

Amikacin (AN), amoxicillin (AMX), ampicillin (AM), ampicillin-Sulbactam (SAM), cefazolin (CZ), cefepime (FEP), cefixime (CFM), cefotaxime (CTX), ceftizoxim (CT), ceftazidime (CAZ), ceftriaxone(CRO), cefuroxime sodium (XM), cephalexin (CN), cephalothin (CF), chloramphenicol (C), ciprofloxacin(CP), co-trimoxazole (SXT), enrofloxacin (NFX), florfenicol (FF), furazolidone (FR), gentamicin (GM), imipenem (IPM), kanamycin (K), nalidixic acid (NA), neomycin (N), nitrofurantoin (FM), ofloxacin (OFX), piperacilin (PIP), streptomycin (S), and tetracycline (TE)

(77.5%) (Firoozeh et al., 2011), which was in agreement with Morshed el al., 2010 investigation on a small group of nine human cases, with (77.8%) resistance rate of nalidixic acid followed by (66.7%) of flumequine. Furthermore In a trend study spanning 2001 till 2005, Ashtiani et al., 2009 showed a gradual increase in resistanc to nalidixic acid which correlate with our results. Our study identified the well-known antimicrobial drug of phenicol group, chloramphenicol, demonstrated no sign of resistance which seems, to be due to strict regulations implemented to ban its use on food animals.

Among those antibiotic with no signs of resistance phenomena, ceftazidime and cefixime, can be mentioned which were not in support of Morshed *et al.* assumption of respectively 6.9% and 24.1% resistance rate in their poultry subgroup of their study. Our findings were in agreement to Mirmahdavi and coworkers to some extent, who worked on antimicrobial resistance of 413 *Salmonella* isolates collected from human clinical cases against five antibiotics, belonged to 3rd generation cephalosporin including ceftizoxime, ceftriaxone, ceftazidime, cefotaxime, and cefoperazone.

All, except cefoperazone with 21% resistance rate were pan-susceptible. In another investigation carried out by Shapouri et al. on 240 chickens and eggs, the maximum susceptibility rate (100%) was observed against Gentamicin, Kanamycin and Nalidixic acid, while our findings were in consistent with the first two antimicrobial drugs but in quite contrast with Nalidixic acid which has been recorded as the second most resistant drug (Shapouri et al., 2009). The results obtained in this study showed a similar pattern of antibiotic resistance in the city of Arak and the city of Qazvin for antibiotics CF, FM, NA, CN; however, there are no patterns of antibiotic resistance in other cities. Although Takestan and Abhar are closer in distance to Qazvin, this similarity in pattern between the city of Oazvin & Arak indicate the poultry transportation between the two cities. Furthermore, antibiotic resistance, antibiotic greater than 7, can be seen in these two cities, which can be a reason for the uncontrolled use of antibiotics in these two cities. In conclusion, our results regarding high resistance rate of nitrofurans group of antibiotics in poultry population of our investigation, along with the potential risk of

Antimicrobials	Agent		Resistant No. (Percentages)		
Class	Acronyme	Disc Content (µg.)	<u>Poultry</u>		
agents	Acro	Disc C (L	<u>No.</u> (%)		
Aminoglycosides					
Amikacin	AN	30	<u>4</u> (6.5)		
Gentamicin	GM	10	0 (0)		
Kanamycin	K	30	$\frac{1}{2}$ (1.6)		
Neomycin	И	30			
Streptomycin	S	10	<u>2</u> (3.3)		
<u>Carbapenem</u>	103.6	10	a (0)		
Imipenem	IPM	10	<u>0</u> (0)		
Cephalosporins	07	20	9 (12.0)		
Cefazolin Combolisthin	CZ CF	30 30	$\begin{array}{cccc} & (13.0) \\ 9 & (14.7) \\ 4 & (6.5) \\ 23 & (37.7) \\ 1 & (1.6) \\ 0 & (0) \\ 0 & $		
Cephalothin			$\frac{9}{4}$ (6.5)		
Cefuroxime sodium Cephalexin	XM CN	30 30	$\frac{4}{23}$ (37.7)		
Cefixime	CFM	30	$\frac{25}{1}$ (1.6)		
Cefotaxime	CTX	30	$\frac{1}{0}$ (0)		
Ceftizoxime	CT	30	Ŭ (0)		
Ceftazidime	CAZ	30	ŏ ŏ		
Ceftriaxone	CRO	30	ŏ ŏ		
Cefepime	FEP	30	ບັ ໄປໃ		
Nitrofuran	1 1.1	20			
Furazolidone	FR	100	11 (18.0)		
Nitrofurantoin	FM	300	<u>11</u> (18.0) <u>55</u> (90.2)		
Penicillins			<u> </u>		
Amoxicillin	AMX	25	<u>8</u> (13.0)		
Ampicillin	AM	10	<u>7</u> (11.5)		
Ampicillin / Sulbactam	SAM	10+5			
Piperacilin	PIP	100	<u>1</u> (1.6)		
Phenicols					
Chloramphenicol	С	30	$\frac{0}{2}$ (0) $\frac{1}{2}$ (3.3)		
Florfenicol	FF	30	2 (3.3)		
<u>Quinolones & Fluoroquinolones</u>		_			
Enrofloxacin	NFX	5 5	$\begin{array}{ccc} \underline{0} & (0) \\ \underline{0} & (0) \\ \underline{1} & (1.6) \end{array}$		
Ofloxacin	OFX	5	$\frac{0}{1}$ (0)		
Ciprofloxacin	CP	5	$\frac{1}{41}$ (1.6)		
Nalidixic acid	NA	30	<u>41</u> (67.2)		
<u>Sulfonamide</u>	CVT	25	2 (3.3)		
Co-trimoxazole	SXT	25	<u>4</u> (c.c)		
Tetracycline Tetracycline	TE	30	<u>7</u> (11.5)		
renacychne	IL	20	<u>/</u> (11.5)		
Total =			<u>61</u> (100)		

Table1. Antimicrobial resistance in Salmonella Enteritidis isolated from Poultry

Table2.	Multi-drug resistance (MDR)	patterns	among 61	Salmonella Enteritidis	isolates

Antibiotype	Antibiotics	No.	%	Source	City
Resistotye 1	AN,FM,NA	3	4.9	Poultry	Arak
Resistotye 2	CF,CN,NA,	1	1.6	Poultry	Ghazvin
Resistotye 3	CF,FM,NA	3	4.9	Poultry	Ghazvin & Arak
Resistotye 4	CF,FM,PIP	1	1.6	Poultry	Shiraz
Resistotye 6	CN,FM,FR	1	1.5	Poultry	Shiraz
Resistotye 7	CN,FM,N	1	1.6	Poultry	Shiraz
Resistotye 8	CN,FM,NA	4	6.5	Poultry	Ghazvin & Arak
Resistotye 9	CZ,NA,TE	1	1.6	Poultry	Arak
Resistotye 10	FM,FR,NA	3	4.9	Poultry	Arak
Resistotye 11	FM,NA,TE	1	1.6	Poultry	Arak
Resistotye 12	FM,TE,XM	1	1.6	Poultry	Abhar
Resistotye 13	AN,CN,FM,NA	1	1.6	Poultry	Takestan
Resistotye 14	CN,CZ,FM,FR	1	1.6	Poultry	Shiraz
Resistotye 15	CN,FM,FR,N	1	1.6	Poultry	Shiraz
Resistotye 16	CN,FM,FR,TE	1	1.6	Poultry	Arak
Resistotye 17	CN,FM,N,NA	1	1.6	Poultry	Takestan
Resistotye 18	AM,AMX,CN,CZ,FM	1	1.6	Poultry	Arak
Resistotye 19	AM,AMX,CN,NA,TE	1	1.6	Poultry	Arak
Resistotye 20	AM,AMX,CZ,FM,NA	1	1.6	Poultry	Arak
Resistotye 21	CN,FM,FR,K,S	1	1.6	Poultry	Shiraz
Resistotye 22	AM,AMX,CF,CN,CZ,NA,SAM	1	1.6	Poultry	Ghazvin
Resistotye 23	AMX,FM,FR,NA,S,SXT,TE	1	1.6	Poultry	Arak
Resistotye 24	AM,AMX,CF,CN,CZ,FF,FM,N,NA,SAM	1	1.6	Poultry	Ghazvin
Resistotye 25	AM,AMX,CF,CN,CZ,FF,FM,N,NA,SAM,XM	1	1.6	Poultry	Ghazvin
Resistotye 26	AM,AMX,CFM,CN,CZ,FM,FR,NA,SAM,SXT,TE,XM	1	1.6	Poultry	Arak

resistance against nalidixic acid, demands new efforts to reduce the prevalence of resistant *Salmonella* in food animals. In view of high rate of sensitivity observed among quinolone class except nalidixic acid, carbapenem group and majority of 3rd generation of cephalosporins. Strategies including adoption of guidelines for the prudent use of antibacterial agents in animals used for food next to enforcing new foodsafety regulations are of great importance. Important investigations that need to be addressed in future include the most frequent serotypes circulating in the region and public health effect of emerging antimicrobial resistance related to those strains.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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