



Advanced Identification and Characterization of *Listeria* Species in Egyptian Local Soft Cheese

Amany N. Dapgh^{1*}, A. A. ELGedawy¹, Hussien A. Abouelhag²,
Asmaa S. Mansour², E. S. Gaber² and Engy Farahat²

¹Animal Health Research Institute (AHRI), ARC, Dokki, Giza, Egypt.

²Department of Microbiology and Immunology, National Research Centre, Dokki, Cairo, Egypt.

Authors' contributions

This work was carried out in collaboration among all authors. Authors AND and HAA designed the study, wrote the protocol. Authors ASM, ESG and EF wrote the first draft of the manuscript. Authors ASM and ESG collected and prepared the samples. Authors ASM, ESG and EF performed the isolation and classical biochemical identification. Authors HAA, ASM, ESG and EF performed the DNA extraction and helped in real time PCR. Authors AND and AAEG conducted the antibiotic susceptibility profile. Author HAA managed the analyses of the study. Author AND managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2020/v8i230188

Editor(s):

(1) Dr. Ana Claudia Coelho, University of Tras-os-Montes and Alto Douro, Portugal.

Reviewers:

(1) Kadhim Fadhil Kadhim, Basrah University, Iraq.

(2) Sanjeev V. Jakati, Maharashtra university of Health Sciences, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/63104>

Original Research Article

Received 02 September 2020

Accepted 09 November 2020

Published 07 December 2020

ABSTRACT

Aims: one of the most important foodborne microorganisms is the Gram's positive environmental wide spread *Listeria spp.* As the *Listeria* may be considered a public health concern so there is in needing to rapid, precise and reliable diagnosis of the organism in consumed food. The present study aimed to survey the presence of *Listeria spp.* among two popular consuming Egyptian white soft cheese using advanced biochemical, antibiotic susceptibility and molecular techniques.

Methodology: *Listeria spp.* was investigated in 155 samples of two white soft cheeses (70 kareish cheese and 85 Damietta cheese) collected from street vendors and retail markets in Giza. The existence of *Listeria spp.* was tested through cultural and the identification was confirmed biochemically by Vitek2 compact system as well as molecular identification via duplex real time PCR using species specific primers.

Results: The results of the study revealed the isolation of two *Listeria spp.* in a total number of 22 from 155 samples (14.19%); 14 isolate out of 70 (20%) Kareish cheese while 8 isolates out of 85

*Corresponding author: Email: amany.nabil.dapgh@gmail.com;

(9.4%) Damietta cheese's samples. The 22 *Listeria* spp. isolates were differentiated into *L. innocua* 15 (68.18%), and *L. monocytogenes* 7 (31.81%) also their antimicrobial susceptibility was declared using advanced Vitek-2 compact system. The two *Listeria* spp. isolates were definitely confirmed by using diplex DNA hybridization real PCR technique.

Conclusion: Soft raw milk based cheese is a popular food in Egypt and looked on as a risk for foodborne bacteria contamination. The data of this study pointed out that there is a potential risk of infection with *Listeria*, especially the public health concern *L. monocytogenes*. The current study presented Vitek-2 compact system as advanced technique for not only for identification and differentiation of *Listeria* strains but also for their antimicrobial susceptibility. Furthermore the using of diplex real PCR technique gives a chance for quick and precise identification.

Keywords: Damietta cheese; Kareish cheese; *Listeria*; real time PCR; Vitek2.

1. INTRODUCTION

Listeria spp. is one of the most significant foodborne bacteria which causes listeriosis; a serious disease in human especially in children, pregnant women and immune-compromised individuals [1].

Listeria infection is either sporadic or in form of outbreaks and primarily occurs as a result of occasional contamination then consumption of ready-to-eat, raw and non-heated food products [2]. Soft white cheeses are widely consumed by Egyptian people due to their high protein content, low price and palatability however, the cross-contamination of such foods with pathogenic microorganisms could occur easily due to human handling and lack of heat processing [3].

Karish cheese as well as Damietta cheese is one of the most popular local types of fresh soft cheese in Egypt. Karish cheese is an ancient cheese affiliated to pharaonic age, traditionally made from defatted (skim) cow or buffalo's milk or a mixture of both. Otherwise, Domiati cheese or Damietta cheese (Gebnah Domiati) is another popular soft white cheese; pickling and salting at the first step in its manufacture give its distinctive flavor [4].

These traditional processes confer many opportunities for microbial contamination. Generally, those cheeses made from raw milk often of poor bacteriological quality and produced under unsatisfactory circumstances. Also, this product is sold exposed without a container, thus the risk of contamination is very high. Therefore, it can be regarded as a convenient medium for the growth of various types of microorganisms [5].

However, there are few published data concerning the prevalence of *Listeria* spp. in Egyptian food. Therefore, the current study was performed to investigate the existence and

distribution of *Listeria* spp. in widely consumed Egyptian white soft cheeses (kareish cheese and Damietta cheese) via advanced, rapid and precise tools.

2. MATERIALS AND METHODS

2.1 Sampling

A total of 155 samples (70 from street vendors' kareish cheese while 85 Damietta cheese samples collected from street vendors and retail markets in Giza rural places, during 2019. All samples were aseptically gathered and transmitted into individual sterile bags or flasks then transported to the laboratory in insulated cold packs within ice box and were analyzed.

2.1.1 Preparation of samples

Twenty five grams of each soft cheese was homogenized in a blender as required for through mixing enrichment steps; aseptically added to 225 ml of 0.1% bacteriological peptone and mixed well, and incubated at 30°C for 24 h.

2.2 Isolation and Identification of *Listeria* spp

The samples were tested for the presence of *Listeria* spp. using selective media "Listeria enrichment broth" (Himedia) supplemented with Listeria selective enrichment supplement (Nalidixic acid, cycloheximide, Acriflavine hydrochloride) (Himedia), followed protocol guided by Markey et al., [6]. A loopful of the incubated broth was streaked onto Oxford agar and PALCAM agar (Oxoid) plates and incubated at 30°C for 48h.

Grey-green colonies surrounded by black zones of esculin hydrolysis were presumed to be *Listeria*. Presumptive *Listeria* colonies were picked from selective agar plate then purified using tryptone soya agar (Himedia) and divided

to two groups. The first group subjected to conventional biochemical identification; Non-spore forming, Gram positive coccobacilli isolates were tested for catalase and umbrella growth in motility test medium at 25°C. Isolates positive for these tests were further examined for hemolysin production using Blood agar base (Sigma- Aldrich) supplemented with 5% sheep blood.

2.3 Advanced Method for Biochemical Identification and Antimicrobial Sensitivity

The second group of purified cultures was exposed for identification and differentiation using the Vitek2 compact systems according to the manufacture instruction. Each isolate was aseptically transmitted to 3.0 ml sterile saline into a polystyrene test tube. The homogenous organism suspension was prepared with a density equivalent to a McFarland 0.50 -0.63 using DensiChek. The suspension tube for each isolate as well as a specific Gram positive identification or an antimicrobial susceptibility card was placed in the cassette. The cassette was loaded into the instrument, sealed and incubated. During the incubation period the cards were read every 15 min. Vitek2 compact system software reported the data automatically according to Biomerieux user guide [7].

2.4 Advanced Identification of *Listeria* Species Using Duplex Real-Time PCR Assay

The isolates were grown for 24 h on solid culture media under the appropriate environment and condition. Crude genomic DNA extract (approximately 5–20 µg ml⁻¹) was obtained in 15–20 min from a single colony using reagent (Sigma-Aldrich, USA) according to the manufacturer's instructions. Each batch of DNA preparation included a non-template control made of the reagent and water. Sequences of the species-specific target genes [8], (Table 1) obtained from GenBank (<http://www.ncbi.nlm.nih.gov/GenBank>; accessed March, 2012) were aligned using StepOne™ software version 2.2.2 (Life Technologies, Thermo Fischer, USA). The oligonucleotides were supplied by (Edificio-Quórum3, Spain). Each duplex was performed in 25 µl total volume, which included 5 µl of DNA extract, 1X Taq MTplexdtec-RT-qPCR (2X), universal PCR Master Mix (Edificio-Quórum3, Spain), 0.4 µmol

l-1 of each primer and 0.1 µmol l-1 of each probe. Forty reaction cycles were performed in 45 min. PCR products were detected by monitoring the elevation in fluorescence of the reporter dye at each PCR cycle. Applied Biosystem software plots the normalized reporter signal determining the threshold cycle (Ct) value.

3. RESULTS AND DISCUSSION

Genus *Listeria* constitutes different species that present everywhere in nature so that easily enter the food chain causing thousands cases of foodborne illness annually. The pathogen can overcome numerous stressful conditions in food environments; refrigeration temperature, at pH values of 5 and above, in high salt concentration (up to 10%) and are relative resistance to freezing and drying [9].

Our investigation discussed the existence of *Listeria* spp. which was surveyed in 155 soft cheese samples. *Listeria* spp. were isolated from 22 (14.19%); 14 out of 70 (20%) Kareish cheese while 8 out of 85 (9.4%) Damietta cheese' samples.

The higher isolation of the organism from Kareish cheese than Damietta cheese samples may be attributed to the street vendors sample origin.

There were two *Listeria* species isolated and confirmed biochemically by conventional and advanced Vitek-2 methods; *L. innocua* 15 (68.18%), and *L. monocytogenes* 7 (31.81%).

Our incidence was near 'either little more or little less' the previous ones recorded in many dairy products surveys; Silva et al. [10] detected *Listeria* in 16.7% of cheese samples, while on another study the incidence was 13.73% [11].

On the other hand, Rahimi et al. [12] reported that (11.2%) of examined samples were contaminated with *Listeria* spp., in which *L. innocua* and *L. monocytogenes* were detected in (72.4%) and (20.7%).

The relative high contamination incidence of *Listeria* spp. in these white soft cheeses may be referred to usage of raw milk, high water activity, environmental contamination and insufficient hygiene during production and handling [13].

Table1. Oligonucleotides' sequences of the duplex designed for the real-time PCR assay to identify two *Listeria* species

Organism	Target gene (accession number)	Forward primer	Reverse primer	Probes 5' → 3'
<i>Listeria monocytogenes</i>	Phosphatidyl inositol phospholipase C	CGGCGCACCTAA CCAAGTAA	CAGTCTGGACAATC TCTTTGAATTTT	(YY)TCAAGATGACTACAAT GGTCCGAGTGTGAAAA(BHQ1)
<i>Listeria innocua</i>	Inhibitor of Apoptosis protein	CTACAAGTAAAC GAGGTTGCTAC	GGAAGTAAGAATG CTGTGGTC	(DFO)CTCCAGCGCCAGAAC GTACATTAAGCC (BHQ2)

L. monocytogenes represents the most important member as its presence in food is of a public health concern, while the presence other than *L. monocytogenes* is an indication that the circumstances are liable to allow contamination with *L. monocytogenes* [14].

It is a Gram-positive bacillus, facultative intracellular pathogen, causes the infection of Listeriosis; a serious disease in children, pregnant women and immunocompromised individuals [15].

The antibiotic susceptibility profile of the two isolated *Listeria* spp. was tested through Vitek 2 compact system cards, as shown in Tables 2 and 3.

The data obtained from Table 2 demonstrated that the *L. innocua* isolates were high susceptible to nitrofurantoin and to trimethoprim/sulfamethoxazole, and ceftioxin intermediate susceptible to levofloxacin but resistant to ciprofloxacin, moxifloxacin oxacillin, vancomycin, erythromycin, clindamycin, gentamycin, rifampicin, tetracycline and doxycycline.

On the other hand, the data obtained from Table 3 demonstrated fortunately, that the *L. monocytogenes* isolates were sensitive to many antimicrobials; ampicillin, ciprofloxacin, levofloxacin, moxifloxacin, vancomycin, erythromycin, tigecycline, nitrofurantoin and linezolid while resistant to gentamycin high level,

streptomycin high level, clindamycin, tetracycline and doxycycline.

These results coincided with that achieved by numerous previous studies; especially the resistance of *Listeria* spp. to both clindamycin and tetracycline as well as their susceptibility to trimethoprim/sulfamethoxazole and vancomycin [2,16-19].

The hardness for the diagnosis of *Listeria* is due to the long time needed for the isolation and affirmation by conventional microbiological methods. So development of reliable advanced technique for detection is greatly needed [20]. Real time PCR is a quick, sensitive, and specific method for the direct detection of *Listeria* spp. especially *L. monocytogenes*.

Our study was conducted through the duplex DNA hybridization PCR designed for identifying *L. monocytogenes* and *L. innocua*'s using specific primers for species-specific genes in the genome and it takes few hours for the confirmation of their existence. All *Listeria* isolates were identified as the expected species by amplification of the predicted targets; with Ct values ranging from 20 to 35; Fig. 1. depending on the amount of starting material used. The negative control did not amplify any of the PCR targets.

The obtained direct real time PCR data harmonized with previous investigations [21-23].

Table 2. Antimicrobial susceptibility of *L. innocua* using vitek 2 compact system

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefotixin Screen	POS	+	Inducible Clindamycin resistance	NEG	-
Ampicillin			Erythromycin	>=8	R
Oxacillin	>=4	R	Clindamycin		R
Gentamicin high level (Synergy)			Linezolid	>=8	
Streptomycin high level (Synergy)			Daptomycin	>=8	
Gentamicin		R	Vancomycin	>=32	R
Ciprofloxacin	4	R	Rifampicin	>=32	R
Levofloxacin	4	I	Doxycycline	>=16	R
Moxifloxacin	>=8	R	Tetracycline	>=4	R
Nitrofurantoin	32	S	Tigecycline	1	
Trimethoprim/sulfamethoxazole	<=10	S			

Table 3. Antimicrobial susceptibility of *L. monocytogenes* using Vitek 2 compact system

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefotixin Screen			Inducible Clindamycin resistance		
Ampicillin	4	S	Erythromycin	<=0.25	S
Oxacillin			Clindamycin	>=4	R
Gentamicin high level (Synergy)	SYN-R	R	Linezolid	1	S
Streptomycin high level (Synergy)	SYN-R	R	Daptomycin		
Gentamicin			Vancomycin	1	S
Ciprofloxacin	<=0.5	S	Rifampicin		
Levofloxacin	0.25	S	Doxycycline	>=16	R
Moxifloxacin	<=0.25	S	Tetracycline	>=16	R
Nitrofurantoin	<=16	S	Tigecycline	<=0.12	S
Trimethoprim/sulfamethoxazole					

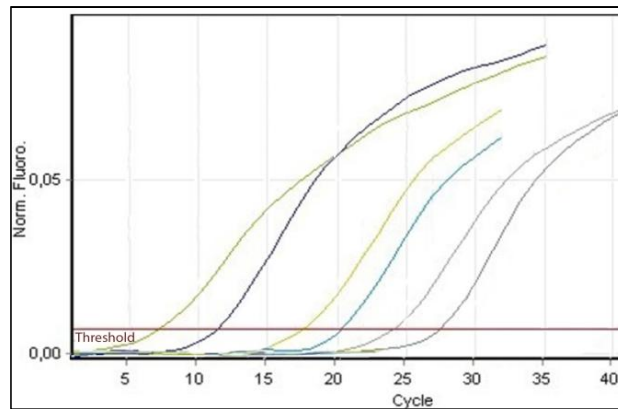


Fig.1. Real time PCR data harmonized

4. CONCLUSION

The results of the current study revealed the existence of *Listeria* spp. in two types of local white soft cheeses; kareish and Damietta cheeses. The usage of advanced techniques as Vitek2 and real time PCR could identify and differentiate the isolates in shorter time than conventional methods. The isolation of *L. monocytogenes* has taken a special attention because of its public health concern.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fan Z, Xie J, Li Y, Wang H. Listeriosis in mainland China: A systematic review. *Int J Infect Dis*. 2019;8:17–24.
2. Mohajeri FA, Derakhshan Z, Ferrante M, Hamidiyan N, Soleymani M, Conti OC et al. The prevalence and antimicrobial resistance of *Listeria* spp. in raw milk and traditional dairy products delivered in Yazd, central Iran (2016). *Food Chem Toxicol*. 2018;114:141-144.
3. FAO/WHO, "Risk assessment of *Listeria monocytogenes* in ready-to-eat foods," Microbiological risk assessment series technical report 5, FAO/WHO; 2004.
4. Abou-Donia SA. Origin, history and manufacturing process of Egyptian dairy products: An overview. *Alex J Fd Sci. and Technol*. 2008;5(1):51-62.
5. Mahmoud I. Safety of traditional food: Regulations and technical aspects. *Scienza Tecnica-Lattiero-Casearia*. 2009; 55(3):159-165.
6. Markey BK, Leonard FC, Archambault M, Cullinane A, Maguire D. *Clinical veterinary microbiology*. Second edition. MOSBY. Elsevier Ltd. Edinburgh London New York, Oxford Philadelphia St Louis Sydney Toronto; 2013.
7. BioMérieux. Vitek 2 product information, document 510769-4EN1.bioMérieux, Inc., Durham, NC; 2006.
8. Hage E, Mpamugo O, Ohai C, Sapkota S, Swift C, Wooldridge D et al. Identification of six *Listeria* species by real-time PCR assay. *Lett Appl Microbiol*. 2014;58(6):535-40.
9. Ozbey G, Icyeroglu A, Muz A. Prevalence of *Listeria* species in raw hamburger meatballs and chicken burgers in eastern Turkey. *Afr J Microbiol Res*. 2013;7(31): 4055–4058.
10. Silva IM, Almeida RC, Alves MA, Almeida PF. Occurrence of *Listeria* spp. in critical control points and the environment of Minas Frescal cheese processing. *Int J Food Microbiol*. 2003;81(3):241-8.
11. Meshref AMS, Mohamed MAZ, Abdel-Atty NS. Occurrence and distribution of *Listeria* species in some Egyptian foods. *Alexandria Journal of Veterinary Sciences*. 2015;46:42-47.
12. Rahimi E, Ameri M, Momtaz H. Prevalence and antimicrobial resistance of *Listeria* species isolated from milk and dairy products in Iran. *Food Control*. 2010;21: 1448–1452.
13. Rogga KJ, Samelis J, Kakouri A, Katsiari MC, Savvaidis IN, Kontominas MG. Survival of *Listeria monocytogenes* in Galotyri, a traditional Greek soft acid-curd

- cheese, stored aerobically at 4°C and 12°C. *Int Dairy J.* 2005;15:59–67.
14. Orsi RH, Den Bakker HC, Wiedmann M. *Listeria monocytogenes* lineages: Genomics, evolution, ecology, and phenotypic characteristics. *Int J Med Microbiol.* 2011;301(2):79-96.
 15. Pagliano P, Ascione T, Boccia G, De Caro F, Esposito S. *Listeria monocytogenes* meningitis in the elderly: Epidemiological, clinical and therapeutic findings. *Le infezioni in medicina.* 2016;24(2):105–111.
 16. Maha AM, AL-Ashmawy, Mayada M, Gwida, Abdelgalil Kh H. Prevalence, detection methods and antimicrobial susceptibility of *Listeria monocytogenes* isolated from milk and soft cheeses and its zoonotic importance. *World Appl Sci J.* 2014;29(7):869-878.
 17. Gómez D, Azón E, Marco N, Carramiñana JJ, Rota C, Ariño A, Yangüela J. Food microbiol antimicrobial resistance of *Listeria monocytogenes* and *Listeria innocua* from meat products and meat-processing environment. 2014;42:61-5.
 18. Escobar C, Gómez D, Del Carmen M, García R, Conchello P, Herrera A. Antimicrobial resistance profiles of *Listeria monocytogenes* and *Listeria innocua* isolated from ready-to-eat products of animal origin in Spain. *Foodborne Pathog Dis.* 2017;14(6):357-363.
 19. Darla SVM, Haubert L, De Fátima SRW, Kroning IS, Cardoso MR, Lopes GV et al. *Listeria monocytogenes* in sliced cheese and ham from retail markets in southern Brazil. *FEMS Microbiol Lett.* 2019;366(22):fnz249.
 20. Amagliania G, Brandiad G, Omicciolib E, Casierebl A, Brucec J, Magnani M. Direct detection of *Listeria monocytogenes* from milk by magnetic based DNA isolation and PCR. *Food Microbiology.* 2004;21(5):597-603.
 21. Ye L, Li Y, Zhao J, Zhang Z, Meng H, Yan H et al. Development of a real-time loop-mediated isothermal amplification assay for the sensitive and rapid detection of *Listeria monocytogenes*. *Lett Appl Microbiol.* 2015;61(1):85-90.
 22. Kędrak-Jabłońska A, Budniak S, Krupa M, Szczawińska A, Reksa M, Szulowski K et al. Detection of *Listeria Spp.* and *Listeria monocytogenes* in Biological Samples by SYBR Green I and TaqMan Probe-based Real-time PCRs. *J Vet Res.* 2017;61(4):427-432.
 23. Torresi M, Ruolo A, Acciari VA, Ancora M, Blasi G, Cammà C et al. Real-time PCR Screening assay for rapid detection of *Listeria Monocytogenes* outbreak strains. *Foods.* 2020;9(1):67.

© 2020 Dapgh et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:

<http://www.sdiarticle4.com/review-history/63104>