Investigating Plasmid Virulence Genes in Salmonella Isolated in Poultry of Ahwaz City

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Abstract
The aim of this study is to identify genes of position of plasmid virulence genes of S. Enteritidis isolated from poultry samples. In this cross-sectional study, 50 samples of Salmonella Enteritidis from poultry samples of Ahvaz city were collected, and after culture on specific medium were confirmed by biochemical tests. Salmonella is from important bacteria of Enterobacteriaceae family, which is very diverse in terms of biochemical and serological and most of them are pathogenic to humans and other animals. Salmonella of Enteritidis is part of three important and dominant serovar in most of countries in the world. Spv virulence plasmids are large and closed plasmids that only are seen in certain Salmonella. The results showed that a total of 50 samples of Salmonella Enteritidis, 18 samples (8.54 percent) had gene spvR and 16 samples (1.45 percent) had gene spvB. The high prevalence of genes spvR and spvB with the bacteria virulence is relevant completely and implies the role of these genes in the pathogenesis. Salmonella genetic change can also be effective in transfer of these plasmid factors between various forms.

Keywords: plasmid virulence genes, Salmonella enteritidis, spv

Introduction:
Salmonella is one of the common pathogenic bacteria agents common between human and poultry. In terms of public health, food poisoning caused by bacteria of salmonella group due to the number of serotypes of different hosts and effective role of natural vectors has particular importance (Nemaity et al., 2009). Salmonella is the important bacteria of the Enterobacteriaceae family that are very diverse in terms of biochemical and serological and most of them are pathogenic to humans and other animals. All Salmonella serotypes can cause disease in animals and in human cause diseases such as Gastroenteritis, enteric fever (typhoid and para-typhoid) and bacteremia. Gastroenteritis is the most common and the most common Salmonella infection in human that is created by serotypes of Salmonella specially Salmonella of typhimurium and enteritidis. Salmonella Enteritidis is part of three important serovar in most of countries in the world. The main tank of serovar is flocks of poultry, raw contaminated egg and meat that is considered the source and important vector of human infections (Amini et al., 2010). Virulence plasmids SPV are large and closed plasmids that only are seen in certain Salmonella. These plasmids carry genes that increase virulence, resistance of serum, cell growth and adhesion, as well as increase the capacity of the survival of the bacteria in the intestinal tissue in infected hosts. Strains lack of the virulence plasmid is very rapidly cleared from the spleen (Boyd and Hartl, 1998). Many serovars such as Salmonella dublin, Salmonella of typhimurium and Enteritidis have virulence plasmids that are common in most of isolates. Although specific plasmids of serovar SSP in multiple serovars in terms of content are not the same, but there is a wonderful area protected as spv (gene of virulence plasmid of Salmonella) that is responsible for virulence. SpvR that is a polypeptide with a weight of 33 kDa, as lysR is the component of regulator proteins. spvR is a protein with positive regulatory effect, it is necessary to express other parts and acts as a transcription of the downstream genes spvA, B, C, D during the stage of growth recession. SpvR protein increases the primary transcription from promoters spvR (Rutger and Kasadsos, 2010). The aim of this study is to identify genes spv position including spvB, spvC and spvR Salmonella Enteritidis isolated from poultry samples of Ahvaz.

Materials and methods
In this cross-sectional study, based on previous studies and confidence level 95% to 150 samples of poultry feces samples were collected in 2015 in Ahvaz city. The samples for culture on Mac Cancan Agar and CHROMagar Salmonella were cultured and incubated at 37 ° C for 24 hours (Frubas
For the extraction of DNA, kit Gram-negative bacteria Cinnagen -Cinna Pure DNA KIT-PR881613 was used. According to the sources used, PCR test program is as follows: primary Denaturation step is 94 °C for 5 minutes, denaturation step 94 °C for 1 minute, the connection stage as the following table in temperatures listed for 1 minute, extension step 72 °C for 1 minute (the number of 35 cycles), final extension step 72 °C for 10 minutes. Primers used in this test are listed in the following table (Huang et al., 2005). Standard strain of Salmonella Enteritidis with RTCC 1621 was used as a positive control. PCR test was performed on TECHNE device and final product was stored at 4 °C. The samples on agarose gel 1.5% have been transferred and after staining in the gel BIORAD dock was evaluated. Statistical data with SPSS-22 software and using descriptive statistics tests were analyzed.

<table>
<thead>
<tr>
<th>Temperature connection</th>
<th>Product Size</th>
<th>Target gene</th>
<th>Nucleotide sequence (5-3)</th>
<th>Name of primers virulence</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>660</td>
<td>spvC</td>
<td>CGGAAATACCATCTACAAATA</td>
<td>SPVC1</td>
</tr>
<tr>
<td>6</td>
<td>1771</td>
<td>spvB</td>
<td>CGGAAATACCATCTACAAATA</td>
<td>SPVB.F</td>
</tr>
<tr>
<td>6</td>
<td>890</td>
<td>spvR</td>
<td>CGGAAATACCATCTACAAATA</td>
<td>SPVR.F</td>
</tr>
</tbody>
</table>

**Table 1.** The sequence of primers of virulence of Salmonella enteritidis used in the Multiplex-PCR test

**Results and discussion**

After culturing and confirmed the presence of salmonella bacteria, biochemical tests performed with environments and Salmonella bacteria were confirmed. To determine the serotype, serotyping of samples with antiserum of Baharafshan was also performed and serum group was determined and samples of Salmonella Enteritidis were identified and finally 50 samples of bacteria were isolated.

Molecular test results showed that from a total of 50 samples of Salmonella Enteritidis isolated from poultry samples, the most frequency is related to spvR gene and gene spvC not recognized in any of the samples. Distribution of frequency of each of the genes separately in terms of the number and percentage in Figure 1 is listed. Results of PCR product on agarose gel and bands made with marker in Figure 1 have been identified.

![Figure 1. Frequency distribution of virulence genes of Salmonella enteritidis Bacteria studied in terms of the number and percentage](image)

In the study of Derakhshande and colleagues that was conducted in 2013 on samples of different serotypes of Salmonella collected from different regions of Shiraz, was specified that among 15 samples of Salmonella Enteritidis, the frequency of genes is spvR 60 percent, 93.3 spvC percent and 46.6 spvB percent. In their study also pointed out that genetic variation in Salmonella can be effective in transfer of virulence plasmid agents between spices. As a result of our research, non-isolating gene spvC can confirm the fact that the changes of genomic of this gene have a direct impact in isolating and identifying it. Coretta et al (2003) stated that Salmonella to create public
infection in mouse models and laboratory needs for the virulence plasmid spv. By removing the genes operon spv, virulence also greatly reduces. Virulence plasmid causes lack of connection of lysosomes to Fagvzm in immune cells, and on the one hand causes also planned death of immune cells, as well. By creating mutation in the area, the effects of virulence decreased. Matsui et al. (2001) stated that any mutation in operon genes spv reduces virulence in experiment of animal model of it and the spvB and spvA play the determinant role in this operon and attributed virulence agent to spvB and spvA. In various studies conducted in the world, the most common strains of Salmonella isolated from eggs contaminated are Salmonella enteritidis (Litel et al., 2006; Litel et al., 2007, Suresh et al., 2006). Hemmatzadeh and Salemi (1998) introduced Salmonella Enteritidis as the most common serovar isolated in humans, poultry and other livestock in the province of Chaharmahal and Bakhtiari in Iran. In the present study also compared with the results of other researchers, the highest frequency is reported of SpvR and spvB genes and higher incidence of these two genes is associated with the virulence of the bacteria completely and indicates the role of these genes in the pathogenicity. Genetic changes in Salmonella can be also effective in transfer of these plasmid factors between various forms.

Reference: