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Full Length Research Paper

Isolation of *Salmonella* spp. from poultry (ostrich, pigeon, and chicken) and detection of their *hilA* gene by PCR method

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Salmonella **serovars are one of the primary food-borne pathogens that cause infections in both animals and humans. The hyperinvasive locus A (*hilA*) gene has an important role in *Salmonella* pathogenicity. This gene encodes an ompR/ToxR transcriptional regulator that activates the expression of invasion gene and facilitates the entry of the bacteria into intestinal epithelial cells. Our goal of this research was to isolate *Salmonella* from poultry and detect their *hilA* gene by Polymerase Chain Reaction (PCR) technique. For this purpose 520 samples were obtained from ostrich, pigeon and chicken in poultry farms of East Azarbayjan province which is located in northwest of Iran. Samples were tested by bacteriological and serological tests. PCR was applied with one pair of primers targeting the *Salmonella hilA* gene. According to the results of bacteriological tests 45 *Salmonella* strains were found among 520 of total samples. The prevalence of *Salmonella* in ostrich, pigeon and chicken were determined as 6.66, 15.55, and 7.25%, respectively. *Salmonella* isolates represented four different serogroups (D1.B.C1, and C2) which *Salmonella* serogroup D1 was the most dominant serogroup. The PCR of the *hilA* gene produced a band of 854 bp with all the *Salmonella* isolates. This research indicated the relatively high prevalence of *Salmonella* spp in pigeon compare with ostrich and chicken and suggested that the *hilA* gene could be present in different *Salmonella* strains from different hosts in poultry.**

Key words: PCR, Ostrich, *Salmonella*, *hilA*, Iran.

INTRODUCTION

Salmonellosis has been considered one of the most important infectious disease in both humans and animals (Keusch, 2002). According to several studies the prevalence of *Salmonella* in poultry were reported in different areas of Iran (Zahraei et al., 2007; Jamshidi et al., 2009). The widespread occurrence of *Salmonella* in natural environment and the intensive husbandry practice used in the meat, fish and shellfish industries has been a significant problem in public health (Michael et al., 2007). *Salmonella* infections are caused by the ingestion of contaminated food or water, after which the bacteria are able to colonize the small intestine and invade intestinal enterocytes (Jennifer et al., 2002). The most common source of human salmonellosis is food of poultry origin. Human *Salmonella* infection can lead to several clinical conditions including enteric fever, enterocolitis and systemic infections (Piyush and Anju, 2008). The World Health Organization has estimated that annually there are close to 17 million cases of typhoid fever, with nearly 600,000 deaths, and 1.3 billion cases of acute gastroenteritis due to nontyphoidal salmonellosis, with 3 million deaths (Ivanoff, 1995; Pang et al., 1995).

In recent years, various molecular techniques that detect specific genes of *Salmonella* have been used as tools in epidemiological typing. During the process of *Salmonella* infection invasion genes are required for bacterial entry into host cells. *Salmonella* has evolved a highly complex regulatory scheme to control the expression of invasion genes. Environmental factors such as high osmolarity, low oxygen pressure and growth phase regulate expression of hyperinvasive locus A (*hilA*) an ompR/ToxR type transcriptional regulator that enhance bacterial invasiveness. In fact the *hilA* gene encodes a regulator that activates the expression of invasion genes in response to both environmental and genetic regulatory factors (Jennifer et al., 2002). *hilA* is

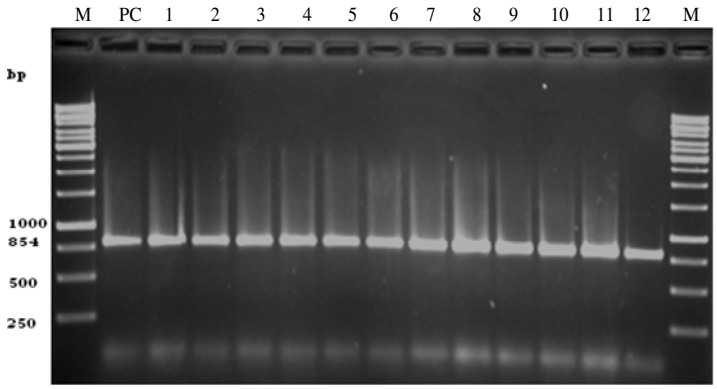

Figure 1. PCR results of hilA genes of different *Salmonella* srogroups, isolated from poultry. M: molecular weight marker PC: positive control (hilA gene of *Salmonella typhimurium* with ATCC 14025). Lanes 1, 2, 3 and 4, belonging to strains no 1, 5, 7, and 12 respectively (four different serogroups isolated from chickens), lanes 5, 6, 7, and 8, belonging to strains no 30, 32, 33, and 39, respectively (four different serogroups isolated from pigeons), lane 9 belonging to strain no 45 (serogroup D1 isolated from ostrich). Strain numbers and related serogroups in Table 1.

important for the regulation of the type III secretion apparatus, which is involved in the invasion of enterocytes (Lostroch et al., 2000). Murray and Lee (2000) have reported that the *hilA* gene is an important feature of *Salmonella* pathogenesis as it is requirepd for bacterial colonization of the extracellular, luminal compartment of the host intestine.

Several studies were carried out about the detection of *Salmonella hilA* gene including: direct PCR amplification of the *hilA* gene (Pathmanathan et al., 2003), validation of PCR for diagnosis of salmonellosis by amplification of *hilA* gene (Sanchez et al., 2004), detection of *hilA* gene sequences in *Salmonella enterica* (Cardona-Castro et al., 2002). But detection of *hilA* gene in *Salmonella* serovars that cause infections in poultry especially in pigeon and ostrich has not been widely studied. Therefore, in the present study we isolated *Salmonella* spp from poultry and detected their *hilA* gene by PCR method.

MATERIALS AND METHODS

Sampling and biochemical test

In all, 520 samples were collected from chickens (400 samples), pigeons (90 samples), and ostriches (30 samples) of poultry farms of Sarab city of Iran from December 2009 to May 2010. The samples were harvested from intestine, spleen and liver of poultry and inoculated into selenite-cystein broth (Merck Germany) for overnight enrichment at 37°C and later plated on MacConkey agar (Merck Germany). After 24 h incubation at 37°C non lactose fermenter colonies were confirmed by biochemical tests (Indole, Methyl Red, Voges-Proskauer, Citrate and urease). Serotyping of isolates was performed in serogroup level by standard agglutination test using O and H antisera (Difco, USA).

PCR amplification

Bacterial DNA extraction was carried out according to the methodology described by Zahraei et al. (2007). PCR was applied by the method of Cardona-Castro et al. (2002), using one pair primers targeting the *hilA* gene. *Salmonella* typhimurium with ATCC 14025 was used as positive control. The sequence of the primers is as follows:

Forward: 5'-CGGAACGTTATTTGCGCCATGCTGAGGTAG-3'
Reverse: 5'-GCATGGATCCCCGCCGGCGAGATTGTG-3'

PCR was carried out in a 50 µl reaction volume consisting of 100 mM Tris-HCl, 500 mM KCl, 0.20 mM dNTPs (Promega, Madison), 20 µm of each primer, 3 µl of genomic DNA, 25 mM $MgCl_2$, and 0.5 U of Taq DNA polymerase (fermentase). Amplification was performed in a thermal cycler (Biosystem). The cycling condition was as follows: initial denaturation 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 60 s, annealing at 65°C for 60 s, elongation at 72°C for 60 s, and final extension 72°C period for 10 min (Cardona-Castro et al., 2002). Amplified products were subjected to electrophresis at 100V on a 1.2% agarose gel and a 250bp DNA ladder was used as a size reference. After staining with ethidium bromide the gel was documented and photographed under UV light.

RESULTS

Out of the 520 samples which were examined by bacteriological tests, 45 samples (8.65%) had positive results for *Salmonella* as follows:

29 of 400 samples (7.25%) from chickens, 14 of 90 samples (15.55%), from pigeons, and 2 of 30 samples (6.66) from ostriches were positive for *Salmonella* strains. According to serological test results, the *Salmonella* isolates represented four different serogroups (D1, B, C1 and C2). Table 2 shows the frequency of the four different *Salmonella* serogroups isolated from poultry. Amplification of *hilA* gene produced 854 bp band with all the *Salmonella* isolates (Figure 1). PCR results showed the presence of *hilA* gene within all the *Salmonella* isolates (Table 1).

DISCUSSION

The epidemiological data in Table 1 and 2 show that *Salmonella* serogroup D1 with 53.3% frequency was the most dominant serogroup in three different hosts (ostrich, pigeon, and chicken). This result could be due to high prevalence of *S. enteritidis* (belonging to serogroup D) in poultry farms of Iran (Madadgar et al., 2008). The second epidemiological conclusion of this study was the relatively high prevalence rate of *Salmonella* in pigeon (15.55%) compare with ostrich (6.66%) and chicken (7.25%). Several studies were carried out in Iran and many different countries which showed the prevalence of *Salmonella* in chickens with different rates (Zahraei et al.,

Table 1. Salmonella serogroups isolated from poultry (ostrich, pigeon, and chicken) and PCR results of their *hilA* gene.

Strain no.	Source	*Salmonella* serogroup	PCR result for *hilA* gene
1	Chicken	D1	+
2	Chicken	D1	+
3	Chicken	D1	+
4	Chicken	D1	+
5	Chicken	B	+
6	Chicken	D1	+
7	Chicken	C1	+
8	Chicken	D1	+
9	Chicken	B	+
10	Chicken	B	+
11	Chicken	D1	+
12	Chicken	C2	+
13	Chicken	D1	+
14	Chicken	C1	+
15	Chicken	D1	+
16	Chicken	B	+
17	Chicken	B	+
18	Chicken	D1	+
19	Chicken	D1	+
20	Chicken	C1	+
21	Chicken	B	+
22	Chicken	D1	+
23	Chicken	D1	+
24	Chicken	C2	+
25	Chicken	C2	+
26	Chicken	D1	+
27	Chicken	B	+
28	Chicken	D1	+
29	Chicken	D1	+
30	Pigeon	B	+
31	Pigeon	B	+
32	Pigeon	D1	+
33	Pigeon	C1	+
34	Pigeon	D1	+
35	Pigeon	B	+
36	Pigeon	C1	+
37	Pigeon	B	+
38	Pigeon	D1	+
39	Pigeon	C2	+
40	Pigeon	D1	+
41	Pigeon	D1	+
42	Pigeon	D1	+
43	Pigeon	B	+
44	Ostrich	D1	+
45	Ostrich	D1	+

+ = presence of *hilA* gene.

2006; Zhao et al., 2001). In general it should be considered that the prevalence of *Salmonella* in chicken is varied depend on the geographic condition and the methods applied.

Table 2. Frequency of *Salmonella* serogroups among 45 *Salmonella* isolates.

Salmonella serogroup	Number	Percent
D1	24	53.3
B	12	26.6
C1	5	11.1
C2	4	8.8
Total	45	100

Authors in different countries have reported the prevalence of *Salmonella* in chicken caracasses with percentage ranging from 3 to 66% (Zhao et al., 2001) which agree with our results. Epidemiological study of salmonellosis in pigeon and ostrich has not been well studied in Iran. Therefore there is no data to compare with our results. In overall as the food of poultry origin is one of the most common source of human salmonellosis therefore the epidemiological result of this research revealed an important public health problem. Detection of the *hilA* genes of *Salmonella* isolates were the main goal of our study. As mentioned, this gene incodes an ompR/ToxR transcriptional regulator that activates the expression of invasion gene and has an important role in *Salmonella* pathogenicity. According to Figure 1 the *hilA* gene produced 854 bp band and was presented in all of the *Salmonella* isolates. As Table 1 and Figure 1 show the *hilA* genes were detected in all four *Salmonella* serogroups which isolated from three different hosts (ostrich, pigeon, and chicken). Several research groups have studied about *hilA* gene in different *Salmonella* serovars that cause human infections. Guo et al. (2000), used PCR to detect *hilA* gene in tomatoes contaminated with *S. enterica* serovar Montevideo. Pathmanathan et al. (2003), studied about the presence of the *hilA* gene in 33 *Salmonella* strains and 15 non-*Salmonella* strains by direct PCR amplification of the *hilA* gene. They detected the *hilA* gene in all the *Salmonella* strains and none of the non-*Salmonella* strains gave any amplification in PCR (indicated 100% specificity for *Salmonella hilA* gene). In the present study we detected the *hilA* gene in *Salmonella* strains belonging to ostrich and pigeon which has not been reported in previous studies.

Our results agree with the above studies and show the presence of the *hilA* gene in four different *Salmonella* serogroups isolated from three different sources with the same PCR bands. Finally the utilization of our results and previous studies which showed high specificity (100%) of the *hilA* gene in *Salmonella*, make more confident that the detection of this gene can be used for rapid diagnosis of *Salmonella* infections in poultry and other hosts by molecular techniques.

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