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Isolation and Determination of Antibiotic Resistance Patterns in Non-typhoid *Salmonella* spp isolated from chicken

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ABSTRACT

Background: Salmonellosis is one of the most common food borne diseases in industrial and developing countries. In recent years, an increase in antimicrobial drug resistance, among non-typhoid *Salmonella* spp has been observed.

Objectives: The aim of this study was to isolate and determine antibiotic resistance pattern in non-typhoid *Salmonella* spp.

Materials and Methods: This descriptive study was done on 100 samples of chickens collected from 196 retail markets and was examined for the presence of *Salmonella* using standard bacteriological procedures and stereotyping kit. Antimicrobial susceptibility testing was performed by disk diffusion methods according to the National Committee for Clinical Laboratory Standards (CLSI). The data were analyzed by using the SPSS software version 18.

Result: Forty- four percent of samples were contaminated with *Salmonella* infection and 56% didn't have any contamination. The stereotyping results showed that 34 of 44 isolates of *Salmonella* belonged to *Salmonella* infantis (79.5 %), one strain (2.3%) of group C and 8 strain (18.2%) of group D. However, all these strains were sensitive to Cefotaxime and Ciprofloxacin, and 100% were resistant to Nalidixic acid, Tetracyclin and Sterptomycin. The most common resistance pattern (34.1%) was towards six antibiotics, and 6.8% of strains were resistant to at least three antibiotics.

Conclusion: High levels of resistance to antibiotics that are used commonly for human and poultry can be a warning for our community health and this information must be used to form important strategies for improvement of infection control.

Keywords: *Salmonella*; Drug Resistance, Microbial; Anti-Infective Agents

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►Implication for health policy/practice/research/medical education:

The results of this study indicates the high prevalence of multiple drug-resistant in *Salmonella* food-borne pathogens in chicken. This information will aid the development of public health interventions designed to limit the spread of antimicrobial resistance in food animal production.

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1. Background

Illnesses from food are one of the most important economic and health problems among industrial and non-industrial countries. In recent years, *Salmonella* has been one of the most common causes of food born disease (1) Salmonellosis is an infectious disease which often occurs through contaminated food, especially food products with an animal origin such as meat, chicken, egg, animal foods and sometimes vegetables in the food chain (2, 3). In developing countries, estimation of Salmonellosis is difficult because there has not been sufficient surveillance (4-8). Therefore, globally, many studies have been performed reporting that the prevalence and kind of *Salmonella* serotypes are different based on geographical regions (9-11). *Salmonella* entericaserovars Enteritidis and Typhimurium were reported to be the two most frequent serotypes of *Salmonella* isolated in Iran and other countries (12-15). During the two past decades, the emergence of antibiotic-resistant *Salmonella* has become a serious problem worldwide. Wide usage of antibiotics in the diet of domestic animals has made drug resistant bacteria which could be transferred to human beings (16). Also, in recent years, problem of resistant strains to multiple drugs (MDR) is increasing and most studies in Iran and other countries have shown high resistance of *Salmonella* strains to several antibiotics (13, 17, 18).

2. Objectives

The aim of this study was to isolate and determine the antibiotic resistance pattern of non-typhoid salmonellae of chicken.

3. Materials and Methods

From November 2009 to December 2010, a total of 100 samples from chickens of a number of different super markets in Babol were tested for the presence of *Salmonella* spp.

The isolates were identified based on biochemical and serological tests according to the Institute of Standards and

Industrial Research of Iran (19).

Bacteriology, biochemistry and serologic tests:

Primary enriching stage

25 grams of chicken was weighed and added to 225 ml of BPW and incubated for 24 hrs at 37°C. Secondary enrichment stage (Selective): 0.1 ml of BPW media was added to 9.9 ml of Broth Rappaport-vassiliadis (R.V Merk), followed by incubations for 24 and 48 hours at 37°C and 43°C, respectively. Isolation: a loop of each enriched medium was cultured on Hecton agar (HE Merk) and incubated at 37°C. Serotyping of *Salmonella* spp, isolates was done by slide agglutination (Bio Merieux and DIFCO kits) and compared with the Kauffmann-White scheme.

Antimicrobial susceptibility test:

Antimicrobial susceptibility test was performed by the disk agar diffusion method (DD) according to the standard protocol of CLSI recommendation (20). The following antimicrobial disks (HiMedia) were used for the antimicrobial susceptibility test; gentamycin (GM 10µg), trimethoprim (SXT 5µg), nalidixic acid (NA30 µg), ciprofloxacin (CP 5µg), cefotaxime (CTX 30µg), imipenem (IPM 10µg), colistin (CL 10µg), ceftazidime (CAZ 30µg), amoxicillin (AMX 30µg), ampicillin (AM 10µg), chloramphenicol (C 30µg), streptomycin (S 10µg), tetracycline (TE 30µg).

Data were analyzed using the SPSS statistical software version 18.

4. Results

Forty-four % of samples were contaminated with *Salmonella* infection and 56% didn't have any contamination. The serotyping results showed that 34 of 44 isolates were related to *Salmonella* infection (79.5 %), one strain (2.3%) was from group C and 8 strains (18.2%) were from group D. However, all these strains were 100% resistant to nalidixic acid, tetracycline and streptomycin respectively (Table 1). Moreover, 34.1% of the isolates had multiple resistance to more than three antibiotics (Table 2).

Table 1. Percentage of Isolates Susceptible, Moderately Susceptible or Resistant to Each Antibiotic Disk Diffusion Method

Antibiotics	Sensitive	Intermediate	Resistance
Nalidixic acid	0.00	0.00	100
Tetracyclines	0.00	0.00	100
Streptomycin	0.00	0.00	100
Amoxicillin	30	0.00	70
Chloramphenicol	28	08.0	64
Trimetoprim	20	14	66
Amoxi/Clavulanic	34	34	32
Ceftazidime	44	28	28
Colistin	76	0.00	24
Ampicillin	80	10	10
Gentamicin	98	0.00	02.00
Imipenem	98	02.00	0.00
Cefotaxime	100	0.00	0.00
Ciprofloxacin	100	0.00	0.00

Table 2. Multiple Antibiotic Resistance Patterns of Salmonella

Antibiotics	Isolated, No.	Multiple resistances, No. (%)
NA, TE, S, AMX, C, SXT, CAZ, CL	02.00	04.00(9.1)
NA, TE,S, AMX, C, SXT, CAZ,GM	01.00	
NA, TE,S, AMX, C, SXT, CAZ,AM	01.00	
NA, TE, S, AMX, C, CAZ, CL	03.00	05.00(11.4)
NA, TE,S, AMX, C, CAZ,AM	01.00	
NA, TE,S, AMX, C,SXT,CL	01.00	
NA, TE, S, AMX, C, SXT	6	12 (27.3)
NA, TE,S, AMX, C,CAZ	03.00	
NA, TE,S, AMX, C,CL	01.00	
NA, TE,S, AMX, SXT,AM	01.00	
NA, TE,S, AMX, SXT,CL	01.00	
NA, TE,S, C, SXT	05.00	15 (34.1)
NA, TE,S,SXT,AMX	05.00	
NA, TE,S, C,AMX	04.00	
NA, TE,S,AMX,CL	04.00	
NA, TE, S, SXT	03.00	03.00 (6.8)
NA, TE, S	03.00	03.00 (6.8)

5. Discussion

Food borne diseases caused by non-typhoid *Salmonella* are found to be a major public health problem in many parts of the world (21). This report shows that 44% of tested samples were contaminated with *Salmonella* spp. There are a vast number of reports in the literature about the isolation of *Salmonella* in poultry products. The high prevalence of *Salmonella* spp in raw chicken samples found in this study agrees with data from other studies (10, 13, 15, 22, 23).

The most common isolated stereotype of this study was *Salmonella entericaserovarInfantis* (79.5%). Although in many performed studies, *Salmonella typhimurium* or *Salmonella interitidis* were separated from meat materials (13, 15, 24, 25) but the prevalence of food contamination by *Salmonella infantis* has been shown to be increasing (21, 26, 27). However, the variety of isolated serotypes are different according to geographical region and food. However, the variety of isolated serotype are different according to geographical region and food. These differences could be created via different ways such as environmental contamination, control system in transferring contamination and methods of sampling.

Antimicrobial susceptibility of *Salmonella* strains is useful for epidemiological purposes. In this study, 100% of isolated strains were resistant to NA, TE, and S. In the survey of Soltan Dalal et al. and Shapori et al. resistance to NA were reported in 90.6% and 57.7% of cases respectively (13, 15). In the different studies, high resistance of *Salmonella* strain to NA has been reported because this

antibiotic is used for the treatment of salmonellosis, other bacterial infections of humans and in the live-stock raising centers.

Our results indicate that among the *Salmonella* strains 88.7% had resistance to more than 3 antibiotics. The most common resistance pattern (34.1%) was towards six antibiotics (including NA, TE, S, AMX, C, SXT) and the second most common resistance pattern was towards eight antibiotics (9.1%) (including NA, TE, S, AMX, C, SXT, CAZ, CL) and 6.8% of strains were resistant to 3 antibiotics, (NA, TE and S) (Table1). Based on the previous literature, resistance to 3 different classes of antibiotics is defined as a multi-drug resistance (28). Previous reports have indicated that the amount of multidrug resistance ranges between 5-60% and this is similar to that found for different regions of Iran (29 - 31). Stevenson et al. (17) demonstrated the increased resistance of *Salmonella enterica* strains to NA and reported that the common resistance pattern was towards NA, AM, C, S, SXT and TE (17); these reports are similar to our findings and the study by Soltan Dalal (13). The reasons behind the increasing resistance of species that cause food born diseases are the uncontrolled use of antibiotics in veterinary medicine that cause destruction of sensitive bacteria and selection of resistant strains to multiple antibiotics. Through food, these strains can directly infect humans or transfer resistance genes to human endogen (25). Limiting the use of antibiotics in human

and animals, doing some antibiotic sensitivity tests for selection of suitable drugs and considering drug dose and treatment duration can decrease the spread of resistant strains.

Our study revealed a high prevalence of *Salmonella infantis*, therefore, continuous supervision for avoiding these kinds of contaminations of food seems necessary. Also we suggest that for recognizing and analyzing multi drug resistance of *Salmonella* strains, genetic investigation of resistant gene in these strains should be performed.

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Authors' Contribution

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