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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 media 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.[1] *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.[2] *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.[3] 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.[4]

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

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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 ×g 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 ×g 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´CGGTCAGCCACCCTCTGAG-3′ and RB88: 5′–CTTGACCGCCTTTCCGATACC-3′ for detection of *Shigella* spp.,[5] 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

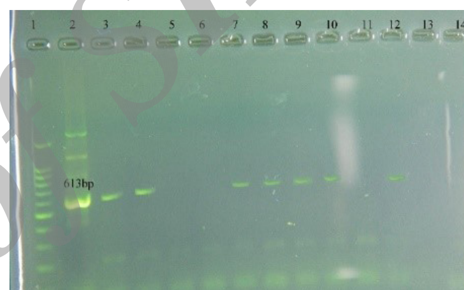

Figure 1. Agarose Gel Electrophoresis of PCR Products. Lane 1: Ladder 100 bp; Lane 2: Standard strain of *Shigella dysenteriae*; Lane 3 ,4, 7-10, 12: Positive samples; Lane 5, 6, 11, 13: Negative samples; Lane 14: Negative control.

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

	Positive Samples	
	Direct PCR	After Enrichment
Manual packaging 78	5 (6.4%)	15 (19.2%)
Automatic packaging 12	2 (16.6%)	5 (41.6%)

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

	Positive Samples	
	Direct PCR	After Enrichment
Chinese lettuce 15	1 (6.6%)	5(33.3%)
Normal lettuce 51	6 (11.7%)	13 (25.4%)
Screw lettuce 24	0 (0%)	2 (8.3%)

Table 1. Thermal Conditions of PCR

Initial Denaturation	Denaturation	Annealing	Extension	Number of Cycles	Final Extension
95°C,15 min	94°C, 30 s	57°C, 60 s	72°C,45 s	35	72°C, 5 min

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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.[7]

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.[8,9] 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 bacterium, 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 shigellosis and improper salad preparation is the important risk factor for acquiring the disease.[10]

In few other published surveys on *Shigella* contamination in salad samples, Mokhtari et al in Tunisia detected the bacterium in 7 out of 67 culture negative samples (10.4%) by real time PCR using primers targeting *ipaH* gene.[11] In the other research from these authors, 2% (4 out of 197) of salad samples were positive based on routine culture-based methods while PCR results showed the presence of *ipaH* gene in 8.6% (24 out of 280) of food samples including salad.[12] Finally, there was no positive sample in evaluation of 100 ready-to-eat salads in a culture-based survey carried out in Turkey by Cetinkaya et al.[13]

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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References

1. Hu K, Renly S, Edlund S, Davis M, Kaufman J. A modeling framework to accelerate food-borne outbreak investigations. Food Control. 2016;59:53-58. doi:10.1016/j.foodcont.2015.05.017
2. Jimenez KB, McCoy CB, Achi R. Detection of *shigella* in lettuce by the use of a rapid molecular assay with increased sensitivity. Braz J Microbiol. 2010;41(4):993-1000. doi:10.1590/s1517-83822010000400018
3. Acheson DWK. Food and Waterborne Illnesses. In: Encyclopedia of Microbiology. Elsevier; 2009:365-381. doi:10.1016/B978-012373944-5.00183-8
4. Zhao X, Lin CW, Wang J, Oh DH. Advances in rapid detection methods for foodborne pathogens. J Microbiol Biotechnol. 2014;24(3):297-312.
5. Binet R, Deer DM, Uhlfelder SJ. Rapid detection of *Shigella*

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and enteroinvasive *Escherichia coli* in produce enrichments by a conventional multiplex PCR assay. Food Microbiol. 2014;40:48-54. doi:10.1016/j.fm.2013.12.001
6. Warren BR. Comparison of conventional culture methods and the polymerase chain reaction for the detection of *Shigella* spp. on tomato surfaces [dissertation]. Florida: University of Florida; 2003.
7. Shiferaw B, Solghan S, Palmer A, et al. Antimicrobial susceptibility patterns of *Shigella* isolates in Foodborne Diseases Active Surveillance Network (FoodNet) sites, 2000-2010. Clin Infect Dis. 2012;54 Suppl 5:S458-463. doi:10.1093/cid/cis230
8. Ashrafi hafez A, Asadolahi E, Havasian M, et al. Study on the parasitic and microbial contamination of vegetables, and the effect of washing procedures on their elimination in Ilam city. J Paramed Sci. 2013;4(4):37-41.
9. Hosseinvand A, Sohrabvandi S, Mortazavian A. Assessment of the microbiological safety of some spices and raw vegetables in Tehran caterings. Arvand J Health Med Sci. 2016;1(2):108-111. doi:10.22631/ajhms.2016.43222
10. Dunn RA, Hall WN, Altamirano JV, Dietrich SE, Robinson-Dunn B, Johnson DR. Outbreak of *Shigella flexneri* linked to salad prepared at a central commissary in Michigan. Public Health Rep. 1995;110(5):580-586.
11. Mokhtari W, Nsaibia S, Gharbi A, Aouni M. Real-time PCR using SYBR Green for the detection of *Shigella* spp. in food and stool samples. Mol Cell Probes. 2013;27(1):53-59. doi:10.1016/j.mcp.2012.09.002
12. Mokhtari W, Nsaibia S, Majouri D, Ben Hassen A, Gharbi A, Aouni M. Detection and characterization of *Shigella* species isolated from food and human stool samples in Nabeul, Tunisia, by molecular methods and culture techniques. J Appl Microbiol. 2012;113(1):209-222. doi:10.1111/j.1365-2672.2012.05324.x
13. Cetinkaya F, Cibik R, Ece Soyutemiz G, Ozakin C, Kayali R, Levent B. *Shigella* and *Salmonella* contamination in various foodstuffs in Turkey. Food Control. 2008;19(11):1059-1063. doi:10.1016/j.foodcont.2007.11.004

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