**Prevalence and genetic detection of *L. monocytogenes* from milk and some milk products**

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**ABSTRACT**

A total of 200 random samples of fresh dairy milk (80), soft cheese (40), kariesh cheese (40) and ice cream (40) were collected from small retails and different shops at Kaliopia and Giza Governates during the period of October 2016 to January 2017 and transferred with minimum delay to laboratory for detection the presence of *Listeria species*.The bacteriological results revealed that, 5/200 (2.5%) were *Listeria monocytogenes* (*L.monocytogenes*) includes 3/80 (3.75 %) from raw milk, 1/40 (2.5%) from each kariesh cheese and ice cream samples and 0/40 (0%) from soft cheese. The results of MicrogenTM Listeria-ID System revealed that all isolates were *L.monocytogenes* (99.92%) .The PCR results for *L. monocytogenes* showed that all 16S rRNA were detected in five studied strains (100.0%) i.e., all studied strains were *L. monocytogenes*.

**Keywords:***L. monocytogenes*, Microgen TM Listeria-ID System, 16S rRNA

**1. INTRODUCTION**

Infectious diseases caused by bacteria affect millions of people worldwide. Today, infectious diseases account for one-third of all deaths in the world; the World Health Organization estimates that nearly 50,000 people die each day throughout the world from infectious diseases (Chanda and Rakholiya,2011).

*L.monocytogenes* is ubiquitous bacteria. It causes listeriosis, a serious infectious disease which occurs as consequence of consumption of food contaminated with this pathogen bacterium. The frequency of incidence of listeriosis is low (1%), but with high mortality rate (30%). In certain countries large outbreaks of listeriosis were associated with consumption of fresh cheeses and milk. In the process of production of milk and dairy products, it most commonly occurs as consequence of post-pasteurization contamination. *L.monocytogenes* has the ability to multiply and grow at low temperatures (4 0 C) and to survive even on freezing temperatures, and as such poses risk for health of consumers, if found in milk, cheese, ice-cream and other dairy products (Kasalica *et al*.,2011).

Members of the genus Listeria are short rods, aerobic to facultative anaerobic, Gram- positive, not forming spores and  capsules, distributed individually and in form of short chains, sometimes in form of the letters V and Y. In direct smear, they can be coccoid, and therefore mistaken with streptococci (Todar, 2009).

*L.monocytogenes* is primarily transmitted via the oral route, after which the organism penetrates the intestinal tract to cause systemic infections. Itcauses infections of the [central nervous system](https://en.wikipedia.org/wiki/Central_nervous_system)  ([meningitis](https://en.wikipedia.org/wiki/Meningitis),  [meningoencephalitis](https://en.wikipedia.org/wiki/Meningoencephalitis%22%20%5Co%20%22Meningoencephalitis) , [brain abscess](https://en.wikipedia.org/wiki/Brain_abscess),  [cerebritis](https://en.wikipedia.org/wiki/Cerebritis%22%20%5Co%20%22Cerebritis)) and  [bacteremia](https://en.wikipedia.org/wiki/Bacteremia%22%20%5Co%20%22Bacteremia) in those who are [immunocompromised](https://en.wikipedia.org/wiki/Immunocompromised%22%20%5Co%20%22Immunocompromised),  pregnant women, and those at the extremes of age (newborns and the elderly), as well as [gastroenteritis](https://en.wikipedia.org/wiki/Gastroenteritis) in healthy persons who have been severely infected. The diagnosis of listeriosis requires the isolation of the organism from the blood and/or the cerebrospinal fluid (Wikipedia, 2017).Therefore, this study was conducted to estimate the prevalence and bacteriological characterization of *L.monocytogenes* in milk, soft cheese, kariesh cheese and ice cream at Kaliobia and Giza Governorates .

**2. MATERIAL AND METHODS**

*2.1. Samples collection:*

Two hundred random samples of fresh dairy milk (80), soft cheese (40), kariesh cheese (40) and ice cream (40) were collected from small retails and different shops at Kaliopia and Giza governates during the period of October 2016 to January 2017 and transferred with minimum delay to laboratory for detection the presence of *Listeria species*. Each examined sample was taken alone in sterile plastic bags and kept in ice box.

*2.2. Bacteriological examination:*

*2.2.1.Isolation of Listeria species* (International Standard Organization, 2004).

*2.2.1.1.Primary enrichment:*

Xg or xml of sample was added to 9ml of half Fraser broth (OxoidCM0895+SR0166) then samples were homogenized and incubated aerobically at 30°C for 24±2 hours.

*2.2.1.2.Secondary enrichment:*

0.1ml of incubated primary enrichment culture were transfered to 10ml of Fraser broth (OxoidCM0895+SR0156) and were incubated at 35°C or 37°C for 48±2 hours.

*2.2.1.3.Selective isolation:*

A loopful from incubated Fraser broth was streaked onto the PALCAM agar plates (OxoidCM0877+SR0150) then incubated at 37°C for 24±3 hours and ,if necessary, for an additional 24±3 hours.

*2.2.1.4.Purification:*

The listeria like colonies were picked and streaked onto Tryptic Soya agar (LAB011) with 0.6% Yeast extract (TSYEA) then were incubated at 35-37°C for 18-24hours.

*2.2.2. Identification of Listeria species:*

*2.2.2.1.Morphological identification:*

 Pinpoint colonies of TSYEA were subjected to identification procedures which included Gram’s staining followed by a microscopic examination(VALUE @ Amrita, 2011). The characteristic Gram-positive, coccobacillary or short rod-shaped organisms were sub-cultured in semisolid media at 25°C for 12-18 h. Subsequently, the cultures showing typical tumbling motility were considered as “presumptive” listeria isolates (Tittsler and Sandholzer ,1936).

*2.2.2.2. Biochemical identification:*

MicrogenTM Listeria-ID System is an identification system for *Listeria species*. Each Microgen Listeria-ID microwell test strip contains 11 dehydrated substrates for the performance of carbohydrate utilization tests and one empty well for the performance of a haemolsin reaction (Rodriguez *et al*., 1986).

Identification of isolates is achieved by recording the results visualized by a colour change after 18-24 hours incubation.These results are then analysed using the Microgen Identification System Software (MID-60) (Lapage *et al*.,1973).

*2.2.2.3 Genotypic detection of isolated L.monocytogenes*

The genomic 16s rRNA gene of five isolated *L.monocytogenes* tested using specific primer (Table 1) for this gene following QIAamp® DNA Mini Kit instructions (Catalogue no. M501DP100), Emerald Amp GT PCR mastermix (Takara) with Code No. RR310A and 1.5%agarose gel electrophoreses (Sambrook *et al*., 1989). The PCR condition have specific sequence and amplify a specific product as shown in Table (1). Temperature and time conditions of the primers during PCR are shown in Table (2) according to specific authors and Emerald Amp GT PCR mastermix (Takara) kit.

**3. RESULTS**

The bacteriological results of the examined samples revealed that, all isolates 5 (2.5%) recovered from 200 samples were *L.monocytogenes* includes 3/80 (3.75 %) from raw milk, 1/40 (2.5%) from each kariesh cheese and ice cream samples and 0(0%) from soft cheese(Table, 3).

The recovered isolates on PALCAM agar were grown well and showed small 2-3 mm in diameter, gray green colonies in color and black hollow surrounded (esculin hydrolysis).They were Gram - positive bacilli or coccobacilli; motile showing umbrella pattern motility.

Biochemical reactions using MicrogenTM Listeria-ID System(Table 4) showed that all strains were *L.monocytogenes* (99.92%).

The PCR results for *L. monocytogenes* showed thatthe genomic 16S rRNA gene was detected in five studied strains (100.0%) .The 16 S rRNAgene was amplified in five strains giving product of 1200 bp as shown in Fig. (1). i.e., all studied strains were *L. monocytogenes*.

**4. DISCUSSION**

*L. monocytogenes* has been involved in many outbreaks and sporadic cases of diseases primarily associated with the consumption of pasteurized milk, cheeses made from unpasteurized milk and other dairy based products that serve as good medium for the growth and survival of many pathogenic organisms in both industrialized and developing countries (Makino *et al*., 2005 and Manfreda *et al*., 2005).

The results of *L.monocytogenes* isolation from raw milk revealed that, 3(3.75%) out of 80 samples were positive. These results came in accordance with that obtained by Meshref *et al*., (2015) and Navratilova *et al*., (2004) who reported prevalence of *L.monocytogenes* in raw milk samples were 3.92% and 3.85% respectively . Meanwhile, these results disagreed with those recorded by Al-Kassaa *et al*., (2016) who mentioned absence of *L. monocytogenes* in all analyzed fresh cow milk samples.

The results of bacteriological examination of 40ice cream samples revealed that prevalence of *L.monocytogenes* was 2.5%.Nearly similar results were recorded by Tantawy, Hasnaa (2011) who stated that incidence of *L.monocytogenes* in 75 ice cream samples was 2.66%. Meanwhile, these results disagreed with those recorded by **Effimia** (2015) who recorded that26% of 127 ice cream samples were positive for *L. monocytogenes .*

The current results indicated absence of *L.monocytogenes* in 40 soft cheese samples.The same results were recorded by Ahmed (2013) and Alzaeem *et al*., (2016) while Chaves and Arias (2009) reported that 27 *L. monocytogenes* strains were isolated from 110 soft cheese samples.

The present results revealed that 1 (2.5%) of 40 Kariesh cheese samples was *L.monocytogenes* positive. These results finding go hand in hand with the finding of Elshinaway, Saadia *et al*., (2017) . Meanwhile, they disagreed with the finding of Hussien*et al*., (2013) who mentioned 20% of 35 kareish cheese samples were contaminated with *L. monocytogenes* and Abd El Tawab *et al.,* (2015) who mentioned that 3(6%) were positive for *L.monocytogenes* in 50 kariesh cheese samples.

**5.CONCLUSION**

 This study indicates that some dairy products (raw milk, soft cheese, kariesh cheese and ice cream) sold in Kaliopia and Giza markets may be considered as a threat to consumers. They are significant vehicles of *L. monocytogenes* which regularly causing listeriosis outbreaks. Therefore clear risk factors and people that are susceptible for acquiring listeriosis should not consume such products. This indicates importance and need for permanent control, and detection of potential sources of contamination. Introduction of HACCP (Hazard Analysis and Critical Control Points), as a way of control in the process of production and processing the risk of contamination of dairy products with this pathogen can be reduced.

**6. REFERENCES**

Abd El Tawab, A.A. :Maarouf, A.A.A. and Mahdy, Zeinab A.M. 2015. Bacteriological and molecular studies of Listeria species in milk and milk products at El-Kaliobia governorate. Benha Veterinary Medical Journal,(29) 2:170‐181.

# Ahmed, Hanaa Salah 2013.Public health significance of Listeria as a foodborne pathogen. Cairo University Faculty of Veterinary Medicine .Zoonoses Department. Master degree.

[Al Kassaa, I](https://inis.iaea.org/search/search.aspx?orig_q=author:%22Al%20Kassaa,%20I%22)., [El Omari, K.h.](https://inis.iaea.org/search/search.aspx?orig_q=author:%22El%20Omari,%20Kh.%22), [Esmail, B.](https://inis.iaea.org/search/search.aspx?orig_q=author:%22Esmail,%20B.%22), [Hamze, M](https://inis.iaea.org/search/search.aspx?orig_q=author:%22Hamze,%20M%22).[Saati, M.](https://inis.iaea.org/search/search.aspx?orig_q=author:%22Saati,%20M.%22) 2016.Prevalence of *Listeria monocytogenes* in raw milk in north Lebanon. Lebanese Science Journal,47(40):37-43.

Alzaeem, I. ,Salama, A. , Sedik, M. Z.2016.Incidence of *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* in fresh white cheese in Gaza city markets. Asian Journal of Agriculture and Food Sciences , 04 (05):258-264.

Chanda, S. Rakholiya, K. 2011. Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. Science against microbial pathogens: Communicating Current Research and Technological advances. 520-529.

# [Chaves, C](https://www.ncbi.nlm.nih.gov/pubmed/?term=Chaves%20C%5BAuthor%5D&cauthor=true&cauthor_uid=19480346). [Arias, M.L](https://www.ncbi.nlm.nih.gov/pubmed/?term=Arias%20ML%5BAuthor%5D&cauthor=true&cauthor_uid=19480346). 2009.Characterization of *Listeria monocytogenes* isolates obtained from raw cheese samples acquired from different Costa Rican producer zones. [Arch Latinoam Nutr.](https://www.ncbi.nlm.nih.gov/pubmed/19480346) , 59(1):66-70.

Effimia, E. 2015.Prevalence of Listeria monocytogenes and Salmonella spp. in ready-to-eat foods in Kefalonia, Greece. Journal of Bacteriology & Parasitology,6(5):1-8.

Elshinaway, Saadia H., Meshref, A.M.S. , Zeinhom, M.M.A. ,Hafez, Dalia A.A. 2017.Incidence of *Listeria species* in some dairy products in Beni-Suef Governate. Assiut Veterinary Medical Journal, 63 (152): 5-13.

Hussien, M.F., Amin, Manal M. ,Sadek, O.A.2013. Comparison between the microbiological quality of kariesh cheese manufactured from raw and pasteurized skimmed milk sold in Assiut city markets. Assiut Veterinary Medical Journal , 59 (138 ):129:137.

International Standard Organization 2004.Thermo scientific microbiology products-Horizontal methods for the detection and enumeration of *Listeria moncytogenes*. Part 1: Detection method. Amendment 1( ISO 11290-1:1996 A1:Detection method).

# [Kasalica](https://www.researchgate.net/scientific-contributions/2062795456_A_Kasalica), A., [Vuković](https://www.researchgate.net/scientific-contributions/2022692769_V_Vukovic),V., [Vranješ](https://www.researchgate.net/scientific-contributions/2057852289_A_Vranjes), A. ,[Memiši](https://www.researchgate.net/scientific-contributions/2012791048_N_Memisi), N. 2011.*Listeria monocytogenes* in milk and dairy products. Biotechnology in Animal Husbandry, 27(3):1067-1082.

Kumar, A., Grover, S. ,Batish, V.K. 2015. Exploring specific primers targeted against different genes for a multiplex PCR for detection of *Listeria monocytogenes*. 3 Biotech ,5:261–269.

Lapage S.P., Bascombe, S., Wilcox, W.R. , Curtis, M.A. (1973): Identification of bacteria by computer: General aspects and perspectives J.Gen. Microbiol., 77:273-290.

Makino, S.I., Kawamoto, K., Takeshi, K., Okada, Y., Yamasaki, M.,Yamamoto, S. 2005.An outbreak of food-borne Listeriosis due to cheese in Japan, during 2001.Int. J. Food Microbiol. 104: 189-96.

Manfreda, G., De Cesare, A., Stella, S., Cozzi, M., Cantoni, C. 2005.Occurrence and ribotypes of Listeria monocytogenes in Gorgonzolia cheese. Int. J. Food Microbiol.. 102: 287-93.

Meshref, A.M.S., Zeinhom, M.M.A. , Abdel-Atty, N.S. 2015. Occurrence and distribution of *Listeria Species* in some Egyptian foods. Alexandria Journal of Veterinary Sciences, 46(1): 42-47.

# Navratilova, P., Schlegelova, J., Sustackova, A., Napravnikova, E., Lukasova, J. , Klimova, E. 2004.Prevalence of *Listeria monocytogenes* in milk, meat and foodstuff of animal origin and the phenotype of antibiotic resistance of isolated strains. Vet. Med. - Czech, 49 (7): 243–252.

Rodriguez, L.D., Vazquez Boland, J.A., Fernandez Garayzabal, J.F., Echalecu Tranchant, P., Gomez-Lucia, E., Rodriguez Ferri, E.F. , Suarez Fenandez, G. 1986. A microplate technique to determine hemolytic activity for routine typing of Listeria strains, 24:99-103.

Sambrook, J., Fritscgh, E.F. , Mentiates 1989. Molecular coloning. A laboratory manual. Vol !., Cold spring Harbor Laboratotry press, New York.

# Tantawy, Hasnaa M.I.M. 2011. *Listeria monocytogenes* in Egyptian milk and dairy products. Alexandria University Faculty of Veterinary Medicine. Milk Hygiene Department.Master of Veterinary Medical Science.

# Tittsler, R.P. , Sandholzer, L.A.1936.The use of semi-solid agar for the detection of bacterial motility. Journal of Bacteriology, 31(6):575-580.

Todar, K. 2009. "Listeria monocytogenes". Todar/s Online Textbook of Bacteriology., htt:// textbook of bacteriology.net/Listeria.html.

## VALUE @ Amrita 2011.Virtual Amrita Laboratories Universalizing Education. Gram stain technique.

Wikipedia 2017.<https://en.wikipedia.org/wiki/Listeriosis> .

Table (1): Oligonucleotide primers sequences

|  |
| --- |
| Primer Sequence Amplifid Reference 5\ - 3\ product  |
| *16S* GGA CCG GGG CTA ATA CCG AAT GATAA 1200 bp Kumar *et* *rRNA* TTC ATG TAG GCG AGT TGC AGC CTA  *al*., *2015* |

Table (2): Cycling conditions of the different primers during cPCR:

|  |
| --- |
| Gene Primary Secondary Annealing Extension No. of Final  denaturation denaturation cycles extension   |
| *16S* 94˚C 94˚C 60˚C 72˚C 35 72˚C*rRNA* 5 min. 30 sec. 1 min. 1 min. 12 min. |

Table (3): Total number and percentage of positive samples of *L.monocytogenes* from the examined samples

|  |
| --- |
| Sample Number of Number of Positive percentage  Samples positive samples %1 %2 %3 |
| Raw milk 80 3 3.75 1.5 60Kariesh cheese 40 1 2.5 0.5 20Soft cheese 40 0 0 0 0Ice cream 40 1 2.5 0.5 20Total 200 5 8.75 2.5 100 |

1Percentage in relation to total number of samples in each row.2Percentage in relation to total number of collected samples n=200. 3Percentage in relation to total number of positive samples n=5.

Table (4):Tests and results of MicrogenTM Listeria-ID System

|  |
| --- |
| Nombre Test Result |
| 1 Esculin Black (+)2 Mannitol Purple (-)3 xylose Purple (-)4 Arabitol Yellow (+)5 Ribose Purple (-)6 Rhamnose Yellow (+)7 Trehalose Yellow (+)8 Tagatose Purple (-)9 Glucose-1-Phosphate Purple (-)10 M-D-Glucose Yellow (+)11 M-D-Mannitol Yellow (+)12 Haemolysis Straw-brown colored homogeneous liquid, no carpet of red cells on the well floor (+) |



Fig. (1): Agarose gel electrophoresis for 16S rRNA genes of *L.monocytogenes*. Lane L: 100-1500 bp Ladder. Neg. : Negative control. Pos. : Positive control (at 1200 bp ). Lanes 1 to 5 :*L.monocytogenes* (16SrRNA) gene positive.