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Prevalence and Genetic Detection of L. Monocytogenes from Milk and some Milk Products

Ashraf, A. Abd-El Tawab[1]; Fatma, I. El-Hofy[1]; Elham, A. Mobarez[2]; Nancy, Y. Tawkol[3]

[1] *Bacteriology, Immunology and Mycology Dep., Faculty of Vet. Med., Benha Uni.*

[2] *Animal Health Research Institute Dokki, Giza*

[3]*Veterinarian*

A B S T R A C T

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 Qaliopia and Giza governorates 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 Microgen ™ 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 ™ Listeria-ID System, 16S rRNA

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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. It causes infections of the central nervous system (meningitis, meningoencephalitis , brain abscess, cerebritis) and bacteremia in those who are immunocompromised, pregnant women, and those at the extremes of age (newborns and the elderly), as well as 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 Qaliobia and Giza governorate.

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 Qaliopia 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:

Microgen^{TM} 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 color change after 18-24 hours incubation. These results are then analyzed using the Microgen Identification System Software (MID-60) (La page *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 QIA amp® DNA Mini Kit instructions (Catalogue no. M501DP100), Emerald Amp GT PCR master mix (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 Microgen™ Listeria-ID System (Table 4) showed that all strains were *L. monocytogenes* (99.92%).

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

Table (1): Oligonucleotide primers sequences

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

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

Gene	Primary denaturation	Secondary denaturation	Annealing cycles	Extension	No. of Final extension
16S	94˚C94˚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 Samples	Number of positive samples	Positive percentage %[1]	%[2]	%[3]
Raw milk	80	3	3.75	1.5	60
Kariesh cheese	40	1	2.5	0.5	20
Soft cheese	40	0	0	0	0
Ice cream	40	1	2.5	0.5	20
Total	200	5	8.75	2.5	100

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

Table (4): Tests and results of Microgen^{TM} 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 (+)

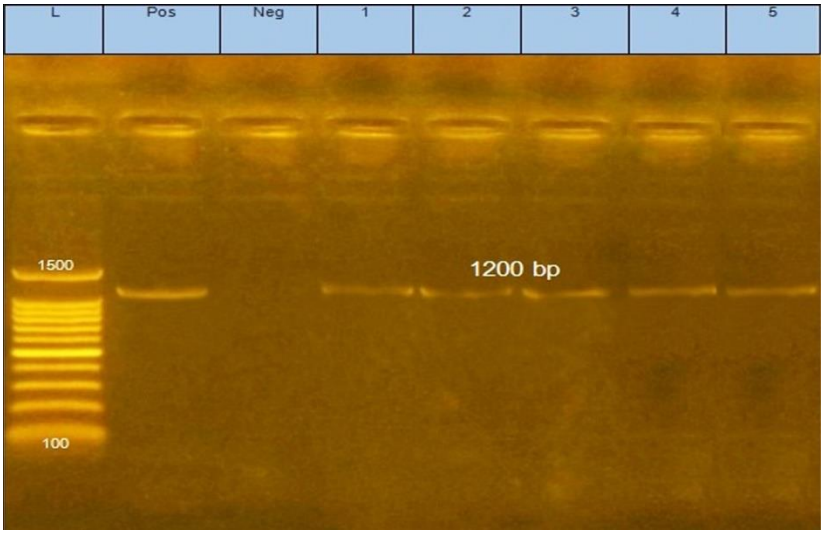

Fig. (1): Agarose gel electrophoresis for 16S rRNA genes of *L. monocytogenes*. Lane L: 100-1500 bp Ladder. Neg.: Negative control. Pos.: Positive control (at 1200 bp). Lanes 1 to 5: *L. monocytogenes* (16SrRNA) gene positive.

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 40 ice 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 that3(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 Qaliobia 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.

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