Molecular Detection of Shigella spp. Contamination in Ready-to-Eat Salad Samples in West of Tehran

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Abstract

Background: Shigella bacteria can infect human body by taking contaminated food and water, and are transmitted from person to person. Human body is the only natural host for these bacteria. Objective: The aim of this study was to detect Shigella contamination in pre-packed samples of salads at restaurants in western regions of Tehran, Iran, using polymerase chain reaction (PCR) method. Materials and Methods: To conduct this research, 90 samples were purchased from the restaurants during the period of June to November 2016. The samples were cut into very small pieces, homogenized and a 25 g portion of these samples was added to 225 mL of Shigella broth medium containing novobiocin and incubated for 24 hours. Then DNA of cultured samples was extracted using DNP™ kit (CinnaGen, Iran). PCR method was optimized for amplification of 613 bp segment of ipaH gene and performed on extracted DNA of all samples (before and after enrichment in Shigella broth). Results: Shigella contamination was detected in 7 (7.8%) and 20 (22.2%) of the tested samples before and after the enrichment, respectively. Conclusion: The results showed the contamination with Shigella bacteria in remarkable percentage of the samples and revealed the necessity of more attention and supervision in the processes of production and distribution of pre-packed salads.

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Background

Foodborne diseases are a global public health problem that affect millions of people every year and are caused by contamination with a variety of pathogens including bacteria, viruses, and parasites. The European Center for Disease Prevention and Control (ECDC) gathers and reports incidence data on common pathogens including Norovirus, Campylobacter, Salmonella, Shigella, Listeria, Escherichia coli, and hepatitis A that cause food-borne illnesses across Europe. Shigella spp. are virulent bacteria that belong to the Enterobacteriaceae family. The infectious dose of Shigella, in some cases, is as low as 10 bacterial cells. The transmission from person to person occurs through the fecal-oral pathway and also by contaminated food and water. The symptoms of shigellosis range from mild watery diarrhea to severe bacillary dysentery with fever, abdominal pain, blood and mucus in a stool sample. Shigella can grow in foods such as potato salad, tuna, shrimp and chicken, as well as raw vegetables, dairy products, meat and poultry. Shigella can also be spread through water sources. Vegetables with high fiber and vitamin content are favorite food of the people who care about proper diet. The consumption rate of such products has been increased in recent years. These foods could be the sources of some kinds of pathogens if they are used in raw forms and sporadic illnesses or outbreaks may be resulted from their consumption, therefore in order to prevent the spread of foodborne diseases, the safety of food must be ensured. Thus, to protect the public health, a rapid diagnostic method to detect foodborne pathogens must be recognized and used.

Objectives

Since there are few published surveys on Shigella contamination in vegetables and salad samples in Iran, the aim of this study was to detect this bacterium in pre-packed samples of salads at restaurants in western regions of Tehran, the capital city of Iran, using polymerase chain reaction (PCR) method.

Materials and Methods

Sampling

Ninety salad samples were purchased from the restaurants in western regions of Tehran during the period of June to...
November 2016.

**Standard Bacterial Strain**
Reference strain of *Shigella dysenteriae* (PTCC 1188) was prepared from IROST, and cultured on BHI medium and used as positive control for culture-based and molecular tests.

**Enrichment**
The samples were cut into very small pieces under sterile conditions. A 25 g portion of these samples was added to 225 mL of *Shigella* broth medium containing novobiocin and incubated at 37°C for 24 hours.

Then, 1.5 mL of medium after incubation was centrifuged at 1000 xg for 3 minutes, the sediment was subjected to DNA extraction using DNP™ kit (Cinna Gen, Iran).

**Preparation of the Samples for Direct PCR**
Twenty-five grams of the samples were cut to the small pieces under sterile conditions. The samples were mixed with 8 mL of distilled water for 1 minute in the test tube and were centrifuged at 1000 xg for 1 minute and the sediment was subjected to DNA extraction.

**Analysis of Extracted DNA**
The quantification and analysis of the extracted DNA was performed using 1.3% agarose gel electrophoresis. The results were visualized using gel documentation (E-Gel imager, UPV, Taiwan).

**Polymerase Chain Reaction**
PCR method was optimized for amplification of 613 bp segment of *ipaH* gene using the specific primer pair of RB87: 5’CGGTCAGCCACCTCTGAG-3’ and RB88: 5’-CTTGACCGCCTTTCCGATACC-3’ for detection of *Shigella* spp., and performed on extracted DNA of all samples (before and after enrichment in *Shigella* broth).

**PCR Mix**
The PCR mixture in a total volume of 25 µL contained: 12.5 µL Taq Mix (2X) with 1×final concentration (DELTA Life Sciences), 1 µL reverse primer (10 µM), 1 µL forward primer (10 µM), 8.5 µL sterile water, and 2 µL template DNA. The PCR amplification was performed for 35 cycles, which had different thermal conditions as shown in Table 1. The PCR products were analyzed using agarose gel electrophoresis with SYBR green staining.

**Results**
Amplification of 613 bp segment and *Shigella* contamination were detected in 7 (7.8%) and 20 (22.2%) of the tested samples before and after the enrichment, respectively (Figure 1). There was not any statistically significant difference between the frequency of contamination in different groups of the samples based on the type of lettuces and packaging (P > 0.05) (SPSS 23.0, using chi-square test; 0.05). The frequency of positive samples in different groups based on the type of packaging and lettuce has been shown in Tables 2 and 3.

**Discussion**
*Shigella* spp. are among the most common agents of foodborne gastroenteritis and are transmitted by consuming contaminated foods.

This study aimed to investigate the frequency of *Shigella* contamination in the pre-packed salad samples collected from the restaurants in the west of Tehran, Iran.

Due to the limitations of culture-based methods for isolation of *Shigella* and high sensitivity of molecular techniques, PCR was used in this study after enrichment

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**Table 1. Thermal Conditions of PCR**

<table>
<thead>
<tr>
<th>Initial Denaturation</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Number of Cycles</th>
<th>Final Extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>95°C, 15 min</td>
<td>94°C, 30 s</td>
<td>57°C, 60 s</td>
<td>72°C, 45 s</td>
<td>35</td>
<td>72°C, 5 min</td>
</tr>
</tbody>
</table>

**Table 2. Contamination of Salad Samples With *Shigella* spp., Based on the Type of Packaging**

<table>
<thead>
<tr>
<th>Positive Samples</th>
<th>Direct PCR</th>
<th>After Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual packaging</td>
<td>5 (6.4%)</td>
<td>15 (19.2%)</td>
</tr>
<tr>
<td>Automatic packaging</td>
<td>2 (16.6%)</td>
<td>5 (41.6%)</td>
</tr>
</tbody>
</table>

**Table 3. Contamination of Salad Samples With *Shigella* spp., Based on the Type of Lettuce**

<table>
<thead>
<tr>
<th>Positive Samples</th>
<th>Direct PCR</th>
<th>After Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese lettuce 15</td>
<td>1 (6.6%)</td>
<td>5 (33.3%)</td>
</tr>
<tr>
<td>Normal lettuce 51</td>
<td>6 (11.7%)</td>
<td>13 (25.4%)</td>
</tr>
<tr>
<td>Screw lettuce 24</td>
<td>0 (0%)</td>
<td>2 (8.3%)</td>
</tr>
</tbody>
</table>

**Figure 1.** Agarose Gel Electrophoresis of PCR Products. Lane 1: Ladder 100 bp; Lane 2: Standard strain of *Shigella dysenteriae*; Lane 3, 4, 7-10, 12: Positive samples; Lane 5, 6, 11, 13: Negative samples; Lane 14: Negative control.
in Shigella broth.

Currently, enrichment procedures use a low carbohydrate medium, Shigella broth (SB) with addition of novobiocin, for the detection or isolation of Shigella spp. Acid produced by other Enterobacteriaceae during the fermentation of carbohydrates have been reported to be toxic to Shigella; however other studies have shown the acid tolerance of Shigella spp. to grow at a pH of 4.5 to 4.75 and to survive at a pH of 4.0, since SB contains very little carbohydrate. The effect of low pH environment on the enrichment of Shigella is limited when this medium is used.\(^6\)

Comprehensive review on the results of previous surveys shows that shigellosis continues to be a major public health problem and remains endemic in many developing and developed countries. Moreover, antimicrobial resistance has been increasing among Shigella isolates and multidrug resistant Shigella infections are widespread.\(^2\)

In many published researches, the focus has been on the detection and isolation of the bacterium in the stool samples of people involved in the production and distribution processes of foods. Although the level of personal hygiene in societies has been increased significantly, there is still a high chance of getting Shigella infection via consumption of contaminated food. Contamination with the bacterium is not limited to the cases of direct contact of persons with each other or consuming the food contaminated during processing. Food materials can be contaminated by Shigella in the farms, therefore it is also important to investigate the infection source in planting and harvesting processes.\(^3,4,5\) The infectious dose of Shigella is as low as 10-200 organisms and the presence of few bacteria may result in acquiring infection from eating contaminated food. Therefore, sample enrichment increases the sensitivity of PCR method to detect the bacteria, as the finding of our study showed the contamination rate of 22.2% in the tested samples after enrichment in Shigella broth compared to the rate of 7.8% before enrichment (direct PCR).

In 1992, Dunn reported an outbreak of shigellosis in 46 patients in Michigan (USA) resulted from consuming tossed salad and declared that raw vegetables are a potential vehicle for transmission of Shigella.\(^1\) The high percentage of the positive samples and rate of contamination with Shigella in this research may be due to poor education and personal hygiene of people involved in the production and distribution of pre-packed salads and probably contaminated water sources for irrigation of the farms and because the human body is the only natural host of Shigella spp. This strengthen the hypothesis of the pollution of the water sources by sewage.

Since there has not been previously published survey on Shigella contamination in salad samples in Iran, more research needs to be carried out to reveal more details.

**Conclusion**

The results showed the contamination with Shigella bacteria in remarkable percentage of the salad samples that may be considered as a public health warning and revealed the necessity of more attention and supervision in the process of production and distribution of pre-packed salads.

Besides, the findings point to the importance of enrichment in increasing the sensitivity of the PCR method to detect the bacteria specially Shigella in food samples.

**Authors’ Contributions**

STT: Enrichment of the samples and molecular detection of Shigella contamination. NH: Supervision of the research and help in writing and editing the manuscript. LJ: Consulting the research.

**Ethical Approval**

This study does not need to have any ethical approval.

**Conflict of Interest Disclosures**

The authors declare that they have no conflict of interests.

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