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Detection of *Listeria monocytogenes* in Several Types of Frozen Meat in Baghdad city

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Abstract

Detection of pathogenic bacteria, such as *Listeria monocytogenes*, in food is crucial for safeguarding public health in Iraq. Forty five samples of frozen meat (15 samples of each of minced red meat, chicken, and fish) were collected from different markets in Baghdad city. Molecular (RT-PCR) and culturing (conventional microbiological examination) methods were used to determine the level of contamination of *L. monocytogenes* in these types of meat.

For the culturing method, TSYEB broth was used as an enrichment medium, whereas BALCAM medium (HiMedia) with the listeria selective supplement FD061 was used as a selective medium, for the isolation and identification of this bacterium. The isolates were confirmed microscopically and biochemically. The results of the culturing method showed that the total number of the isolates of *L. monocytogenes* was 14/45 (31.1%). The incidence of this bacterium was high in fish (11/15, 73.3%), while it was low in the other two types of meat. 2/15 (13.3%) in red meat and 1/15 (6.7 %) in chicken.

Molecular detection of each sample of the bacteria was performed using RT-PCR technique after preparing the Genomic DNA extraction of these samples according to the protocol provided by ReliaPrep™ Blood gDNA Miniprep System kit (Promega, USA). The PCR primers and the hybridization probe ((Macrogen, Korea) were used to target the *inlA* gene sequence (specific for *L. monocytogenes*). The results of the RT-PCR assay showed that 10/45 (22.2%) of the samples were positive for *L. monocytogenes*, which was detected only in fish samples ((10/15, 66.7%), while not found in minced red meat and chicken. However, our results showed differences when compared to other previous works because there were many studies found that the highest contamination rate was in red meat products.

We conclude that the PCR kit used for the detection of *L monocytogenes* appears to give accurate results in the diagnoses of this bacterium in meat products and in comparison with the other routine diagnosis methods in the laboratory, which included culturing and doing biochemical tests which last for approximately 7 days, the RT-PCR technique was able to confirm the findings within 48 hours.

Keywords: Isolation; *Listeria monocytogenes*; Real time polymerase chain reaction; frozen meats; Baghdad

تشخيص اللستيريا المستوحدة في عدة انواع من اللحوم المجمدة في مدينة بغداد

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الخلاصة

للكشف عن البكتيريا المسببة للأمراض مثل الليستريا المستوحدة في الغذاء أمر بالغ الأهمية لحماية الصحة العامة في العراق. تم جمع 45 عينة من اللحوم المجمدة (15 عينة من كل نوع من اللحوم مثل اللحوم الحمراء المفرومة والدجاج والأسماك) من أسواق مختلفة في مدينة بغداد. تم استخدام الطريقة الجزيئية (RT-PCR) وطريقة التزريع (الفحص الميكروبيولوجي التقليدي) لتحديد مستوى تلوث *L. monocytogenes* في هذه الأنواع من اللحوم.

لطريقة التزريع ، استخدم مرق TSYEB كوسط اغنائي وكذلك تم استخدام وسط BALCAM (HiMedia)) مع إضافة المكملات (المكملات الانتقائية الليسترية FD061) كوسط انتقائي لعزل وتحديد هذه البكتيريا. تم فحص العزلات مجهريا وبالفحوصات الكيميائية الحيوية لتأكيدها ، وأظهرت نتائج طريقة التزريع أن العدد الإجمالي لعزلات *L. monocytogenes* كان 45/14 (31.1٪) وكانت نسبة الإصابة بهذه البكتيريا عالية في الأسماك 15/11 (73.3٪). بينما كانت منخفضة في أنواع اللحوم الأخرى: 15/2 (13.3٪) من اللحوم الحمراء و 15/1 (6.7٪) من الدجاج.

تم إجراء الكشف الجزيئي للبكتيريا لكل عينة باستخدام تقنية RT-PCR بعد تحضير استخراج الجينوم (DNA) لهذه العينات وفقًا لبروتوكول: ReliaPrep ™ Blood gDNA Miniprep System kit (Promega، USA). تم استخدام مسبار التهجين the hybridization probe والبرايمرات (Macrogen ، كوريا) لاستهداف تسلسل جين *inlA* (خاص بـ *L. monocytogenes*) , وأظهرت نتائج اختبارات RT-PCR أن 45/10 (22.2٪) من العينات كانت إيجابية لـ *L. monocytogenes* وتم اكتشافها فقط في عينات الأسماك: 15/10 (66.7٪) بينما لم تكتشف في اللحم الأحمر المفروم والدجاج ، لقد أظهرت نتائجنا فروقات مختلفة مقارنة بالأعمال السابقة الأخرى لان هنالك كثير من الدراسات وجدت ان النسبة الاعلى من التلوث كانت في منتجات اللحم المفروم .

من خلال هذا العمل ، استنتجنا ان مجموعة المواد لل kit التي تم استخدامها في ال PCR للكشف عن *L monocytogenes* اعطت نتائج دقيقة لتشخيص هذه البكتيريا في منتجات اللحوم, ومقارنة بالطرق التشخيصية الروتينية الاخرى في المختبر والتي تضمنت التزريع واجراء الفحوصات البايوكيميائية التي استغرقت حدود 7 ايام , فان تقنية RT-PCR كان لها القدرة على اثبات النتائج و التشخيص خلال 48 ساعة .

Introduction

Listeria monocytogenes is a pathological, Gram-positive, facultative, intracellular bacterium that has the capacity of causing serious illnesses in animals as well as humans [1]. It is associated with a spectrum of diseases in humans, such as meningoencephalitis, septicemia, and abortion, especially in the more vulnerable parts of the population, including pregnant women, infants, and geriatric and immunocompromised people. In addition, *L. monocytogenes* can cause mild to severe febrile gastroenteritis, which is a noninvasive illness in adults. However, in persons with weak cell-mediated immunity, Listeriosis may lead to meningitis, being an uncommon cause of this disease in adults [2].

The importance of pathogenicity of *L. monocytogenes* is based on the ability of this bacterium to survive and multiply in phagocytic host cells. Apparently, it can invade the gastrointestinal epithelium as an intracellular infection by the action of a protein that is called internalin (InlA/InlB), which allows the bacteria to attach to the cadherin protein on the intestinal cell membrane through a zipper mechanism [3]. *L. monocytogenes* strains differ in the number of internalin genes encoded in their genomes [4]. Null mutations in four internalin genes (*inlA, inlB, inlC,* and *inlJ*) resulted in reduced invasion or virulence in tissue cultures or animal models [5].

L. monocytogenes is widely distributed in nature, as it is recognized as a primary inhabitant of the soil and decomposing vegetation [6]. It is able to grow at acidic pH, high concentrations of NaCl, and cold environment, as well as being considered as a psychrotrophic species [7]. These, beside other factors, conferred a great importance to this species in the food industry sector [8].

Listerial infections were reported to be increased, mainly those associated with food consumption, such as meals ready-to-eat (MRE) [9- 12]. Even some epidemiologically monitored flare-ups were recorded [13], leading this bacterium to be declared as a main food-borne pathogen.

L. monocytogenes is a major concern to the public health and food industry, because of its prevalence in the environment and adaptability to survive or even thrive under very harsh circumstances [14]. The increasing rate of *L. monocytogenes* contamination , especially in modern eating style, has led to the rapid action of the discovery of new and fast methods for testing food products.

Almost all listeriosis cases are foodborne. A high variety of foods could be contaminated by *L. monocytogenes*, including raw poultry meat, ground beef, soft dairy products, and fish. Most of these items are widely consumed in Iraq [15].

Many bimolecular methods and techniques were established for the diagnosis of *L. monocytogenes*, including DNA probes and PCR (polymerase chain reaction) techniques [16, 17]. In addition, a direct method for the detection of *L. monocytogenes* in food products by PCR was recorded in many studies [18].

Because of high sensitivity, specificity, and low time consumption of PCR, this and other molecular methods have been recommended [19- 21]. However, the sensitivity and specificity of the PCR are dependent upon many factors, such as DNA extraction procedures, the target genes, and the primer sequences [22]. In addition, a PCR assay can only determine the presence or absence of bacteria rather than the quantify of bacterial cells. Thus, the real-time PCR (RT-PCR) is recommended, which can adapt to any specific gene sequence used in the conventional PCR method [23].

The current study was designed to detect *L. monocytogenes* in different types of meat products by the molecular detection method (RT-PCR) in comparison with the routine diagnosis method (microbiological examination) in order to investigate the level of contamination in these types of food in Baghdad city.

Materials and methods

This study was conducted on a set of some types of frozen meat samples that were purchased from the local markets of different areas of Baghdad city. In a total of 45 samples, three types of frozen meat distributed as: 15 minced red meat, 15 chicken, and 15 fish (three different brands of each type and five replicates for each brand) were investigated. These brands (e.g. Al-Hasanat minced red meat , AL-Baraka minced red meat, Al-Halal minced red meat , Al-kafil chicken , Al-dur al abiad chicken , Al-sadia chicken, Alo-Sea fish, Al-Fakhir fish, and Carp fish) were collected randomly during the period from June to July, 2019. These samples were tightly sealed by polyethylene bags.

The isolation of *L. monocytogenes* :

Frozen meat samples were transferred to the laboratory and 1g of each sample was thoroughly homogenized. The samples were preserved in 20ml of TSYEB broth (tryptic soya yeast extract), as an enrichment medium, at 4°C for five days (cold incubation method) in order to reduce other bacterial contamination, because only listeria can grow at low temperatures, overgrowing other organisms which grow more slowly if at all [24, 25] . Then, the growth was initially streaked on listeria agar (Balcam /HiMedia, India) supplemented with listeria selective supplement FD061 containing Polymyxin B sulphate, Ceftazidime, and Acriflavine hydrochloride [26].The media was incubated at 37° for 24 hours. After purification by sub culturing the bacteria, the pure isolates were examined microscopically by using Gram stain and tested by some biochemical tests, such as catalase, oxidase, sugar fermentation , Esculin hydrolysis test and motility at 22°c [27]. Also, the blood haemolysis test was performed to confirm the diagnosis [28].

Molecular detection using RT- PCR

DNA extraction was conducted for each sample of meat and genomic DNA was isolated according to the protocol of ReliaPrep™ Blood gDNA Miniprep System (Promega, USA). The set of PCR primers and the hybridization probe (Macrogen, Korea) were appropriate for this work, as shown in (Table-1), in order to target the *inlA* gene sequence specific for *L. monocytogenes* and the specificity of these primers as well as the hybridization probe were verified according to a previous work [29].

Run started on (Mic qPCR Cycler ,Bio Molecular System, Australia) Serial no. "mic M0000336" S/W, v2.4.0.

During this work, a positive control was used in order to optimize RT- PCR technique and to match with the PCR primers and hybridization probe. This control was represented by a DNA extract of *L. monocytogenes* (accession numbers MH092995.1) confirmed previously by PCR and DNA

sequence obtained from the BLAST-N program (National Center for Biotechnology Information) and recorded by a previous study on carp fish sample [30].

Table 1-Primers and hybridization probe used in this study .

Name	Purpose	Sequence (5`- 3`)
inlA-F	Forward primer	5`-TCGCAAACAGATCTAGACCAAGTT-3`
inlA-R	Reverse primer	5`-GTTCAAGTATTCCAATCCATCGATAG-3`
inlA-P	*L. monocytogenes* hybridization probe	FAM-5`- CAACGCTTCAGGCGGATAGATTAGGGAT-3`-TAMRA

Annealing temperature (60ºc)

Each RT-PCR assay was performed in a total volume of 10µl of the mixture (7 µl of Master mix and 3 µl of template) . Master mix consisted of the forward and reverse primers , hybridization probe, and template DNA. Thermal cycling was performed at 95º C for 5 minutes and 50 cycles as follows: 95 ºC for 15 s and 60 ºC for 1 minute.

Results

For the culturing method, BALCAM medium was used as a selective medium for the isolation of Listeria. Bacterial colonies appeared as greyish/black colonies with black halos (Figure-1).The bacteria was examined microscopically and showed gram positive rods, whereas some were arranged as V- and Y-shaped.

Figure 1- Greyish/black colonies of *L. monocytogenes* on listeria agar

Biochemical tests were conducted for each isolate. The bacterium was catalase positive, oxidase negative, mannitol fermentation negative, esculin hydrolysis positive, and motile at 22ºc. In addition, β-haemolysis test was positive.

The results of the culturing method of different types of frozen meats showed that ,out of 45 samples , the total number of bacterial isolates of *L. monocytogenes* was 14 with a percentage of 31.1%. These isolates were distributed as 2/15 (13.3%) , 1/15 (6.7%) , and 11/15 (73.3%) of minced red meat ,chicken, and fish, respectively (Table-2) .

The same samples were tested by RT-PCR and the results demonstrated that 10 (22.2%) were positive, being detected only in fish samples 10/15 (66.7%), whereas no isolates were found in minced red meat and chicken samples (Table-2). The cycle quantity (Cq) of fish samples ranged from 35.47 to 38.30, while that of the positive control sample was 11.12, as shown in Figure -2.

Table 2-Results of detection of *L. monocytogenes* in different types of meat samples:

Sample	No. of Sample	Diagnoses by culturing	Amplifying by PCR
Red meat	15	2(13.3%)	0
chicken	15	1(6.7%)	0
fish	15	11(73.3%)	10 (66.7%)
Total	45	14(31.1%)	10(22.2%)

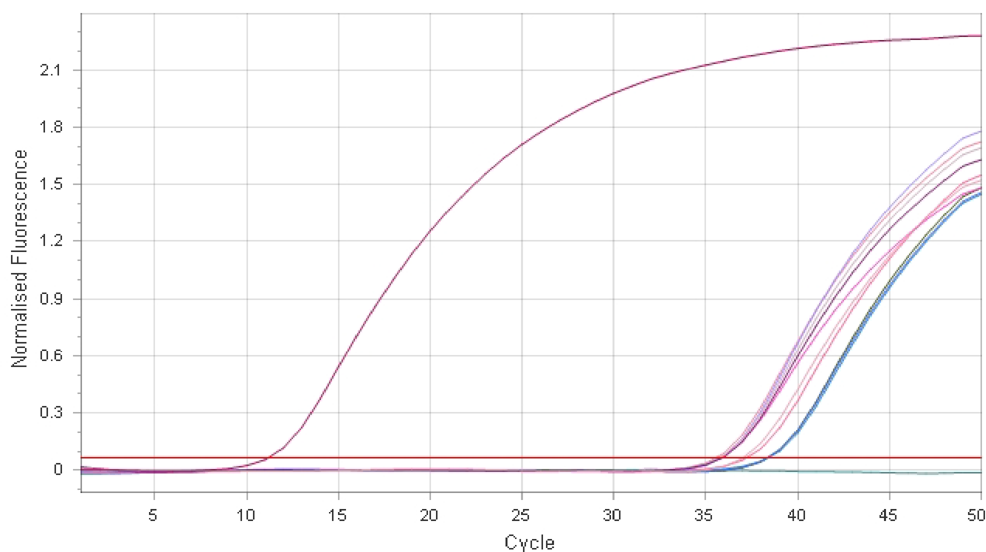

Figure 2-Cycle quantity (Cq) of positive samples and positive control as shown at amplification curves reported by the PCR program software.

Discussion

L. monocytogenes can be isolated from raw milk, meat, poultry, fish, vegetables, cheese, ice cream, and ready-to-eat (RTE) products [31- 34]. Most of these items are widely consumed in Iraqi cities. This was found to be a serious public health problem because this bacterium can spread through the consumption of these products, causing different infections, including Human listeriosis [35].

Only few studies that are concerned with this field, especially in Baghdad, were published. Therefore, our aim was to detect the prevalence of *L. monocytogenes* in different types of frozen meat samples found in the markets of Baghdad city.

L. monocytogenes was diagnosed in the samples by the culturing method as well as by using a molecular assay (RT-PCR).

The results of the culturing method showed that 14/45 (31.1%) of the samples were positive for the growth of this type of bacterium, while 10 /45 (22.2%) were subsequently identified as *L. monocytogenes* by using RT-PCR method. This bacterial isolate was detected only in fish samples 10/15 (66.7%), while not found in minced red meat and chicken samples.

During this investigation, no contamination was found by this type of bacteria in some raw frozen meat samples, such as minced red meat and chicken, in contrast to fish meat which is considerably more likely to be contaminated with *L. monocytogenes*. These results disagree with those reported by other studies conducted in Iraq [36, 37]. Our values also showed differences when compared to other studies performed in other countries. In Isfahan, Iran, a research was conducted on various food products, including dairy products, meat, and ready-to-eat food, and found a 4.7% contamination rate with *L. monocytogenes* [38], which is lower than that found by our study. However, the occurrence of *L. monocytogenes* in the current research was lower than that found in an earlier study [39]. Another research [40] found that the highest contamination rate was in red meat products.

Our explanation for the differences between our results and those of the previous works is that a wide range of animal species can become infected with *L. monocytogenes*, including mammals and domesticated animals [41]. However, cows are very rarely infected with this bacterium [42], in the opposite to sheep which is more sensitive to be infected with listeria [43]. In Baghdad markets, frozen

minced red meat is mostly from cow origin, which might be the reason for the negative detection of these bacteria in the types of food tested in this work. In addition, the low contamination may be due to the collection of the samples during the summer (hot season), which provides suitable conditions for the infection or the growth of listeria [44]. Another research conducted in Iraq found that the highest incidence of listeriosis occurred in the cold seasons [45]. Regarding chicken meat, our results showed the lowest incidence of *L. monocytogenes* compared with those found in other studies [46,47] However, the numbers of samples of the previous studies were higher than those investigated in the current study.

L. monocytogenes cells that contaminate raw meats are likely to be damaged in some samples studied due to the effects of processing. In raw and processed meats, other bacteria, such as lactic acid bacteria, may become the dominant population and preserves the product from contamination [48] .This bacteria may cause competition for nutrients, production of toxic end products, or production of specific antibiotics against pathogenic bacteria like listeria [49].

A number of molecular biological methods were described for the detection of *L. monocytogenes*, including DNA probes and PCR techniques. Direct detection of *L. monocytogenes* in food products by PCR was reported in several cases [50 -54].

Many studies described a qPCR assay for the specific detection of *L. monocytogenes* in food samples. Positive deviation was observed in ten analyzed samples. A possible explanation for these discordant results is that DNA from dead or viable, but non cultivable, *L. monocytogenes* cells was detected by the alternative method in the food matrix [55, 56]. Furthermore, negative deviation was not detected, which demonstrates the robustness of the alternative method, as food components such as organic compounds, calcium ions, glycogen, and lipids were demonstrated to inhibit PCR [57].

One to two stages of enrichment processes are used in the real-time PCR detection; these processes utilize different selective media. These processes are used by different enrichment and DNA preparation approaches in order to achieve increasing numbers of live cells to be in detectable level, decrease the numbers of dead *L. monocytogenes* cells, and reduce the foodborne PCR inhibitors [58].

Detection of *L. monocytogenes* in food by the conventional cultivation method involves the growth in a pre-enrichment medium, then on a selective medium, followed by a series of biochemical and serological tests to confirm the diagnoses [59]. These work-intensive methods are time-consuming as they may require up to 10 days to be finished. Real-time (RT)-PCR is rapid and allows an accurate identification and precise nucleic acid sequences quantification [60, 61]. The lack of post-PCR steps minimizes the cross-contamination risk and allows for a high output [62, 63].

To our knowledge, there are few published data regarding the prevalence of *L. monocytogenes* in food samples, especially frozen meat, in Baghdad areas. In fact, *L. monocytogenes* is rarely tested in food products by using PCR. Therefore, this work was conducted to assess the use of PCR for the direct detection of *L. monocytogenes* in meat products and determine the level of contamination in Baghdad city.

L. monocytogenes was diagnosed successfully by RT- PCR technique in some types of meat samples. The use of the kit designed specifically to the target *inlA* gene (internalin gene), including primers and hybridization probe, provided satisfactory results for the detection of this bacterium. In comparison with the routine diagnosis methods, including culturing and biochemical tests which last for approximately 7 days, the RT-PCR technique was able to confirm the findings within 48 hours.

The RT- PCR assay had high sensitivity and specificity and it can provide quick and reliable results for the diagnosis of a large number of foodborne pathogens that contaminate various types of food products. Improved methods for the diagnosis of the bacteria in food need to be available, including those based on the use of DNA probes, or the polymerase chain reaction, in order to prevent and control human infections.

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References

1. Farber, J.M. and Peterkin, P.I. **1991** .*Listeria monocytogenes*, a food-borne pathogen.*Microbiological Reviews*, PubMed/US National Library of Medicine National Institutes of Health . **55**: 476–511.
2. Almudena H.-M**.** , Antoni P.-C. **2014 .** What Is New in Listeriosis? .*Biomed Res Int*. 2014 Apr **14**: 358051.
3. Hamon M, Bierne H, Cossart P. **2006.** *Listeria monocytogenes*: A multifaceted model. *Nat Rev Microbiol,* **4**(6): 423–34.
4. Nelson KE , Fouts DE, Mongodin EF, Ravel J. ,DeBoy RT., Kolonay JF, Rasko DA., Angiuoli SV., Gill SR., Paulsen IT., *et al.* **2004.** Whole genome comparisons of serotype 4b and 1/2a strains of the food-borne pathogen *Listeria monocytogenes* reveal new insights into the core genome components of this species. *Nucleic Acids Res*. **32**: 2386–2395.
5. Sabet C., Lecuit M., Cabanes D., Cossart P., Bierne H. **2005** . LPXTG protein InlJ, a newly identified internalin involved in *Listeria monocytogenes* virulence. *Infect. Immun.* **73**: 6912–6922.
6. Bakardjiev AI,Theriot JA,Portnoy DA. **2006** .Correction:*Listeriamonocytogens* Traffics from Maternal Organs to the Placenta and Back. *PLoS Pathogenes*, **2**(7): e80
7. Shabala L, Lee SH, Cannesson P, Ross T. **2008** . Acid and NaCl limits to growth of *Listeria monocytogenes* and influence of sequence of inimical acid and NaCl levels on inactivation kinetics. *Food Prot.J.* **71**(6): 1169-77.
8. Bajard S, Rosso L, Fardel G, Flandrois JP. **1996** The particular behaviour of *Listeria monocytogenes* under sub-optimal conditions. *International Journal of Food Microbiology*. **29**(2-3):201–211.
9. Bannister, B.A. **1987** .*Listeria monocytogenes* meningitis associated witheating soft cheese. *Journal of Infection*,**15**: 165–168.
10. Barnes R., Archer P., Stack J. and Istre G.R. **1989 .**Listeriosis associated with consumption of turkey franks.Morbidity andMortality .*Weekly Reports*, **38**: 267–268.
11. Kaczmarski E.B. , Jones D.M. **1989** .Listeriosis and ready cooked chicken. *Lancet*1, 383–384.
12. Jacquet C., Catimel B., Brosch R., Buchrieser C., Dehaumont P.,Coulet V., Lepoutre A., Veit P. , Rocourt J. **1995** .Investi-gations related to the epidemic strain involved in the frenchlisteriosis outbreak in 1992.*Applied and Environmental Microbiology,* **61**: 2242–2246.
13. Kathariou, S. **2000** .Pathogenesis determinants of *Listeria monocytogenes* .InMicrobial Foodborne Diseasesed. Cary, J.W., Linz*, J.E. andBhatnagar, D. Lancaster, PA: Technomic publishing*, pp. 295–314.
14. Aznar R, Alarcón B. **2003**. PCR detection of *Listeria monocytogenes*: a study of multiple factors affecting sensitivity.*J Appl Microbiol,* **95**: 958-966
15. Saman S T S A, Bizhar AT, Alind MA, Sowaila MM, Yousif H M S. **2017**. Isolation and Molecular Detection of *Listeria monocytogenes*in Minced Meat, Frozen Chicken and Cheese in Duhok Province, Kurdistan Region of Iraq. *Food Microbiol Saf Hyg* , **2**(1): 118.
16. .Bansal NS, McDonell FHY, Smith A, Arnold G, Ibrahim GF. **1996 .** MultiplexPCR assay for the routine detection of Listeria in food. *Int J Food Microbiol.* **l33**: 293-300.
17. O'Connor L, Joy J, Kane M, Maher M. **2000** .Rapid polymerase chain reaction/DNA probe membrane-based assay for the detection of Listeria and *Listeria monocytogenes* in food. *J Food Prot,* **63**: 337-342.11.
18. Hudson JA, Lake RJ, Savill MG, Scholes P, McCormick RE. **2001**. Rapiddetection of *Listeria monocytogenes* in ham samples using immunomagneticseparation followed by polymerase chain reaction. *J Appl Microbiol,* **90**: 614-621.
19. Hough, A., Harbison S., Savill M., Melton L., Fletcher G.. **2002**.Rapid enumeration of *Listeria monocytogenesin* artificially contam-inated cabbage using real-time polymerase chain reaction. *J. FoodProt*. **65**: 1329–1332.
20. Niederhauser, C., Candrian U., Hofelein C., Jermini M, Buhler H. P., Luthy J. **1992**. Use of polymerase chain reaction for detection *of Listeria monocytogenes* in food.Appl. *Environ. Microbiol*. **58**: 1564–1568.
21. Wang, R. F., Cao W. W., Johnson M. G.. **1992**. 16S rRNA-basedprobes and polymerase chain reaction method to detect *Listeria monocytogenes* cells added to foods.Appl. *Environ. Microbiol*. **58**: 2827–2831.

22. Yamamoto, Y. **2002**. PCR in diagnosis of infection: detection ofbacteria in cerebrospinal fluids.*Clin. Diagn. Lab. Immunol*. **9**: 508–514.

23. Hellyer, T. J., DesJardin L. E., Hehman G. L., Cave M. D, .Eisenach K. D. **1999**. Quantitative analysis of mRNA as a marker forviability of Mycobacterium tuberculosis. *J. Clin. Microbiol*. **37**: 290–295

24. Gillespie, Stephen H . Medical microbiology illustrated . **1994** , Amember of the reed Elsevier plc group ,*Butterworth- Heinemann Ltd* , .pp: 36-38.

25. Geo, F.; Janet, S., and Stephan A. (**2001**). Medial Microbiology Twenty. Second Edition , *McGraw-Hill companies,* p. 192-193.

26. Anthony D. Hitchins . **2001**. *Listeria monocytogenes*. U.S. Food & Drug Administration ,FDA, Center for food safety and Applied Nutrition/ *bacteriological analytical manual* ,pp.197-212 .

27. McClain, D., Lee, W.H. **1988**. Development of USDA-FSIS method for isolation of *Listeria monocytogenes* from raw meat and poultry. *J. AOAC*., **71**: 660-664.

28. Cowan, S.T. **1993**. In: *Cowan and Steel's manual for identification of medical bacteria*. Barrow, S.I. and Feltham, R.K. (Eds). Cowan Cambridge University Press. pp. 317.

29. Heo E, Jeong, Bora S., Hyun J. P. ,Young J., Jin San M., Sung H. W., Jin-Seok K., Yohan Y. **2014**. Rapid Detection of *Listeria monocytogenes* by Real-Time PCR in Processed Meat and Dairy Products.*Journal of Food Protection*, **77**(3): 453–458.

30. Al-Gburi N.M . **2020** .Detection and pathogenicity of *Listeria monocytogenes* in common carp (Cyprinus carpio) fish in Baghdad, Iraq . *Iraqi Journal of Veterinary Sciences*, **34**(2): 311-316.

31. Berrada H, Soriano J, Pico Y, Manes J. **2006**. Quantification of *Listeria monocytogenes* in salads by real time quantitative PCR. Int. *J. Food Microbiol*. **107**: 202–206.

32. Duodu S, Mehmeti I, Holst-Jensen A, Loncarevic S. **2009**. Improved sample preparation for real-time PCR detection of *Listeria monocytogenes* in hot-smoked salmon using filtering and immunomagnetic separation techniques. *Food Anal. Methods,* **2**: 23–29.

33. Rantsiou, K, Alessandria V, Urso R, Dolci P, Cocolin L. **2008.** Detection, quantification and vitality of *Listeria monocytogenes* in food as determined by quantitative PCR. Int. *J. Food Microbiol*. **121**: 99–105.

34. Schuchat A, Swaminathan B, Broome C. **1991**. Epidemiology of human listeriosis. *Clin. Microbiol. Rev.* **4**:169–183.

35. Shoai-Tehrani, M., Pilmis, B., Maury, M. M., Robineau, O., Disson, O., Jouvion, G., .. Listeria Endovascular Infections Study Group.**2019**. *Listeria monocytogenes*-associated endovascular infections: A study of 71 consecutive cases. *The Journal of Infection*, **79**(4): 322–331.

36. Ahmed SSTS, Tayeb BH, Ameen AM, Merza SM, Sharif YHM . **2017** . Isolation and Molecular Detection of *Listeria monocytogenes* in Minced Meat, Frozen Chicken and Cheese in Duhok Province, Kurdistan Region of Iraq. *J Food Microbiol Saf Hyg,* **2**: 118.

37. Adil AM , Ahmed IA. **2018** . Isolation and Diagnosis of *Listeria monocytogenes* from some Types of Frozen Meat . Tikrit University, *Journal for Agricultural Sciences,* **18**(2).

38. Jalali M, Abedi D. **2008** . Prevalence of Listeria species in food products in Isfahan, *Iran. Int J Food Microbiol,* **122**: 336-340.

39. Goh SG, Kuan CH, Loo YY, Chang WS, Lye YL, *et al*. **2012** . *Listeria monocytogenes* in retailed raw chicken meat in Malaysia. *Poultry Science,* **91**: 2686-2690.

40. Zhou X, Jiao X. **2006** . Prevalence and lineages of *Listeria monocytogenes* in Chinese food products. *Lett Appl Microbiol,* **43**: 554-559.

41. Alsheikh AD, Mohammed GE, Abdalla MA, Bakhiet AO. **2014**. First Isolation and Identification of *Listeria monocytogenes* Isolated from Frozen and Shock Frozen Dressed Broiler Chicken in Sudan. *British Microbiology Research Journal,* **4**: 28-38.

42. Al-Zubaidi K.I. **2006** . Natural and Experimental Study for the Localization of the *Listeria monocytogenes* in some of the Internal and its Role in the Spread of the Disease. *M.Sc Thesis*. Baghdad University. .

43. Al-Dughaym A.M., Fadl Elmula A., Mohamed G. E., Hegazy A. A.,Radwan Y. A., Housawi F. M. T. , Gameel A. A. **2001** . Firstreport of an outbreak of ovine septicaemic listeriosis in Saudi Arabia.Rev. *Sci. Tech. Off. Int. Epiz*., **20**: 777–783.

44. Bonardi, M. *et al*., **2002** . High specific activity radioactivity tracers: a powerful tool for studying very low level and long term exposure to different chemical forms of both essential and toxic elements: *Microchem J,* **73**: 153- 166.

45. Hussien OA, Huda AA. **2015**. Isolation and Identification of *Listeria .monocytogenes* From Human and Animal in Al- Qadissiya Province . *Al- Qadissiya J. For pure science*, **12**(1): 1122.

46. Mahmood MS, Ahmed AN, Hussain I.**2003** . Prevalence of *Listeria monocytogenes* in Poultry Meat, Poultry Meat Products and Other Related Inanimates at Faisalabad. *Asian Network for Scientific Information,* **2**: 346-349.

47. Ayaz ND, Kaplan YZ, Kaplan YZ, Dogru AK., Aksoy MH. **2009** . Rapid detection of Listeria monocytogenes in chicken carcasses by IMS-PCR. *Annals of Microbiology,* **59**: 741-744.

48. Stiles, M.E. **1996**. Biopreservation by lactic acid bacteria. *Antonie van Leeuwenhoek*, **70**: 331–345.

49. Nilsson, L., Huss, H.H. & Gram, L. **1997**. Inhibition of *Listeria monocytogenes* on cold-smoked salmon by nisin and carbon dioxide atmosphere. *International Journal of Food Microbiology,* **38**: 217–228.

50. Bansal NS, McDonell FH, Smith A, Arnold G, Ibrahim GF. **1996.** Multiplex PCR assay for the routine detection of Listeria in food. *Int J Food Microbiol,* **33**: 293-300.

51. Manzano M, Cocolin L, Ferroni P, Cantoni C, Comi G. **1997**. A simple and fast PCR protocol to detect *L. monocytogenes* from meat. *Journal of the Science of Food and Agriculture,* **74**: 25-30.

52. Agersborg A, Dahl R, Martinez I. **1997**. Sample preparation and DNA extraction procedures for polymerase chain reaction identification of L. monocytogenes in seafood. Int *J Food Microbiol,* **35**: 275-280.

53. O'Connor L, Joy J, Kane M, Smith T, Maher M. **2000**. Rapid polymerase chain reaction/DNA probe membrane-based assay for the detection of Listeria and *Listeria monocytogenes* in food. *J Food Prot,* **63**: 337-342.

54. Hudson JA, Lake RJ, Savill MG, Scholes P, McCormick RE. **2001**. Rapiddetection of *Listeria monocytogenes* in ham samples using immunomagnetic separation followed by polymerase chain reaction. *J Appl Microbiol,* **90**: 614-621.

55. O' Grady J, Sedano-Balbás S, Maher M, Smith T, Barry T. **2008**. Rapid real-time PCR detection of *Listeria monocytogenes* in enriched food samples based on the ssrA gene, a novel diagnostic target. *Food Microbiol,* **25**: 75-84.

56. O'Grady J, Ruttledge M, Sedano-Balbás S, Smith TJ, Barry T, *et al*. **2009** Rapid detection of *Listeria monocytogenes* in food using culture enrichment combined with real-time PCR. *Food Microbiol,* **26**: 4-7

57. Rodríguez-Lázaro D, Hernandez M. **2006**. Molecular methodology in Food Microbiology diagnostics: trends and current challenges. *IUFoST World Congress,* pp. 1085-1099.

58. Oravcová K, Trncíková T, Kaclíková E. **2007** . Comparison of three real-time PCR-based methods for the detection of *Listeria monocytogenes* in food. *J Food Nutr Res,* **46**: 63-67.

59. Rodríguez-Lázaro D, Hernández M, Scortti M, Esteve T, Vázquez-Boland JA, *et al*. **2004** . Quantitative detection of *Listeria monocytogenes* and Listeria innocua by real-time PCR: assessment of hly, iap, and lin02483 targets and AmpliFluor technology. *Appl Environ Microbiol,* **70**: 1366-1377

60. Klein D. **2002** .Quantification using real-time PCR technology: applications and limitations. *Trends Mol Med,* **8**: 257-260

61. Norton DM .**2002** . Polymerase chain reaction-based methods for detection of *Listeria monocytogenes*: toward real-time screening for food and environmental samples. *J AOAC Int,* **85**: 505-515.

62. Gillespie, Stephen H . **1994** Medical microbiology illustrated ., Amember of the reed Elsevier plc group ,*Butterworth- Heinemann Ltd* , .pp: 36-38.

63. Geo F.; Janet S., Stephan A. **2001**. Medial Microbiology Twenty. Second Edition , *McGraw-Hill companies,* p. 192-193..