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Contamination of Household Refrigerators by *Listeria* Species in Ahvaz, Iran

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A B S T R A C T

Background: *Listeria monocytogenes* has been isolated from various foods and environments in temperate areas, tropical countries and different parts of Iran. The bacterium as a psychrotrophic organism is capable of growth at refrigeration temperatures.
Objective: The current study was conducted to determine the incidence of *Listeria* spp. on the surfaces of domestic refrigerators in Ahvaz city as a tropic area, to provide insights in to true burden of, and the risks posed by the bacterium in domestic refrigeration systems.
Materials and Methods: During December 2009 – June 2010, 180 refrigerators located at student accommodations and private homes in Ahvaz, were sampled for the presence of *Listeria* spp. The temperature of each refrigerator was measured and owners were asked to fill out a questionnaire regarding the method of cleaning. All samples were tested by culture in *Listeria* enrichment broth (LEB), Oxford agar and PALCAM agar using standard methods. Suspected colonies were identified by biochemical tests.
Results: *L. monocytogenes* was present in 1 domestic refrigerator out of the 180 investigated (0.5 %) and *L. innocua* was also isolated from 2 refrigerators (1.2%). It was demonstrated that a significant number of the investigated refrigerators were operating at a temperature that can compromise the safety of the foods stored inside them. Also, most owners used mixture of water and dishwasher and some of them used water alone to clean their refrigerators.
Conclusions: Although the incidence of *L. monocytogenes* in domestic refrigerators in Ahvaz is low contamination of the stored food in refrigerator by the bacterium is still a concern. Two of the isolated *Listeria* were from student accommodations. It was found that most of the refrigerators used in student accommodations in comparison to private homes, were not cleaned in low frequency and had higher temperature.

Keywords: Listeria; Incidence; Refrigerator; Ahvaz; Tropical

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Implication for health policy/practice/research/medical education: The results of the current study indicated that some household refrigerators may be contaminated with pathogenic bacteria like *Listeria monocytogenes*. The study findings highlighted the importance of adequate temperature control and thorough and regular cleaning of domestic refrigerators with appropriate cleaning products in high frequency to ensure food safety

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1. Background

Listeria spp. are widespread in the environment and commonly found in soil, sewage, dust and water (1, 2). One particular species, *Listeria monocytogenes*, has been recognized as a food-borne pathogen since 1981, and is well known for its survival and growth at refrigeration temperatures (3).

The bacterium has been isolated from various foods and environments in temperate areas (4-9) and even in tropical countries (10-12) and Iran (13-15). The ability of the bacterium to adhere to various surface materials such as stainless steel, rubber, glass and polypropylene has been documented (16-18). Also, colonization of refrigerators by *L. monocytogenes* has been reported(19, 20). Refrigerators can support the growth of the bacterium, which can therefore increase to clinically significant numbers in foods stored for extended periods in domestic refrigerators (21, 22).

2. Objectives

The current study aimed to determine the incidence of *Listeria* spp. on the surfaces of domestic refrigerators, to provide insights into true burden, and the posed risks of the bacterium in domestic refrigeration systems.

3. Materials and Methods

During the period December 2009–June 2010, 180 refrigerators located at student accommodations and private homes in Ahvaz city, South-West of Iran, were sampled for the presence of *Listeria* spp. Before sampling, the temperature of each refrigerator was measured using a normal thermometer and the refrigerator owners were asked to fill out a questionnaire about cleaning time, method and the last date of washing, type of food stored in the refrigerator and type of packing food before the food stored in the refrigerator. From each refrigerator, three samples were taken from the interior surfaces (≈100 cm^2) including shelves, and bottoms of meat and vegetable drawers with sterile cotton swabs, previously immersed in sterile normal saline.

The swabs were transferred to 10 ml of *Listeria* enrichment broth (LEB, Merck, Germany) and incubated at 30 °C for 48 h. A loop full of each enrichment culture after 24 h and 48 h of incubation, was streaked separately onto PALCAM agar (Merck, Darmstadt, Germany) and Oxford agar plates (Merck, Darmstadt, Germany) containing Polymixin B (10 mg/L), Acriflavin (5 mg/L) and Ceftazidime (20 mg/L). After incubation at 37 °C for 48 h, at least 5 suspected colonies were sub-cultured on Brain Heart Infusion Agar (BHIA, Hispanlab) and incubated at 37° C for 48h. All the isolated colonies were characterized by Gram staining, motility at 25° C and 37° C, catalase and oxidase test and acid production from xylose and mannitol. For further confirmation, other biochemical reactions, β-haemolytic activity on 5% sheep blood agar (Merck) and CAMP test were performed according to Bergey's Manual of Systematic Bacteriology (23).

4. Results

As indicated in *Table 1* , *L. monocytogenes* was present in 1 domestic refrigerator and *L. innocua* was isolated from 2 refrigerators out of the 180 investigated. The questionnaire showed that most of the people who participated in the study used to put the fruits, vegetables and eggs without packing in shelves, but they kept ready-to eat or remaining foods, cheese, meat and other kinds of foods in a container or bowl and then stored in the refrigerator.

Data showed that a significant number of the investigated refrigerators were operating at a temperature that could compromise the safety of the foods stored inside them *(Figure 1)*. A temperature above 8°C was measured in about 25% of the investigated refrigerators. It must be mentioned that no correlation was found between the presence of *Listeria* spp. in refrigerators and their temperature (*Table 1*).

As shown in *Figure 3* , about 50% of people cleaned their refrigerators monthly or less and 20% of people every 2-3 months. Visual findings showed that most of these refrigerators were contaminated with molds and it may be due to cleaning frequency or detergent type. To the best of our knowledge, there is limited data regarding prevalence of *Listeria* spp.

Table 1. *Listeria*spp. in 180 Domestic Refrigerators in Ahvaz

Organisms	Place of Isolation	Frequency of Cleaning	Last Cleaning Prior to Sampling	Products Used for Cleaning	Temperature (°C)
L. monocytogenes	Student accommodations	Monthly	3 weeks	Water	8
L. innocua	Student accommodations	Each 2-3 months	2 weeks	Water	11
L. innocua	Private home	Each 2-3 months	1 month	Water + dishwasher	10

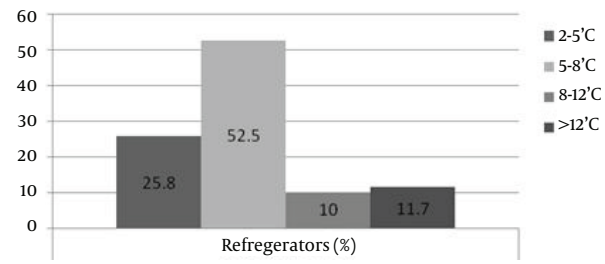

Figure 1. Operating Temperatures of 180 Domestic Refrigerators in Ahvaz

The questionnaire also showed that more than 88% of the owners preferred to clean their refrigerators by water or mixture of water and dishwasher. Normal detergents, vinegar or bicarbonate components were less popular *(Figure 2)*. In the food consumed in Iran and no information exists on the incidence of *Listeria* spp. in Iranian refrigera producttors. *Listeria* spp. was detected in 6.7% of meat and meat samples, 1.3% of dairy samples, 1.2% of

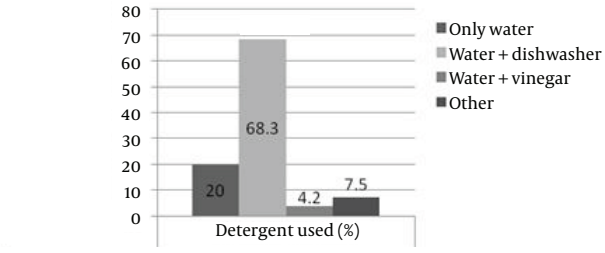

Figure 2. Products Used in the Cleaning of 180 Domestic Refrigerators in Ahvaz

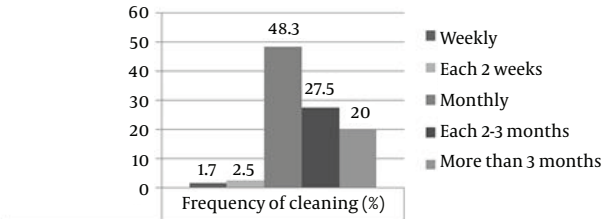

Figure 3. Frequency of Cleaning of 180 Domestic Refrigerators in Ahvaz

vegetable samples and 12% of ready to eat samples in Isfahan (13). In another study in Shahrekord the overall incidence of *Listeria* species in raw milk was 2.2%. *L. monocytogenes* was found in 1.6% of the raw milk samples, while *L. innocua* was found in 0.6% of the samples (15). Also, based on few documents regarding the prevalence of *L. monocytogenes* in the foods consumed in Khuzestan, *L. monocytogenes* was only detected in 4% of farmed tropical fish(14), 1.4 % of shrimp (24), and 5% of raw milk samples (25).

The incidence of *Listeria* in refrigerators has also been investigated in other countries. The data showed that the low incidence of L. monocytogenes in domestic refrigerators in the current study is in agreement with previous reports in other countries, where *L. monocytogenes* has been isolated from 2 of 136, 1 of 35, 3 of 86 and 4 of 342 household refrigerators tested in Greece, Holland, Portugal and United Kingdom, respectively(19, 20, 26, 27). Meanwhile the bacterium was not recovered from any of the 195 domestic refrigerators sampled in the USA (28).

It should be noted that *L. monocytogenes* is a psychotropic organism and capable of growth at refrigeration temperatures, which means that low numbers of initially contaminating cells may proliferate and become hazardous if present on or transferred to ready-to-eat foods stored in refrigerators (13). It has been shown that the bacterium is capable of adherence to many kinds of surfaces such as glass, stainless steel and plastics which are normally used in interior refrigerators (17). Ability of the bacteria to stick to surfaces can also increase the resistance to disinfectants and detergents and it is recommended to clean the surfaces prior to disinfection (29-31).

The ability of *Listeria* to contaminate the interior surfaces of the refrigerators may reflect the fact that food such as meat, cheeses and vegetables stored in the refrigerators can be contaminated with the bacteria (32-35). Consumption of these foods either raw or undercooked, may pose a health risk, particularly in immunocompromised hosts such as pregnant women and the elderly people. Therefore, it can be concluded that the presence of *L. monocytogenes* in domestic or commercial refrigerators even in low number is a significant public health concern.

It must be mentioned that since both *L. monocytogenes* and *L. innocua* share ecological niches, the isolation of both bacteria is not surprising, and isolation of *L. innocua* in some samples is considered as an indicator of possible contamination with *L. monocytogenes* . So, presence of any *Listeria* spp. in domestic refrigerators may indicate poor hygiene and cross-contamination scenarios which could favor the persistence of *L. monocytogenes*.

As indicated in *Table 1* , all of the contaminated refrigerators had the temperature between 8-11°C and the 2 isolated *Listeria* spp. were isolated from student refrigerators. The point is that measurement of inside temperature of refrigerators showed that 21.7 % of them were working at temperatures higher than 8°C *(Figure 1)* and most of these refrigerators were situated in student accommodations. So, it may be realized that the contamination rate is bigger than the obtained result, which could compromise the safety of the foods stored inside them. Temperature abuse is also reported in other countries in the cold chain, both in commercial and domestic situations. Sergelidis (20) reported that 25% of the 136 domestic refrigerators and 13.6% of the 228 supermarket refrigerators investigated in Greece were operating at temperatures higher than 10 °C. In another study in Portugal 12% of domestic refrigerators in 86 households were operating at the same temperature (26).

As it can be observed in *Figure 2* , most owners use mixture of water and dishwasher and some of them use water alone to clean their refrigerators. As observed in *Table*

1, the 2 refrigerators that tested positive for *Listeria* spp. were normally cleaned only with water. Moreover, in the current study almost all refrigerators were contaminated with molds. Although refrigerator manufacturers recommend that the plastic interiors of domestic refrigerators should be cleaned with solutions of bicarbonate, partly to restrict the growth of moulds, but it has been shown that some bacteria and especially *L. monocytogenes* are quite sensitive to anionic detergent products which could be used to control the pathogens in surfaces, environment or refrigerators (36).

As observed in *Figure 3* , more than 95% of the investigated refrigerators were cleaned only monthly or less frequently. It could affect the refrigerator sanitation and allow the pathogens as well as moulds to contaminate and persist on internal surfaces of refrigerators.

5. Discussion

Different kinds of foods, especially raw materials, frequently contain spoilage and pathogenic organisms including *L. monocytogenes* (13-15). Putting unwrapped raw materials in refrigerators may allow these organisms to enter the refrigerators and pose a health risk in kitchen. As mentioned above, most people in the current study used to put the fruits, vegetables and eggs without packing in shelves and it might be the way that this pathogen enters the refrigerators from these sources, which needs more investigation.

Although the number of samples positive for *Listeria* spp. was too low to allow valid conclusions, but the gathered data showed that the only *L. monocytogenes* and one of the *L. innocua* were isolated from student refrigerators. This may have happened due to students` negligence in cleaning their fridges, put unwrapped food in their refrigerators, use water alone for cleaning or due to the fact that most of the refrigerators used in student accommodations in comparison to private homes were not working properly and had higher temperature. Therefore, it is recommended that owners always check their refrigerator temperature, use an appropriate disinfectant, clean the refrigerators in high frequency and do not keep all kinds of food without packing in refrigerator.

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Authors' Contribution

None declared.

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