

# Prevalence of food contamination with *Listeria* spp. in Kermanshah, Islamic Republic of Iran

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## معدل انتشار تلوث الطعام بأنواع الليستريات في كرمنشاہ في جمهورية إيران الإسلامية

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**الخلاصة:** إن الليستريات المُسْتَوْجِدَة هي إحدى العوامل المرضية للبشر والتي تسبب لديهم أمراضاً وخيمة. وقد هدف الباحثون من هذه الدراسة إلى التعرف على تلوث الطعام بأنواع الليستريات في كرمنشاہ في جمهورية إيران الإسلامية. وتضمنت عينة الدراسة 185 من منتجات الألبان و187 من منتجات اللحوم و158 من الأطعمة الجاهزة للأكل مثل السلطات؛ وقد تم اختيار العينات عشوائياً من الأسواق. وقد زُرعت العينات في مَرَق فريزر النصف وفي مَرَق فريزر ثم استزرعت على سب آكسفورد وعلى وسط بالكام. وأجريت اختبارات التأكد بها فيها استخدام الكربوهيدرات على المستفردات للتعرف على الأنواع. وتم استفراد الجراثيم من 66 عينة من أصل 530 عينة (12.5%). وأظهرت منتجات اللحوم أعلى معدلات التلوث (27.2%) ومنتجات الألبان أخفضها (3.8%). وعثر الباحثون على الليستريات الحميدة في 56 عينة (10.6%). وتشير النتائج إلى أن معدل التلوث بالليستريا المُسْتَوْجِدَة كان منخفضاً حتى في الأطعمة الجاهزة للأكل، إلا أن أنواع الليستريات الأخرى ولاسيما ذراي الليستريا الحميدة توافقت بمعدل تلوث أعلى مما يدل على ضرورة اتخاذ المزيد من إجراءات المراقبة على التصحيح الغذائي.

**ABSTRACT** *Listeria monocytogenes* is a human pathogen causing serious diseases. We aimed to determine food contamination with *Listeria* spp. in Kermanshah, Islamic Republic of Iran. Samples (185 dairy, 187 meat products and 158 ready-to-eat foods such as salads) were randomly collected from markets. After processing, samples were cultured in half-Fraser and Fraser broth followed by cultivation on PALCAM and Oxford media. Confirmatory tests including carbohydrate utilization were performed on isolates to determine species. Bacteria were isolated from 66/530 samples (12.5%). Meat products showed the highest (27.2%) and dairy products the lowest (3.8%) contamination rates. *L. innocua* was found in 56 (10.6%) samples, but *L. monocytogenes* was only found in 3 samples (0.6%). The results indicate that the rate of contamination with *L. monocytogenes*, even for ready-to-eat foods, was low but for other *Listeria* spp., in particular strains of *L. innocua*, the rate of contamination was higher, suggesting that more control on food sanitation is required.

## Prévalence de la contamination alimentaire par *Listeria* spp. à Kermanshah (République islamique d'Iran)

**RÉSUMÉ** *Listeria monocytogenes* est un agent pathogène humain à l'origine de maladies graves. Nous avons cherché à établir la prévalence de la contamination alimentaire par *Listeria* spp. à Kermanshah (République islamique d'Iran). Des échantillons (185 produits laitiers, 187 produits carnés et 158 plats prêts à consommer, comme des salades) ont été collectés au hasard sur les marchés. Après traitement, les échantillons ont été mis en culture dans un bouillon de Fraser-demi et de base Fraser puis repiqués sur gélose PALCAM et en milieu Oxford. Des tests de confirmation, y compris l'utilisation des glucides, ont été réalisés sur des isolats afin de déterminer l'espèce. Des bactéries ont été isolées dans 66 échantillons sur 530 (12,5 %). Les produits carnés étaient les plus fréquemment contaminés (27,2 %) tandis que les produits laitiers étaient les moins fréquemment contaminés (3,8 %). *L. innocua* a été identifiée dans 56 échantillons (10,6 %), alors que *L. monocytogenes* n'a été retrouvée que dans trois échantillons (0,6 %). Ces résultats indiquent que le taux de contamination par *L. monocytogenes*, même dans les plats prêts à consommer, était faible, mais que pour les autres *Listeria* spp., en particulier les souches de *L. innocua*, le taux de contamination était plus élevé, suggérant qu'un contrôle plus strict de l'hygiène alimentaire est requis.

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## Introduction

*Listeria* are Gram-positive, non-spore forming, facultatively anaerobic bacilli that are widespread in natural environments [1]. Although environmental reservoirs for *Listeria* spp. have not been determined, they are frequently found in soil, water, decaying plants and food processing environments [2,3]. Among *Listeria* species, *L. monocytogenes* is well known as a human pathogen [2]. It infects humans through ingestion of contaminated foods and can cause non-invasive diseases (gastroenteritis) and/or serious invasive systemic diseases [4].

Given the increase in the elderly population in both developing and developed countries and the changes in food habits toward ready-to-eat products the incidence of listeriosis is increasing worldwide [5]. At the same time, increased numbers of international travellers and of food exchanges between countries means that food safety is a major health priority. We aimed to study the prevalence of contamination with *Listeria* spp. in a variety of food products obtained in Kermanshah, a city in the west of the Islamic Republic of Iran.

## Methods

A total of 530 food samples were analysed: 185 dairy products, both pasteurized and non-pasteurized (various milks, cheese and ice creams), 187 meat products [minced meat, sausages and *kielbasa* (a type of Eastern European sausage or Polish sausage which is usually served uncooked)] and 158 ready-to-eat foods [*oloveyh* salad (a mayonnaise-based salad containing cooked chicken meat, potatoes, sour cucumber and green beans), green salads and fruit juices]. Samples were randomly collected from various food suppliers and distributors in Kermanshah. The method of storage at the outlet (refrigerated or non-refrigerated) and the general quality of samples at

collection (appearance, colour and smell) were determined (scoring from 1 to 5, representing bad to excellent conditions). We also classified the samples according to the general conditions of cleanness and sanitation of the market outlet (scoring from 1 to 5 score representing bad to excellent conditions).

Samples (200 g) were collected in sterile plastic bags and taken to the laboratory. A 25 g sample of each was aseptically weighed and homogenized and mixed with 225 mL of half-Fraser broth (Merck). Solid samples were aseptically blended and homogenized [1] before adding to the half-Fraser broth medium. All equipment was sterile. The mixture (culture) was incubated at 30 °C for 24 hours. From the mixture, 0.1 mL was added to 9.9 mL of Fraser broth medium (Merck) and incubated at 37°C for 24 hours. In order to isolate *Listeria* spp. colonies, the cultures were plated out on polymixin acriflavin lithium chloride cef-tazidime asculin mannitol (PALCAM) agar and Oxford media (Merck) and incubated at 37 °C for 24 to 48 hours. Five colonies with the characteristics of *Listeria* spp. (grey-green colonies with a black halo) were selected from each

plate set and cultured on tryptone soy agar medium enriched with 0.6% yeast extract. The cultures were incubated at 37 °C for 24 to 48 hours. In order to characterise the *Listeria* spp., differential tests including Gram staining, catalase, oxidase, motility at 25°C, sheep-blood haemolysis, Christie-Atkins-Munch-Petersen (CAMP), methyl red, Voges-Proskauer (MRVP) and carbohydrate utilization were carried out.

The data was analysed using *Stata*, version 11 software. Results are presented as mean and standard deviation (SD) and percentages. Student *t*-test and chi-squared tests were used for statistical analysis.

## Results

Of the 530 samples the proportions scoring 1, 2, 3, 4 and 5 for general quality, i.e. from bad to excellent, were 2.3%, 18.7%, 34.7%, 29.6% and 14.7% respectively. The lower scoring groups showed a significantly higher contamination rate ( $P < 0.05$ ). However, no significant difference was found in contamination rates according to the sanitation score of the market outlets (Table 1).

**Table 1** General quality of food samples and marketplace sanitation contamination with *Listeria* spp.

Variable	No. of samples	Contamination with <i>Listeria</i> spp.		<i>P</i> -value <sup>a</sup>
		No.	%	
<b>Food quality score</b>				0.001
1	12	6	50.0	
2	99	14	14.1	
3	184	22	12.0	
4	157	18	11.5	
5	78	6	7.7	
<b>Marketplace sanitation score</b>				> 0.05
1	31	7	22.6	
2	66	8	12.1	
3	188	22	11.7	
4	193	23	11.9	
5	52	6	11.5	
<b>Total</b>	530	66	12.5	

<sup>a</sup>Chi-squared test.

A total of 66 samples (12.5%) were contaminated with *Listeria* spp. From these 66 samples, 3 strains of *L. monocytogenes*, 56 strains of *L. innocua*, 5 strains of *L. grayi* and 2 strains of *L. welshimeri* were isolated.

Nearly two-thirds (63.8%) of the 185 dairy product samples were pasteurized and the contamination rate for pasteurized and non-pasteurized dairy products was 1.5% and 5.0% respectively ( $P = 0.45$ ). Overall, only 3.8% of the dairy samples were contaminated. The contamination rate of milk (10.2%) was higher than ice cream (1.5%) and cheese (0%) (Table 2).

Of the 187 meat product samples 27.3% were contaminated with *Listeria* spp. (Table 2); minced meat papered for kebab showed the highest contamination rate (60.3%).

Overall 5.1% of the 158 ready-to-eat foods were contaminated with *Listeria* spp., 18.2% of meat-containing *oloveyh* salads, 5.4% of green salads and 1 sample (1.8%) of fruit juice. However, none of them was contaminated with *L. monocytogenes* (Table 2).

## Discussion

The results of this study showed that meat products were significantly more likely to be contaminated with *Listeria* spp. than other products. The higher contamination rate of meat products (for both *Listeria* spp. and *L. monocytogenes*), particularly minced meat such as sausages, suggests that the processing and preparation of meat may be carried out in poor hygienic conditions. Other factors such as equipment and additives may also play a role in contamination. Fortunately, this type of food is usually cooked thoroughly before use and consequently this process reduces the risk of listeriosis. The majority of samples tested were fresh products and this may reduce the likelihood of detectable contamination since there will be less time for bacterial growth.

The results showed a significant association between the contamination rate and the general quality of food products. The contamination rate was highest for products with low quality scores, indicating that the food materials and preparation play a key role in *Listeria* contamination.

Our results can be compared with the results of other studies in Islamic Republic of Iran and Asian countries. For instance, a study in Isfahan on different food products including meat, vegetables, dairy and ready-to-eat foods reported a 4.7% contamination rate with *Listeria* spp., which is lower than our result (12.5%). However, contamination with *L. monocytogenes* was 1.2% in Isfahan, which is higher than our results (only 3 samples, 0.6%) [6]. In a study in Pakistan, 10%–12.5% of fresh meat products were contaminated with *Listeria* spp. and this is similar to our result [7].

Our findings for the contamination level of dairy products are consistent with the results reported in Isfahan. In both studies dairy products showed a low contamination level (3.7% in our study and 1.1% in Isfahan) and none of them were contaminated with *L. monocytogenes* [6]. In other research in the Islamic Republic of Iran, the rate of contamination of 500 raw milk samples with *Listeria* spp. was 2.2% [8], which is similar to our results. However, *L. monocytogenes* was found in 1.6% of raw milk samples and this strain was not found in our study. In that study the samples

Table 2 Type of foods tested and their rate of contamination with *Listeria* spp.

Type of foods	Total samples	Contamination with <i>Listeria</i> spp.		Contamination with <i>L. monocytogenes</i>		P-value <sup>c</sup>
	No.	No.	%	No.	%	
<b>Dairy products</b>						
Ice cream	67	1	1.5	0	0.0	0.007
Milk	59	6	10.2	0	0.0	
Cheese	59	0	0.0	0	0.0	
<b>Meat products</b>						
Kielbasa <sup>a</sup>	58	4	6.9	2	3.5	< 0.001
Sausages	56	3	5.4	0	0.0	
Minced meat	73	44	60.3	1	1.4	
<b>Ready-to-eat foods</b>						
<i>Oloveyh</i> salad <sup>b</sup>	11	2	18.2	0	0.0	0.08
Fruit juice	55	1	1.8	0	0.0	
Green salad	92	5	5.4	0	0.0	
<b>Total</b>	530	66	12.5	3	0.6	< 0.001

<sup>a</sup>Type of sausage, usually served uncooked; <sup>b</sup>Mayonnaise-based salad containing cooked chicken meat, potatoes, sour cucumber and green beans.

<sup>c</sup>Chi-squared (Fisher exact) test.

tested were raw and non-pasteurized milk, which can explain the higher rate of contamination. In studies in Northern Ireland and Brazil the contamination rates of *L. monocytogenes* were reported to range from 10%–15.3% for raw milk and cheese and 9.5%–18.3% for raw food materials [9,10]. The difference may reflect the food habits in industrialized countries in terms of using ready-to-eat products particularly processed meats. The consumption of ready-to-eat foods, particularly meat products, is generally not popular in Islamic Republic of Iran. Nevertheless, 18.2% of *oloveyh* salad samples, which

contain meat and are not subsequently cooked, were contaminated.

Studies in Asian countries also indicated low *Listeria* spp. contamination rates in food products. For instance, in a comprehensive study on 2686 food samples in China, 2.3% of the samples were contaminated with *L. monocytogenes*, which is slightly higher than our results. Moreover, similar to our findings, the contamination rate was found to be highest in meat products [11].

In conclusion, the rate of *Listeria* spp. contamination of food samples was comparatively low in Kermanshah. Meat products showed the highest and

contamination rate and dairy products the lowest. *L. monocytogenes* was found in only 3 samples. Further studies on *Listeria* spp. are recommended to provide a better view of contamination rate and the routes of transmission for this foodborne pathogen.

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## References

- Allerberger F. *Listeria*: growth, phenotypic differentiation and molecular microbiology. *FEMS Immunology and Medical Microbiology*, 2002, 35:183–189.
- Vazquez-Boland JA et al. *Listeria* pathogenesis and molecular virulence determinants. *Clinical Microbiology Reviews*, 2001, 14:584–640.
- Akya A, Thomas C, Pointon A. *Listeria monocytogenes* does not survive ingestion by *Acanthamoeba polyphaga*. *Journal of Microbiology*, 2010, 156:809–818.
- Farber JM, Peterkin PI. *Listeria monocytogenes*, a food-borne pathogen. *Microbiological Reviews*, 1991, 55:476–511.
- Koch J, Stark K. Significant increase of listeriosis in Germany—epidemiological patterns 2001–2005. *Eurosurveillance : European Communicable Disease Bulletin*, 2006, 11:85–88.
- Jalali M, Abedi D. Prevalence of *Listeria* species in food products in Isfahan, Iran. *International Journal of Food Microbiology*, 2008, 122:336–340.
- Mahmood MS, Ahmed AN, Hussain I. Prevalence of *Listeria monocytogenes* in poultry meat, poultry meat products and other related inanimates at Faisalabad. Pakistan. *Journal of Nutrition*, 2003, 2:346–349.
- Moshtaghi H, Mohamadpour A. Incidence of *Listeria* spp. in raw milk in Shahrekord, Iran. *Foodborne Pathogens and Disease*, 2007, 4:34–42.
- Harvey J, Gilmour A. Occurrence of *Listeria* species in raw milk and dairy products produced in Northern Ireland. *Journal of Applied Bacteriology*, 1992, 72:119–125.
- Da Silva MC, Hofer E, Tibana A. Incidence of *Listeria monocytogenes* in cheese produced in Rio de Janeiro. Brazil. *Journal of Food Protection*, 1998, 61:354–356.
- Zhou X, Jiao X. Prevalence and lineages of *Listeria monocytogenes* in Chinese food products. *Letters in Applied Microbiology*, 2006, 43:554–559.