

## ORIGINAL ARTICLE

# Prevalence of *L. monocytogenes* and *E. coli* O157:H7 in Some Dairy Products in Beni Suef City, Egypt

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### Abstract

The present study aimed to determine the prevalence of *Listeria monocytogenes* and *E. coli* O157:H7 in some milk products. 100 samples of dairy products (25 each of Kareish cheese, Talaga cheese, small-scale ice cream, and small-scale yoghurt) were collected from retail outlets, farmers' markets and dairy shops in Beni Suef city, Egypt. Microbiological analysis of the samples was performed to detect the presence of *E. coli* O157:H7 and *L. monocytogenes* and the isolates were further confirmed by PCR method using specific primer of *L. monocytogenes* (hlyA gene). The overall prevalence of *Listeria spp.* was 1(4%), 1(4%), 2(8%) and 1(4%) in small-scale yoghurt, Kareish cheese, Talaga cheese and small-scale ice cream respectively. None of the examined milk products yielded an isolation of *E. coli* O157:H7. Coordinated efforts aimed at enhancing the safety of dairy products in Egypt must take into account the application of appropriate hygiene and production standards as well as the evaluation of measures aimed at reducing contamination in the dairy supply chain.

### Keywords

Dairy Products, *E. coli* O157:H7, *L. monocytogenes*, PCR

## 1. Introduction

Due to the high nutritional content of dairy products, they are a significant source of nutrients such as protein, fat, lactose, calcium, vitamins, and other necessary elements that are abundant in milk and milk products, they offer a favorable medium for the development and proliferation of several microorganisms (Pal and Awel, 2014).

The most popular native soft cheese in Egypt is often Kareish cheese, which is distinguished by its high concentration of water-soluble vitamins, calcium, protein, and phosphorus and low-fat content (El-Bagoury and Mosaad, 2002). It is made from raw skim milk either buffalo's or cow's milk. Also, it may be sold uncovered and without container showing it to be suitable medium for the growth of spoilage and pathogenic microorganisms (Dawood et al., 2006).

Yoghurt is a highly favoured fermented milk product in the world, yoghurt is manufactured from pasteurized milk and is produced by lactic acid bacteria under regulated conditions by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Tamime and Robisons, 2007). It is regarded as a nutrient-dense diet since it is rich in nutrients, quickly absorbed, and contains over ten essential nutrients, as well as several minerals and vitamins, the presence of living bacteria in yoghurt has been demonstrated to have quantifiable nutritional benefits (Guarner et al., 2005; Weerathilake et al., 2014).

One of the most widely consumed "luxury" foods in the world is ice cream, which is made from frozen pasteurized milk and other dairy products. Condensed milk products, whole milk, skim milk, cream, frozen cream, and milk solids are the primary components of ice cream.

Despite being a dairy product, ice cream's composition and storage temperature create ideal conditions for the elimination of certain organisms, particularly those that are sensitive to low temperatures. It has been suggested that the ice cream mix's raw ingredients, like milk powder, cream, skimmed milk, flavoring, coloring agents, stabilizers and air during processing, could be the source of these microorganisms (Osamwonyi et al., 2011).

Foodborne pathogens such as *L. monocytogenes* and *E. coli* O157:H7 in milk and dairy products are the most common possible pathogens which represent the main microbiological dangers connected to raw milk (Kousta et al., 2010; Yang et al., 2012) and raw cheese (Verreaes et al., 2015). Worldwide, every year, *E. coli* O157:H7 causes diarrheal illness in around 1.7 billion cases.

The second leading cause of death for children under five is diarrheal illness. Approximately 760,000 children under five pass away from diarrheal diseases annually (Chowdhury et al., 2015). Numerous nations have seen significant listeriosis epidemics, including the USA, Japan, China, and the European Union (including Austria, Denmark, Finland, Sweden, the United Kingdom, Australia, and Spain). Approximately 1060 cases of listeriosis with laboratory confirmation were reported to the National Institute of Communicable Diseases (NICD), with 216 deaths reported as well (Tchatchouang et al., 2020).

Because it is found in the gut, *Escherichia coli* is regarded as a sign of fecal contamination in food. Because certain strains of *E. coli*, known as enterohaemorrhagic *E. coli* (EHEC), may be pathogenic as *E. coli* O157:H7 serotypes. Regarding the presence of *E. coli* in food is categorized as verotoxin-producing *E. coli* (VTEC), which is known to

be the primary cause of hemorrhagic colitis (HC) and the hemolytic-uremic syndrome (HUS) type linked with diarrhea. It is also accountable for around 73,000 episodes of disease each year, including HUS and death. Also associated with outbreaks and sporadic cases of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) Thrombotic Thrombocytopenic Purpura (TTP), bloody and non-bloody diarrhea and other enteric infections all over the world especially in children younger than five years old.

*E. coli* O157:H7 outbreaks have been related to the consumption of unpasteurized milk, as well as raw milk-based dairy products. Humans are susceptible to *E. coli* O157:H7 infection primarily through the consumption of undercooked meat, vegetable, water, raw milk or raw milk products contaminated by feces of healthy carrier animals or dairy environment (Oliver et al., 2005).

Common food-borne pathogens include *Listeria monocytogenes*, which can be found in fruits, vegetables, dairy products, and processed foods. The bacterial pathogen *L. monocytogenes* causes human listeriosis, which can also produce symptoms like headache, stiff neck, drowsiness, nausea, diarrhea, loss of balance, or convulsions. Fever, muscle aches, and occasionally gastrointestinal infections are the symptoms of listeriosis. The disease has a high fatality rate in those who are susceptible, and occasionally the infection can spread to the nervous system as well (Garrido et al., 2010). Fresh, soft and white cheeses are also beneficial to *L. monocytogenes* growth. Additionally, semi-hard cheeses are great for *L. monocytogenes* multiplication. *L. monocytogenes* can be transmitted by the consuming of homemade cheeses which are produced from raw milk (Arslan and Özdemir, 2008).

The hlyA gene is only found in one copy in the genome of pathogenic *L. monocytogenes*, which is useful for gene-based detection, this gene is required for virulence and is used to distinguish *L. monocytogenes* from other strains of *Listeria*, it also codes for the pathogen's toxin. Even with the culture and plating detection method, the final step in confirming the existence of *L. monocytogenes* is to plate the bacteria on blood agar, which demonstrates Listeriolysin O (LLO) activity that is generated from the hlyA gene (Sharma and Mutharasan, 2013).

The current study was conducted to screen the existence of *E. coli* O157:H7 and *Listeria monocytogenes* in some dairy products.

## 2. Materials and Methods

### 2.1. Samples Collection

One hundred samples of dairy products -25 each of Kareish cheese, Talaga cheese, small-scale ice cream, and small-scale yoghurt - were collected from Beni Suef city, Egypt from retail outlets, farmers' markets and dairy shops. The samples were transferred to the laboratory in clean, dry, and sterile insulated ice box with minimum of delay for the following investigations.

### 2.2. Isolation and Identification of *E. coli* O157:H7 (Ahmed and Shimamoto, 2014)

#### 2.2.1. Enrichment

Twenty-five grams of cheese, yoghurt and melted ice cream samples were added to 225ml of tryptone soy broth (Oxoid, UK) supplemented with 20mg/L novobiocin (Oxoid, UK) and incubated for 18-24hrs at 37°C.

#### 2.2.2. Isolation on Sorbitol MacConkey Agar

A loopful from enrichment broth was streaked onto a plate of Sorbitol MacConkey agar (Oxoid, UK) containing tellurite supplement and cefixime (Oxoid, UK) for the purpose of isolating *E. coli* O157:H7 specifically. After inoculation, the plates were incubated for 18 to 24 hours at 37 °C. The plates were checked for growth of pale yellow or straw color colonies indicating non-sorbitol fermenters. Presumptive colonies of *Escherichia coli* O157:H7 were biochemically identified using API 20E strips (Bio Merieux, France).

### 2.3. Isolation and Identification of *L. monocytogenes* (Ismaiel et al., 2014)

#### 2.3.1. Enrichment

Twenty-five grams of each cheese, yoghurt and melted ice cream samples were added to 225ml of *Listeria* enrichment broth with *Listeria* selective supplement (Biolab), then incubated at 30°C for 48hrs.

#### 2.3.2. Selective Plating

A loopful from enrichment broth was streaked onto *Listeria* oxford agar plates (Biolife) with oxford *Listeria* supplement (Himedia) and incubated at 35°C for 24-48hrs. *Listeria* was indicated as characteristic colonies of greyish green to brownish green in color measuring 1-3mm in diameter with black haloes due to aesculin hydrolysis.

### 2.4. DNA Extraction and PCR Conditions

The Patho Gene-spin™ DNA/RNA Extraction Kit was used to extract genomic DNA (Intron Bio., Korea) according to the manufacturer specifications. Conventional PCR was used for the detection of hlyA virulence gene using hlyA-F1 5- GCAGTTGCAAGCGCTTGGAG TGAA-3 as the forward and hlyA-R1 5- GCAACGTATCC TCCAGAGTGATCG -3 as the reverse primers (Metabion international AG, Germany) that target the hlyA virulence gene of product size 465bp (Pospo et al., 2023). PCR amplification was accomplished using COSMO PCR RED Master Mix (Willowfort.co. Birmingham, UK, Lot.N; 0018789, CAT; ND-1289-50) in a 25µL total volume made up of 12.5µL of the Master Mix and 0.5µL of the forward and reverse primers (20pmol working concentration), 6.5µL of nuclease free water, and 5µL of extracted DNA. The reactions were run in Multigene Gradient TC9600-G-230V thermal cycler (Labnet co., USA) with a first denaturation for 2min at 95°C, then 35 cycles of secondary denaturation for 15sec at 95°C, annealing for 20sec at 60°C, and extension for 30min at 72°C; finally, a final extension step for 10min at 72°C. The Spectrophotometer T80 (PG instrument, UK) was used to measure DNA concentration. The amplified products underwent electrophoresis in a 1.5% agarose gel, ethidium bromide staining and UV trans-illumination visualization.

## 3. Results and Discussion

### 3.1. Prevalence of *Listeria* Spp. and *E. coli* O 157:H7 in the Examined Dairy Products

The obtained results indicate that out of 100 examined dairy product samples 5(5%) demonstrated a positive presence of *Listeria* spp. prevalence in small-scale yoghurt, Kareish cheese, Talaga cheese and small-scale ice cream was 1(4%), 1(4%), 2(8%) and 1(4%) respectively. On the other hand, *E. coli* O 157:H7 couldn't be isolated from any of the examined dairy product samples (Table, 1). *Listeria* microorganisms contaminate various dairy products during the processing or ripening stages of cheeses, as well as through post-processing environmental contamination and cross-contamination in dairy plants and/or retail stores (Aygün and Pehlivanlar, 2006).

**Table 1.** Prevalence of *Listeria spp.* in the examined dairy products.

Dairy products	No. of the examined samples	Positive samples	
		No	%
Small scale yoghurt	25	1	4%
Kareish cheese	25	1	4%
Talaga cheese	25	2	8%
Small scale Ice-cream	25	1	4%
Total	100	5	5%

In our study, 1(4%) of small-scale yoghurt samples were contaminated with *Listeria spp.* Results that were almost identical were identified by [Hosseini et al., \(2014\)](#) that only one sample (10%) of yoghurt from total 10 examined samples were positive for *Listeria spp.* Also, [Seyoum et al., \(2015\)](#) found the same result 1(5%) from 20 of the tested samples, while the study performed by [Ismail et al., \(2014\)](#), [Shamloo et al., \(2015\)](#) and [El-Shinawy et al., \(2016\)](#) reported that yoghurt samples were negative for *Listeria spp.* and higher result 2(8%) was postulated by [Gohar et al., \(2017\)](#). Egyptian cheeses have long been an essential component of the contemporary Egyptian diet because of their longer shelf life and higher degree of self-stability compared to milk ([Fox et al., 2004](#)). According to [Table \(1\)](#) there were 2(8%) Talaga cheeses and 1(4%) Kareish cheese samples that were contaminated with *Listeria spp.* A lower incidence of *Listeria spp.* was found by [Ismail et al., \(2014\)](#), while higher incidence was reported by [Metwally and Ali \(2014\)](#), [Abd El-Twab et al., \(2015\)](#) and [Meshref et al., \(2015\)](#).

In Talaga cheese, the higher results were recorded by [Rahimi et al., \(2010\)](#) but lower results were detected by [Arslan and Ozdemir \(2008\)](#), [Kahraman \(2010\)](#), [Al-Ashrawy et al., \(2014\)](#) and [\(El-Etriby, 2016\)](#). On the other hand, some researchers fail to detect *L. monocytogenes* in Talaga cheese samples ([Ahmed, 2013](#); [Akya et al., 2013](#)).

*Listeria spp.* are highly contaminated in Kareish cheese due to the traditional handmade method and inadequate hygienic measures in cheese marketing. The growth of *Listeria* species in Talaga cheese may be due to using raw milk, unhygienic production methods, and improper food storage practices as well as in conditions with low salt conc. and low temp. (4°C) ([El-Shinawy et al., 2016](#)). As a result, cheese could be the appropriate medium for the development and proliferation of the many *Listeria* species, such as *L. monocytogenes* ([Ewida et al., 2022](#)).

*Listeria spp.* are frequently found in several ready-to-eat foods, such as ice cream, due to their psychrotrophic genetic capacities, which increases the risk to public health.

In the present study, about 25 of the examined ice-cream samples, only one sample (4%) yielded growth of *Listeria spp.* ([Rahimi et al., 2012](#); [Shamloo et al., 2015](#); [Abd El-Twab et al., 2015](#); [El-Shinawy et al., 2016](#)) all showed higher levels of *Listeria spp.* in ice-cream roughly 16.7%, 19.04%, 6% and 20% respectively.

Contrary to our results, the tested ice cream samples did not yield any isolates of *Listeria spp.* as showed in ([Ambily and Benna, 2012](#);

[Kevenk and Gulel, 2016](#)). Also, [Metwally and Ali \(2014\)](#) found that none of the examined ice cream samples contained *Listeria spp.*

The increased prevalence of *Listeria spp.* in samples of ice cream may be caused by tainted raw milk, improper handling and processing procedures, low-quality ingredients utilized in production, and incomplete pasteurization, particularly in small-scale ice cream ([El-Shinawy et al., 2016](#)). An ageing phase in the production of ice cream entails refrigerating the ice cream mixture overnight, which promotes the growth of psychrotrophic microbes such *Listeria spp.*

In the current investigation, none of the dairy products samples that were tested yielded an isolation of *E. coli* O157:H7 while higher results were recorded by [Abd El-Atty and Meshref \(2008\)](#), [Elbastawesy et al., \(2016\)](#) and [Elafify et al., \(2019\)](#) in the examined Kareish cheese samples. Similar results were achieved by [El-Bagory and Hammad \(2004\)](#), [Ahmed and Shimamoto \(2014\)](#), [Elbastawesy et al., \(2016\)](#), and [Elafify et al., \(2019\)](#).

The primary cause of the inability to identify *E. coli* O157:H7 in the examined dairy products is that the isolation of *E. coli* O157: H7 is frequently challenging due to its intermittent presence at very low levels among extremely high levels of competing species ([Siriken, et al., 2006](#)).

### 3.2. PCR Technique for *L. monocytogenes* hlyA Gene Detection

The goal of this work was to identify one of *L. monocytogenes*' virulence factors in order to further confirm the isolates. Listeriolysin O (LLO), a virulence factor exclusive to virulent strains of the species and is encoded by the hlyA gene, was the target of PCR ([Suriyapriya et al., 2016](#)). Listeriolysin O (hlyA) is a toxin that is dependent on cholesterol that makes it easier for bacteria to proliferate in the cytosol, escape from phagocytic vacuoles, and infiltrate host cells ([Hassanien and Shaker, 2021](#)). In our investigation, the PCR method's result was shown in [Table \(2\)](#) and [Fig. \(1\)](#) verified that *L. monocytogenes* was present in only Talaga cheese sample and couldn't be confirmed in other dairy products. [Derra et al., \(2013\)](#) found that only one sample (1.7%) of cottage cheese tested sample was positive for *L. monocytogenes* using PCR, however ([Al-Ashrawy et al., 2014](#)) studied the hlyA gene in 28 and 32 of the analyzed Domiati and Kareish cheeses, respectively. ([El-Shinawy et al., 2016](#)) verified that *L. monocytogenes* was present in 2(5%), 3(7.5%), and 1(2.5%) of butter milk cheese, ice-cream and Kareish cheese samples respectively.

**Table 2.** Confirmation of *Listeria monocytogenes* isolated by PCR technique.

Dairy products	No. of dairy samples	No. of samples analyzed for PCR	Positive samples for <i>L. monocytogenes</i>	
			Positive PCR samples	%
Small scale yoghurt	25	1	0	0%
Kareish cheese	25	1	0	0%
Talaga cheese	25	2	1	4%
Small scale Ice-cream	25	1	0	0%
Total	100	5	1	1%



Fig. (1). PCR-assisted Agarose gel electrophoresis of the hlyA gene specific to *L. monocytogenes*: Lane L: Ladder, Lane 1-5: Isolates from various samples, Lane PC: positive control for hlyA at 456bp, Lane NC: negative control.

#### 4. Conclusion

According to this study, several dairy products offered in Beni Suef marketplaces can pose a risk to customers. They are important carriers of *L. monocytogenes*, which frequently results in outbreaks of listeriosis. As a result, those with known risk factors and those who are prone to contracting listeriosis ought not to use these products. This highlights the significance of ongoing oversight and the necessity of identifying possible contamination sources. By implementing Hazard Analysis and Critical Control Points (HACCP) during the production and processing stages, the likelihood of pathogen microorganisms contaminating products is decreased.

#### 5. Authors Contributions

All authors participated equally to the design of the research, methodology, and writing of the manuscript.

#### 6. Conflict of Interest

The authors declare no conflict of interest.

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