African Journal of Microbiology Research Vol. 6(7), pp. 1485-1488, 23 February, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1339
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Antimicrobial resistance in *Salmonella* isolated from broiler chicken carcasses

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Accepted 9 December, 2011

The development of antimicrobial resistance has been a public health issue worldwide. The aim of this study was to determine the antibiotic resistance pattern of *Salmonella* species isolated from broiler chickens in Iran. 37 isolates of *Salmonella* recovered from broiler chicken carcasses were analyzed for resistance to 14 antimicrobial agents. *Salmonella* isolates were serogrouped by standard agglutination test using O and H antisera before antibiotic susceptibility testing. Serogroup screening showed 4 different serogroups (D1, B, C1 and C2) among 37 *Salmonella* isolates. *Salmonella* serogroup D1 with frequency 48.64% was the most prevalent serogroup. Antimicrobial susceptibility testing was performed by the standard disc diffusion method according National Committee for Clinical Laboratory Standards. The results showed that Amikacin, Cephalotin, Tylosin,and Chloramphenicol, were the most effective antibiotics (100% sensitivity). Antibiotics for which *Salmonella* isolates exhibited resistance were: Neomycin (10.81%), Amoxicillin (18.91%), Streptomycin (29.72%), Chlortetracycline (32.43%), Trimethoprim (13.51%), Nalidixic acid (18.91%), Tetracycline (29.72%), Kanamycin (16.21%), Ampicillin (13.51%). Also 51% of resistant isolates belonged to serogroup D1 and serogroups B, C1, and C2 included 37, 9 and 3% of resistant isolates respectively. Overall 28% of *Salmonella* isolates were resistant to more than 4 antibiotics (MDR). Findings of present study indicated that the development of antimicrobial resistance in *Salmonella* is an increasing problem in Iran and resistant strains are widespread.

Key words: Resistance, antimicrobial, *Salmonella*, chicken.

INTRODUCTION

Salmonellosis is an important health problem and a major challenge worldwide. *Salmonella* spp are recognized as the most causative agents of food poisoning. These organisms are Gram negative and rod shape which have been divided into over 2700 serotypes based on somatic, flagellar and capsular antigens (Gallegos et al., 2008). *Salmonella enterica* seovar Typhimurium and *S. enterica* serovar Enteritidis are the most frequent isolated serovars worldwide (Chiu et al., 2010). Foods of poultry origin such as poultry meat and poultry egg have been implicated as important sources of human *Salmonella* infections (Doyle et al, 2007). Poultry are commonly infected with different *Salmonella* serovars. *Salmonella pullourum* and *Salmonella* gallinarum are two host specific serovars in poultry. Since the antibiotics discovery in the 1940, they have been widely used in both animals and humans. However, many important human and animal pathogens have developed resistance to these compounds (Musgrove et al., 2006, Yismaw et al., 2007). The widespread and indiscriminate use of antibiotic in the treatment of poultry diseases has lead to an increase in the number of resistant *Salmonella* strains isolated from poultry (Enabulele et al., 2010). In Iran factors such as insufficient control of drug prescription, and widespread use of antibiotics in the treatment and prophylaxis of salmonellosis, promote and favour drug resistance. Therefore the antibacterial resistance in *Salmonella* is an increasing problem in Iran .The R-plasmid has clinical importance in the acquisition of antibiotic resistance among bacteria. The antibiotic sensitive bacteria may acquire resistance traits from antibiotic resistant strains and thus become resistant to antibiotics (Mandal et al., 2003). Antibiotic resistant organisms lead to increase health costs and mortality and have therefore become an important health concern due to serious consequences of the treatment of disease. In

Table 1. The results of serogroup screening of *Salmonella* isolated from broiler chicken carcasses in Ardebil province, Iran.

Salmonella serogroup	Frequency	
	No	Percentage (%)
D1	18	48.64
B	11	29.72
C1	5	13.51
C2	3	8.10
Total	37	100

developed countries effective control programs of antibiotic use, have successfully reduce the prevalence of antibiotic resistant bacteria (Enabulele et al., 2010). Several published studies in Iran and other countries have reported on antimicrobial resistance in *Salmonella* (Zahraei et al, 2005; Pokharel et al., 2009; Wei et al., 2011; Mohanty et al., 2006; Murugkar et al., 2005; Selvaraj et al., 2010). Knowledge of the epidemiology of *Salmonella* antibiotic resistance and understanding their risk factors for infection are necessary for control of drug resistance in both animals and humans. The objective of the present study was antibiogram of different *Salmonella* strains isolated from broiler chickens in poultry farms in Ardebil province, Iran.

MATERIALS AND METHODS

Serogroup screening

37 *Salmonella* strains which were isolated from broiler poultry farms in Ardebil province, Iran were serogrouped in this study. Serogroup screening of isolates was performed by standard agglutination test using O and H antisera (Difco, USA) as described by Quinn et al. (1994).

Antibiotic susceptibility test

Antibiotic susceptibility testing of the *Salmonella* isolates was performed by the standard disc diffusion method according National Committee for Clinical Laboratory Standards (NCCLS, 1990, MA-A4) (Queen et al., 1994). Pure colonies of the *Salmonella* isolates was taken into 5 ml of normal saline and then was adjusted to McFarland standard turbidity No 0.5. The bacterial suspension was cultured over the entire surface of Muller Hinton agar (Merk) with sterile cotton swab. Antibiotic disc was placed into the inoculated plate surface and then was incubated for 24 h at 37°C. The antibiotic sensitivity of each *Salmonella* isolates was determined by measuring the zone of inhibition on the plate. 14 antimicrobial agents were used as follow: Neomycine (Ne 30 µg), Amoxycillin (Am 10 µg), Streptomycin (10 µg), Chlortetracycline (Ct 30 µg), Gentamicin (Gm 30 µg), Nalidixic acid (Na 30 µg), Trimethoprim (Tr 30 µg), Kanamycin (Km 30 µg), Tetracycline (Tc 30 µg), Ciprofloxacin (Cf 5 µg), Cephalotin (Ce 30 µg), Ampicillin (Ap 10 µg), Tylosin (Tyl 30 µg), Enrofloxacin (Ex 5 µg), (Queen et al, 1994).

RESULTS

Based on serological tests which carrid out by *Salmonella* specific antisera, four different serogroups (D1, B, C1 and C2) were found among 37 *Salmonella* isolates. As Table 1 shows the frequency of serogroup of D1, B, C1and C2 were 18 (48.64%), 11 (29.27%), 5 (13.51%), 3 (8.10%) respectively. *Salmonella* antibiogram results are summarized in Table 2. These results showed that Amikacin, Cephalotin, Tylosin and Chloramphenicol, were the most effective antibiotics (100% sensitivity). Antibiotics for which *Salmonella* isolates exhibited resistance were: Neomycin (10.81%), Amoxicillin (18.91%), Streptomycin (29.72%), Chlortetracycline (32.43%), Trimethoprim (13.51%), Nalidixic acid (18.91%), Tetracycline (29.72%), Kanamycin (16.21%), Ampicillin (13.51%). Intermediate sensitivity was found towards 11 antibiotics as showed in Table 2. Also 51% of resistant isolates were belonged to serogroup D1 and serogroups B, C1, and C2 included 37, 9 and 3% of resistant isolates respectively. Overall 28% of *Salmonella* isolates were resistant to more than 4 antibiotics (Multi Drug Resistant= MDR).

DISCUSSION

The results of serogroup screening of 37 *Salmonella* isolates showed that strains of serogroup D1 with 48.64% prevalence rate and strains of serogroup B with 29.72% of frequency were the most dominant isolates. Moreover antibiotic susceptibility testing showed that the most of resistant isolates were belonged to serogroups D1 and B. As the main pathogen to cause food borne disease, *Salmonella* has been frequently reported among different animal sources especially more divergent *Salmonella* serovars found in chickens (Chiu et al., 2010). Other studies which were carried out in different geographic area in Iran and other countries agree with the results of present study. Zahraei et al. (2005) and Akbarmehr (2010) have reported *Salmonella* serogroups D1and B, as two dominant serogroups in Fars and East Azarbayjan provinces in Iran respectively. This may be due to dominant role of *S.* enteritidis (belongs to serogroup D1) and *S.* typhimurium (belongs to serogroup B) as two main zoonotic and broad-host range pathogens in poultry salmonellosis (Chiu et al., 2010; Madadgar et al., 2008; Akbarmehr, 2010). It must be considered that the prevalent *Salmonella* serovars are different between chickens and associated with chicken lines and geographic area. Factors influencing the prevalence of chicken-associated *Salmonella* are feeds and growth environment, transportation process, and chicken sources (Chiu et al., 2010). Antibiotc susceptibility of 37 *Salmonella* isolates was performed by 14 different antimicrobial agents. As Table 2 shows *Salmonella* isolates were most sensitive towards Amikacin, Cephalotin,

Table 2. Antibiotic sensitivity of *Salmonella* spp. isolated from broiler chicken carcasses in Ardebil province, Iran (n=37).

Antibiotic	Sensitive		Intermediate		Resistant	
	Frequency	(%)	Frequency	(%)	Frequency	(%)
Neomycin	27	72.97	6	16.21	4	10.81
Amoxicillin	25	67.56	5	13.51	7	18.91
Streptomycin	22	59.45	4	10.81	11	29.72
Chlortetracycline	25	59.45	0	0	12	32.43
Gentamicin	34	91.89	3	8.10	0	0
Trimethoprim	28	75.67	4	10.81	5	13.51
Nalidixic acid	21	56.75	9	24.32	7	18.91
Chloramphenicol	37	100	0	0	0	0
Enrofloxacin	35	94.59	2	5.40	0	0
Tetracycline	22	59.45	4	10.81	11	29.72
Cephalotin	37	100	0	0	0	0
Kanamycin	24	64.86	7	18.91	6	16.21
Amikacin	37	100	0	0	0	0
Ampicillin	20	54.05	12	32.43	5	13.51
Ciprofloxacin	34	91.89	3	8.10	0	0
Tylosin	37	100	0	0	0	0

Tylosin, and Chloramphenicol, followed by Ciprofloxacin, Gentamicin and Enrofloxacin. The highest resistance was observed for Chlortetracycline, Streptomycin, and Tetracycline, followed by Amoxicillin, Nalidixic acid, Kanamycin, Trimethoprim, Ampicillin, and Neomycin. The antimicrobial resistance of the *Salmonella* isolated from poultry may show misuse of antibiotics in animal husbandry or may be due to presence of integron or plasmid in *Salmonella* (Chiu et al., 2010; Zahraei et al., 2005). Also multidrug-resistant strains were found in 28% of total *Salmonella* isolates. MDR strains of *Salmonella* are now encountered frequently and the rate of MDR has increased considerably in recent years. Zahraei et al. (2005) in a study which was carried out in Fars province determined the antibiotic sensitivity of 30 *Salmonella* strains isolated from poultry. They reported that 20.6% of *Salmonella* strains exhibited multi-drug resistance which show lower rate compared to our results. Bhattacharya et al. (2011) who studied about antibiogram of *S. typhi* and *S. paratyphi* have found MDR in 12.75% of *Salmonella* isolates. They also reported the resistance to chloramphenicol in 8.97 and 23.44% of *S. typhi* and *S. paratyphi* A isolates respectively. Our results in the present study showed relatively more MDR strains compared with the above research. Moreover in our study, all the *Salmonella* strains were sensitive to chloramphenicol (100% sensitivity) which implies the limited use of chloramphenicol in veterinary medicine in Iran. In another study, Musgrove et al. (2006) reported that *S. typhimurium* was the most prevalent serotype and demonstrated the greatest multiple resistance. Pokharel et al. (2009) in a study which was carried out in Nepal reported that *S. paratyphi* was most sensitive towards Amikacin and Ciprofloxacin which agree with our results. Although it must be considered that the incidence of resistant, susceptible and intermediately resistant isolates differed from procedure to procedure following the performance of disc diffusion assay (Cotter et al. 2001). In Iran the use of antimicrobial drugs are not well controlled. Thus most of microbial resistance may be due to widespread use of antibiotics in treatment of animal disease. Many reports support the hypothesis that the presence of plasmids together with the usage of antimicrobial agents in human medicine and veterinary may largely contributed to the spread of antibiotic resistance (Carattoli, 2003). Findings of present study indicated that the development of antimicrobial resistance in *Salmonella* is an increasing problem in Iran and resistant strains are widespread. Hence antimicrobial susceptibility test must be performed before using antibiotics in the treatment or prophylaxis of disease.

ACKNOWLEDGEMENTS

This work was supported by the Islamic Azad University of Sarab Branch. We are thakfulful to Mr. S. S. Taheri and Mr. Rezanavaz for their valuable collaboration.

REFERENCES

Akbarmehr J, zahraei Salehi T, Nikbakht GH (2010). Identification of *Salmonella* isolated from poultry by MPCR technique and evaluation of their hsp *groEL* gene diversity based on the PCR-RFLP analysis. Afr. J. Microbiol. Res., 4(15): 1599-1604.

Bhattacharya SS, Das U, Choudhury BK (2011). Occurrence and

antibiogram of *Salmonella typhi* and *S. paratyphi* A isolated from Rourkela, Orissa. Indian J Med Res., 133: 431-433.
Carattoli A (2003). Plasmid-Mediated antimicrobial resistance in *Salmonella enterica* Mol. Biol., 5: 113-122.
Cotter G, Adley CC (2001) Comparison and evaluation of antimicrobial susceptibility testing of enterococci performed in accordance with six national committee standardized disk diffusion procedures. J. Clin. Microbiol., 39(10): 3753-3756.
Doyle MP, Beuchat LR (2007). Food Microbiology.third edition ASM Press.Washington DC, pp. 187-219.
Enabulele SA, Amune PO, Aborisade WT (2010). Antibiograms of *Salmonella* isolates from poultry in Ovia North East local government area Edo State, Nigeria. Agric. Biol. J. North Am., 1(6): 1287-1290.
Gallegos R, Loredo A, Ojeda G, Vega A (2008). Identification of *Salmonella* serotypes isolated from cantaloupe and chile pepper production system in Mexico using PCR-RFLP. J. Food Protect., 71(11): 2217-2222.
Lan-Ho C, Cheng-Hsun C, Yan-Ming H, Chien-Shun C, Chien-Yu L (2010). Characterization of 13 multi-drug resistant *Salmonella* serovars from different broiler chickens associated with those of human isolates.BMC Microbiology., 10(86): 1471.
Madadgar O, Zahraei ST, Tadjbakhsh H, Mahzounieh M, Feizabadi M (2008). Genomic and phenotypic evaluation of *Salmonella typhimurium* and *Salmonella enteritidis* in Iran. Comp. Clin. Pathol., 17: 229-235.
Mandal S, Mandal MD, Pal NK (2003). R-Factor in *Salmonella enterica* serovar Typhi: transfer to and acquisition from *Escherichia coli*. Jpn. J. Infect. Dis., 56: 65-67.
Mohanty S, Renuka K, Sood S, Das BK, Kapil A (2006). Antibiogram pattern and seasonality of *Salmonella* serotypes in a North Indian tertiary care hospital. Epidemiol. Infect., 134: 961-966.
Murugkar HV, Rahman H, Kumar A, Bhattacharya D (2005). Isolation, phage typing, and antibiogram of *Salmonella* from man and animals in northeastern India. Indian J. Med. Res., 122: 237-242.
Musgrove MT, Jones DR, Nurthcutt JK, Cox NA, Harrison MA, Fedorka PJ, Ladely SR (2006) Antimicrobial resistance in *Salmonella* and *Escherichia coli* isolated from commercial shell eggs. Poult. Sci., 85(9): 1665-1669.
Pokharel P, Rai SK, Karki G, Katuwal A, Vitrakoti R, Shrestha SK (2009). Study of enteric fever and antibogram of *Salmonella* isolates at a teaching hospital in Kathmandu Valley. Nepal Med. Coll. J., 11(3): 176-178.
Quinn PJ, Carter ME, Markey B, Carter GR (1994). Clinical Vet Microbiology. Wolf Publishing., pp. 209-236.
Selvaraj R, Das R, Ganguly S, Ganguli M, Dhanalakshmi S, Mukhopadhayay SK (2010). Characterization and antibiogram of *Salmonella* spp. from poultry specimens. J. Microbiol. Antimicrobials, 2(9): 123-126.
Wei LS, Wee W (2011). Antibiogram and heavy metal resistance pattern of *Salmonella* spp. Isolated from wild Asian Sea Bass (Lates calcalifer) from Tok Bali, Kelantan, Malaysia. Jordan J. Biological Sci., 4(3): 125-128.
Yismaw G, Negeri C, Kassu A, Tiruneh M, Mulu A (2007). Antimicrobial resistance pattern of *Salmonella* isolates from Gondar university hospital, Northwest Ethiopia. Ethiopian Pharmaceut J., 25(2): 85-90.
Zahraei Salehi T, Mahzounieh M, Saeedzadeh A (2005). The isolation of antibiotic-resistant *Salmonella* from intestine and liver of poultry in Shiraz province of Iran. Int. J. Poul. Sci., 4(5): 320-322.