Surveillance for Contamination of Salad with Listeria Monocytogenes in Some of Baghdad Restaurants

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ABSTRACT

Background: *Listeria monocytogenes*, a member of the genus *Listeria*, is widely distributed in agricultural environments, such as soil, manure and water. The genus of *Listeria* bacteria is about 15-17 species. It is a pathogenic bacterium that can cause a rare but dangerous infection called listeriosis.

Objectives: Studying the rate of salads contaminated with *Listeria* bacteria. and *Listeria monocytogenes* according to International, Arabic and Iraqi specifications and finding the correlation between commitments of restaurants to standard health conditions with contamination with these bacteria

Methods: The study included 152 samples of salads taken from 39 restaurants chosen randomly and of different levels and places in Baghdad from the period between 1/9/2014 to 20/1/2015. The laboratory tests were carried out on samples based on internationally approved methods in addition to methods of the International Standards Organization.

Results: The study revealed that 23 samples (15.13%) from the 152 samples taken from the restaurants were contaminated with *Listeria* species. of these, 3 (2%) were contaminated with *Listeria monocytogenes* and 20 (13.2%) were contaminated with other types of different and non-pathogenic *Listeria* as follows; (*Listeria welshimeri*, *Listeria seeligeri*, *Listeria* ivanovii, *Listeria grayi*, *Listeria innocua*) with the following prevalence (7(4.6%), 6(3.9%), 3(2%), 3(2%), 1 (0.7%) respectively).

Conclusions: Contamination of salads taken from restaurants with Listeria bacteria is not uncommon. This indicates that routine examination is necessary and should be added to the Iraqi standard for salads.

Keywords: *Listeria* spp, *Listeria monocytogenes*, Salad, Vegetables.

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INTRODUCTION

Salads are ready-to-eat foods that are common in Iraq and the most common types are vegetables and fruits such as tomatoes, cucumbers, peppers, onions, carrots, lettuce and others. It is common to have salads served with meals to all age groups and all social levels. There is a potential for contamination of this type of food with many types of pathogenic microorganisms, including *Listeria monocytogenes*, and because this food is usually prepared from raw material, this increases the probability of contamination by already existing microorganisms or through the stages of its preparation [(1).]

Listeria is a small (0.5 µm in diameter and 1 to 2 µm in length), regular gram-positive rod with rounded ends. Cells are found as single units or in short chains and it does not produce spores and capsules. It is motile due to the presence of a few peritrichous flagellae specially when cultured at 20 to 25°C, and shows a characteristic tumbling motility [(2, 3)].

What increases the probability of food contamination with these bacteria is that it can survive and multiply at refrigeration temperatures (1 to 8°C) [(4)], even if the food is contaminated with very small amounts of this bacterium [(5)]. It can also tolerate high salt concentrations (20-30%), and can adapt to and survive acid stress (PH 4.4), the best environment for its growth multiplication is an acidic medium of PH equal to 7. It can also tolerate an alkaline medium equivalent to 9.4 [(5),] It is able to resist drying and deep freezing even if the temperature drops to -20°C [(3,6).]

L. monocytogenes can have severe consequences for particular groups of people including pregnant women and their foetuses, newborn babies, the elderly and immunocompromised individuals. It can cause miscarriages in pregnant women [(7)].

This study was carried out because the Iraqi standards have not included this type of bacteria, although most of the International specifications have.

METHODS

The study was designed as a cross-sectional study over a period of six months from September 2014- January 2015. One hundred and fifty two (152); samples were taken from randomly chosen restaurants (39 restaurants) the samples were chosen depending on what type of salad the restaurant served. These salads consisted of different types; Caesar, Coleslaw, Fattoush, Jajeek, Tabouli, and vegetable salad; (some containing fried eggplant and onion) other preparation components were also found to be added like meat, cheese, fruit, grains & beans. The samples were taken from exhibition areas at each restaurant by using the spoon in each exhibited container. The samples taken from each salad were from multiple sites of the exhibited container. Each sample taken was placed in separated sterile nylon sacs. The required information was written on each sac (sample) (the name of the salad, the date of the sample and information related to the form).

These samples were transferred on the same day and within 3 hours of collection in cool boxes to the Nutrition Research Institute; the Research & Studies & Development Unit. The laboratory tests were carried out on each sample of salad upon arrival to the lab.

Samples were analyzed according to the method described in the International Standard EN 11290-1:2017- Part 1[(8)] and part 2[(9)].

Using ALOA in parallel with the prescribed Oxford Agar (Oxoid). In summary a primary enrichment was carried out in fraser broth (Oxoid) containing half concentration of supplement at 30°C for 24 h followed by a subculture of 0.1ml in 10ml full fraser broth (Oxoid), inoculated for 48 h at 37°C. Confirmation was carried out using the Real Time – Polymerase Chain Reaction and the Bacterial genomic DNA was extracted from the *L. monocytogenes* isolates using the foodproof® StarPrep Two Kit, following the manufacturer's instructions. Real time PCR was used to detect *L. monocytogenes* isolates using foodproof® *Listeria monocytogenes* Detection LyoKit, 5' nuclease.

* Although *Listeria ivanovii* are primarily pathogenic to animals, there are strains which have been shown to cause infection in humans[10]

Subsequently, 10 µ of this second enrichment broth was incubated in Oxford and ALOA plates. Oxford plates were incubated for 48h at 37°C whereas ALOA plates were incubated at 37°C for 24h,Further characterization was done using Microbact TM 12L Listeria identification kit (Oxoid, MB 1128) and the procedure was carried out according to the manufacturer's instructions.

Statistical Analysis

The data obtained was analyzed using the Statistical Package for Social Sciences (SPSS) and the results were presented in tables and charts. Frequency distribution and chi square test were calculated for the prevalence. P value of 0.05 was considered statistically significant with confidence of 95%.

RESULTS

A- Results of samples: The results of this study were as follows:-

1- *Listeria monocytogenes.* The number of samples contaminated with *Listeria monocytogenes* were three samples out of 152 samples examined, which is about 2%.
2- Other types of *Listeria* bacteria: The numbers of samples contaminated with other types of *Listeria* bacteria were 20 out of 152, i.e. 13.2%.

Table 1: The prevalence rate of non-pathogenic listeria in salad

No	Type of non-pathogenic Listeria	No. (%)
1	*Listeria seeliger*	7 (4.6)
2	*Listeria welshimeri*	6 (3.9)
3	*Listeria* ivanovii* [(10)]	3 (2)
4	*Listeria grayi*	3 (2)
5	*Listeria innocua*	1 (0.7)

The number of restaurants in which contamination occurred with types of Listeria in general, was 20 out of 39 restaurants equal to 51.3%, which is considered a large number and a negative indicator of food safety provided to the citizen,

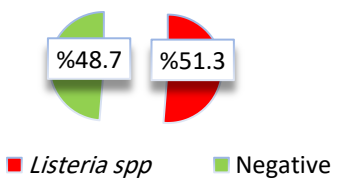


Figure (1): growth of *Listeria monocytogenes* on ALOA agar

as shown in figure5 Figure (2): The red curves indicate multiplication of *Listeria monocytogenes* DNA.

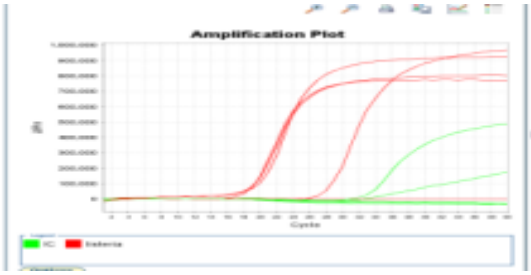


Figure (3): The Multicomponent Plot using FAM (reporter) pigment to authenticate the results obtained in figure 2.

This shows that *Listeria monocytogenes* does not constitute the highest percentage of the contaminated samples. This study revealed that the percentage of salads contaminated with *Listeria monocytogenes* was less than other types of non-pathogenic *Listeria* which is the opposite of what was found in other published studies [19],[20].

1. 5.

Figure (5): comparison between percentages of restaurants with salads contaminated with *listeria* spp. & other not contaminated.

2. The prevalence of restaurants with salads contaminated with *Listeria monocytogenes* was three out of 39 restaurants, (7.7%) as shown in Figure 6.

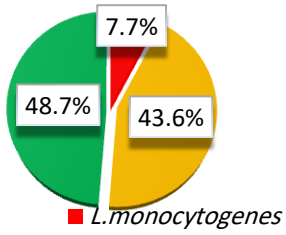


Figure (6): comparison between percentages of restaurants with salads contaminated with *Listeria monocytogenes* & others contaminated with *Listeria* spp, and restaurants with no contaminated salads.

A- Results of questionnaire

There was no statistically significant relationship found between the other factors in the questionnaire and the percentage of contamination. This was due either to the small size of the sample which did not enable showing any clear relationship, or the lack of credibility of restaurant owners.

DISCUSSION

The causes of contamination of salads with the different types of *Listeria* are due to the use of raw or contaminated food. The substances that were added to improve their taste, such as sauce, broccoli, cooked meat, toast, legumes and others may also be contaminated and may encourage the growth of bacteria already existing in these salads, in addition to the possibility of lack of cleanliness of the place, surfaces or the workers, or tools used in the shredder [11],[12].

This is considered a major source of risk for community health because its complications can lead to diseases that do not present as acute conditions, such as abortion, which is not correlated to the presence of this bacterium, due to lake of routine surveillance for it, both in public and private sectors. Although they are few in existence, but the dangers they impose on human beings requires that strict measures be taken regarding contaminaton with these species.

Use of the ALOA culture media was preferred due to its ability to diagnose *Listeria monocytogenes* from other species after 24 hours of incubation, if compared to using the Oxford agar, which takes 3-4 days of incubation [13] (as shown in figure 1). Also, this latter agar cannot differentiate the physical characterizations of *Listeria monocytogenes* colonies.

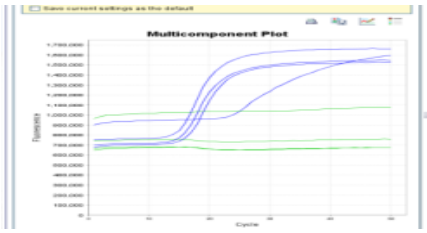
This was also found in other studies which clarified the maximum preference for using ALOA media compared to Oxford Agar media regarding isolation of *Listeria monocytogenes* bacteria [14, 15, 16].

The best way to detect these bacteria is to conduct the methods of counting and detection

both, because the prior is more distinguished compared to the second with the factor of time (faster), whilst the latter is more accurate in detection of these bacteria, but in this case the *Listeria* spp. may require growth boosters which helps them grow [17].

From the above, we can conclude the importance of applying the use of ALOA growth media for the routine investigations for detection and diagnosis of different types of *Listeria* bacteria species which are considered as indicators for presence of pathogenic *Listeria monocytogenes*, as it increases the precision of diagnosis and reduces time and cost [18].

The diagnosis of *Listeria monocytogenes* was confirmed using Real Time-PCR technique, which was identical to the results obtained from the routine tests (using the Analytical Profile



Index *Listeria*). The results were as clear and accurate as the picture shown in the Applied Biosystems 7500 Real-Time PCR, as shown in figure 2 & 3.

One of the explanations of this difference could be due to the limited number of restaurants chosen as a sample; as they were only 39 restaurants in Baghdad region, as this study did not include small food kiosks or mobile carts/vehicle selling ready-made food (street food). The sample was choosing the restaurant randomly, but the salads taken from each restaurant were as a convenient sample.

The other reason may be that our studied salads were not heat-treated upon preparation, as *Listeria monocytogenes* bacteria resists temperature changes and heat treatment more than the rest of the types of *Listeria.* The reason behind this, is the presence of a preventive biological layer (biofilm) which protects it from all external effects [21].

Recommendations:

- Preparation of microbial limits for ready-to-eat foods within the Iraqi standard for Microbiological limits in foods no. 2270.
- Introduction of the *Listeria monocytogenes* and *Listeria* species within the Microbiological limits of Iraqi Standard No. 2270 / Part XII (microbiological limits for tomato, Appetizer, vinegar and pickles) and consider it an important indicator for human consumption.
- Strengthening the monitoring of restaurants to ensure the application of conditions for sanitation and proper washing of vegetables and fruits before mincing in addition to cleanliness and sanitation of the workplace and workers and tools used, guided by the recommendations of the World Health Organization.

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