

## Evaluation the antibacterial effect of hydroalcoholic coffee extract on *L. monocytogenes* isolated from milk and milk products

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### Abstract

*Listeria spp.* is a very resilient bacteria that can persist hostile dairy-processing environments and consequently, food safety challenges appear here with respect to control its growth. *L. monocytogenes* is the most important species that cause a great concern in both food processing and public health. Therefore, this study was performed to investigate the incidence of *Listeria spp.* in 450 samples of raw milk, pasteurized milk, flavored milk, dominate cheese, kareish cheese, small scale ice cream, large scale ice cream, small scale yoghurt, and large scale yoghurt (50 each) were collected from dairy farms, local dairy shops and street vendors in Qena city, Egypt. *Listeria spp.* were isolated from 27 (6%) out of the total samples examined. They were recovered from the tested market raw milk, kareish cheese, small scale ice cream and domiati cheese samples with percentages of 20, 16, 14% and 4%, respectively. However, *Listeria spp.* was absent in other analyzed samples. The most prevalent species was *L. monocytogenes* 10 (2.22%), followed by *L. grayii* 9 (2%), *L. welshimeri* 4 (0.89%) and *L. innocua* 3 (0.67%) with an overall 5.7% verified by PCR. Eight out of 10 *L. monocytogenes* isolates were confirmed by the presence of the 16S rRNA gene that implies a risk of food-borne listeriosis among dairy product consumers. Furthermore, a unique approach was used for the assessment of antibacterial activity of different concentrations of gentamicin as a reference antibiotic standard and hydroalcoholic coffee extract (H. A. coffee extract) (1, 3, 10 and 30 µg/ml) against local isolated *L. monocytogenes* strain in vitro using a microplate in ELISA reader is provided in the current work. The most Potent antimicrobial activity of gentamicin and H. A. coffee extract was obtained at 30 µg/ml against the isolated *L. monocytogenes* strain. At which not exhibit *listeria* growth up to 98.3 % and 96.1 %, respectively. H. A. coffee extract was screened using gas chromatography–mass spectrometry (GC-Mass). This analysis showed that the antibacterial impact of H. A. coffee extract against *L. monocytogenes* is attributed to the active components (caffeine and eugenol).

**Keywords:** Dairy products, Gas chromatography–mass spectrometry, Hydroalcoholic coffee extract, *Listeria species*, PCR.

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## Introduction

Milk and dairy products including yoghurt, cheeses and ice cream are highly perishable food stuff. They are widely accepted among all ages at different stages of society. Unlucky, they are an optimal media for the replication of pathogenic bacteria (Jordan et al., 2016).

Microbial activity is a fundamental mode of deterioration of abundant foods. Thanks to very resilient organisms, one of them are *Listeria* genus that can resist hostile surrounding conditions and consequently, food safety challenges appear here with respect to control its growth (Neha et al., 2018).

Among genus *Listeria*, *L. monocytogenes* is the most important species that cause a great concern in both food processing and public health. It is the primary etiological agent of a fatal zoonotic illness called listeriosis (Thakur et al., 2018). Several listeriosis outbreaks and sporadic cases have been linked to the ingesting contaminated milk and milk products (EFSA, 2018). *L. monocytogenes* possess specific characteristics to persist in sever harsh environmental conditions (El-Shinaway et al., 2017a). Consequently, the behavior of this pathogen elevates its risk of transmission of through foods to consumers causing listeriosis.

Patients at risk of *L. monocytogenes* are often immunocompromised and vulnerable, and guidelines for empiric antibiotic treatment may therefore recommend broad-spectrum antibiotics like gentamicin.

Globally, there is a growing use of plant extracts to reduce public health hazards and economic losses in food industry due to foodborne microorganisms (Mostafa et al., 2018). Coffee is one of the most popular beverages attributed to its attractive taste, stimulant effect, and numerous beneficial medicinal properties including antibacterial, antifungal, anti-

viral and antioxidant activities thanks to its some biomedical components (Gloria et al., 2020). Many studies proved that coffee has an inhibitory activity against *L. monocytogenes* (Martínez-Tomé et al., 2011). Therefore, this study was carried out to detect and identify *Listeria spp.* in milk and some milk products conventionally and by PCR technique and to determine the antibacterial activity of gentamicin and hydroalcoholic coffee extract against *L. monocytogenes*.

## Materials and methods

### Ethical approval:

All experimental procedures in the present study were performed and approved in accordance with the Ethics Committee of the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

## Materials and methods

### Collection of samples:

A total of 450 samples of raw milk, pasteurized milk, flavored milk, dominate cheese, kareish cheese, small scale ice cream, large scale ice cream, small scale yoghurt and large scale yogurt (50 each) were collected from various locations within Qena city from May 2018 to December 2019 and transported to the laboratory in insulated coolers containing cold packs and were analyzed immediately. They were prepared according to FDA (2002).

### I. Isolation and identification of the *Listeria species*:

The isolation of *Listeria spp.* is adopted according to FDA (2002). About 25 ml/g of each sample was aseptically added to 225 ml of listeria selective enrichment broth (CM0862, Oxoid, England) supplemented with listeria selective enrichment agents (SR0141, Oxoid, England) and incubated in 30°C for 24-48 h. A loopful of the incubated broth was streaked onto PALCAM agar (CM0877, Oxoid, England) supplemented

with PALCAM selective supplement (SR0150E 0, Oxoid, England) which incubated for 48 hrs. at 30°C according to (Cutris et al., 1989). Typical grey green colonies with a black halo against a cherry red medium background 1-3 mm in diameter of aesculin hydrolysis were presumed to be *Listeria*. Non spore forming, Gram positive coccobacilli isolates were tested for catalase test, umbrella growth in motility test medium at 25°C,  $\beta$ . haemolysis activity, CAMP test (synergistic lyses of red blood cells) against *S. aureus* and acid production from fermentation of rhamnose, xylose and mannitol according to McLauchin and Rees (2008).

## II. Molecular confirmation of the isolated *Listeria spp.* by PCR methods:

Extraction of DNA was carried out using Veterinary DNA/RNA Extraction Kit (RUO REF. 17159, Intron, Korea), according to manufacturer's instructions for 16S rRNA gene for genus *Listeria*. Whereas, for 16S rRNA gene specific for *L. monocytogenes*, DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen GmbH, Germany) according to the manufacturer's instructions. The PCR amplification and thermal cycling parameters were done according to Beasley and Saris (2004) and Wiedmann et al. (1993) using the following primers as illustrated in Table 1. Conditions of PCR were illustrated in Table 2.

**Table 1. The sequences of the primers used in the molecular confirmation of the isolated *Listeria spp.*:**

Primer Name	Target gene	Sequence(5'-3')	Amplified product	Reference
27 F	16S rRNA for <i>Listeria</i> genus	AGAGTTTGATCCTGGCTCAG	900 bp	Beasley and Saris (2004)
907 R		CCGTCAATTCCTTTGAGTTT		
Lm3 F	16S rRNA species-specific for <i>L. monocytogenes</i>	GGACCGGGGCTAATACCGAATGAT AA	1200 bp	Wiedmann et al., (1993)
Lm5 R		TTCATGTAGGCGAGTTGCAGCCTA		

**Table 2. Conditions of the PCR methods used in the molecular confirmation of the isolated *Listeria spp.*:**

Gene	Primary denaturation	Secondary denaturation	Ann.	Ext.	No. of cycles	Final ext.
16S rRNA for <i>Listeria</i> genus	95°C/5 min	95°C/ 30sec	48°C/ 30 sec	72°C/ 1 min	40	72°C/ 10 min
16S rRNA species-specific for <i>L. monocytogenes</i>	94°C/5 min	94°C/30 sec	60°C/ 1 min	72°C/ 1 min	35	72°C/ 12 min

Ann. = Annealing, Ext. = Extension.

## III. Antibacterial effect of gentamicin and hydroalcoholic coffee extract and against *L. monocytogenes*:

### a) Preparation of inoculum:

To prepare the inoculum, one colony from the PALCAM agar plate was transferred to TSB and incubated for 24 h at 37°C. The inoculum was standardized until

an absorbance of 0.1 at 620 nm (Cava et al., 2007).

### b) Extraction of light roasted coffee bean (hydroalcoholic extract):

Extraction of coffee powder was carried out in duplicate with 60% ethanol. Briefly, 20 g of spent light roasted grounds

dispersed in 200 ml of solvent. After that, samples were shaken for 20 minutes at room temperature (20°C). The extracts were then filtered and concentrated under vacuum in a rotavapor. In order to obtain dry powder, the residue was then freeze dried.

**c) Determination of antibacterial effect of gentamicin and H. A. coffee extract on local isolated *L. monocytogenes* by the microtiter plate method** according to Kavanagh (1963); Kavanagh (1972) and Lourenço and Pinto (2011):

Four different concentrations of roasted (light) coffee bean extract (1, 3, 10, and 30 µg). An aliquot of 50 µl of the stock solution of the extract (1, 3, 10, 30 and 100 µg concentrations) was added separately to 950 µl of inoculum (standardized inoculum using spectrophotometer of 0.1 at 600 nm) prepared in tryptone soya broth (TSB). Negative control of TSB bacterial free and positive control of cultured bacterial broth (standardized inoculum of 0.1 at 600 nm). By using a 96-well microplate, each concentration, positive control and negative control was equally distributed in five wells separately.

The same concentrations of gentamicin (1, 3, 10, 30 and 100 µg) is also tested and in the same time, they used as a control reference in this study to evaluate the H. A. coffee extract against *L. monocytogenes* growth. With optical density at 620 nm (OD 620) readings recorded at 0 and 24 h using ELISA reader by microtiter plate.

**d) Gas chromatography–mass spectrometry (GC-MS) analysis for H. A. coffee extract is done** according to Abd El-Kareem et al. (2016):

The chemical compositions of your samples were performed using Trace GC-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven

temperature was initially held at 50°C and then increased by 5°C /min to 250 °C hold for 2 min. increased to the final temperature 300°C by 30°C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 270, 260°C respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 14 mass spectral database.

#### **Statistical analysis:**

The results expressed as the mean ±SE. Data analysis was preformed GraphPad Prism. Multiple intergroup comparisons and If differences were found, mean comparisons were made by one-way analysis of variance followed by Tukey's multiple comparison test.

#### **Results**

Results presented in Table 3 revealed that 10 (20%) of all the examined milk samples were contaminated with *Listeria spp.* four strains (2.7%) were identified as *L. monocytogenes* that was the most prevalent species isolated followed by *L. welshimeri* 3 (2%), *L. grayii* 2 (1.3%) and *L. innocua* 1 (0.67%). *Listeria spp.* couldn't be isolated from any of pasteurized milk and flavored milk conventionally.

In the examined milk products samples, Table 4 illustrated that the highest incidence of *Listeria spp.* was detected in kareish cheese samples (16%). Followed by 14 % and 4% were isolated from small scale ice cream and domiati cheese samples, respectively. Whereas, *Listeria spp.* was failed to be detected in large scale ice

cream, large scale yoghurt and small-scale yoghurt. *L. monocytogenes* was isolated from 4 %, 6 % and 2% of small-scale ice cream, kareish cheese and domiati cheese, respectively; *L. innocua* was detected in 4% from small scale ice cream samples; *L. welshimeri* were isolated of 2% from small scale ice cream samples. *L. grayii* were recovered from 4, 10 and 2% of small-scale

ice cream, kareish cheese and domiati cheese, respectively.

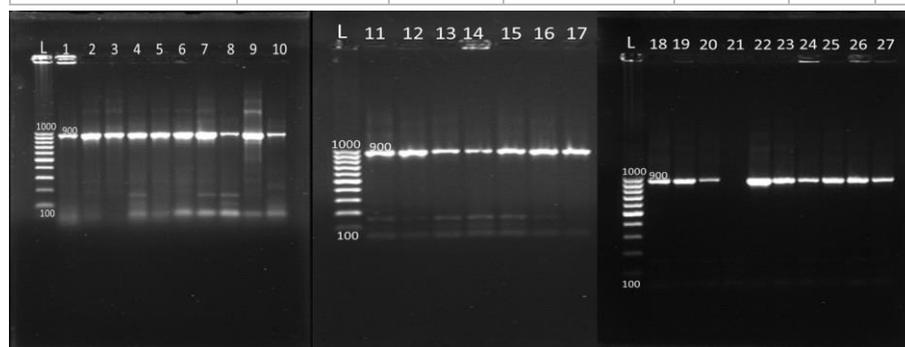
All the twenty-seven isolates of *Listeria spp.* which identified biochemically were genetically tested by PCR technique and designated that 26 out of 27 isolates were PCR positive Fig. 1.

**Table 3. Incidence of *Listeria spp.* isolated from milk samples.**

Type of samples	Positive samples		<i>Listeria spp.</i>							
			<i>L. monocytogenes</i>		<i>L. innocua</i>		<i>L. welshimeri</i>		<i>L. grayii</i>	
	No./50	%	No./50	%	No./50	%	No./50	%	No./50	%
Raw milk	10	20	4	8	1	2	3	6	2	4
pasteurized milk	–	–	–	–	–	–	–	–	–	–
Flavored milk	–	–	–	–	–	–	–	–	–	–
<b>Total</b>	<b>10</b>	<b>6.7</b>	<b>4</b>	<b>2.7</b>	<b>1</b>	<b>0.67</b>	<b>3</b>	<b>2</b>	<b>2</b>	<b>1.3</b>

**Table 4. Incidence of *Listeria spp.* isolated from milk products samples.**

Type of samples	Positive samples		<i>Listeria spp.</i>							
			<i>L. monocytogenes</i>		<i>L. innocua</i>		<i>L. welshimeri</i>		<i>L. grayii</i>	
	No./50	%	No./50	%	No./50	%	No./50	%	No./50	%
Large scale ice cream	–	–	–	–	–	–	–	–	–	–
Small scale ice cream	7	14	2	4	2	4	1	2	2	4
Kariesh cheese	8	16	3	6	–	–	–	–	5	10
Domiati cheese	2	4	1	2	–	–	–	–	1	2
Large scale yoghurt	–	–	–	–	–	–	–	–	–	–
Small scale yoghurt	–	–	–	–	–	–	–	–	–	–
<b>Total</b>	<b>17</b>	<b>5.6</b>	<b>6</b>	<b>2</b>	<b>2</b>	<b>0.67</b>	<b>1</b>	<b>0.33</b>	<b>8</b>	<b>2.6</b>



**Fig. 1.** Agarose gel electrophoresis of 16S rRNA gene (Genus-specific) for *Listeria spp.* by PCR assay.

**Fig. 1. legend** continued

Lane L: 1000 bp DNA ladder (cat. no. 239045).

Lanes 1-27: are representative run for PCR results of 27 extracted DNA expressed all examined samples showing positive except lane 21 was negative amplicon 16S rRNA.

Lanes (1-10): *L. monocytogenes* isolates; (1-4) raw milk, (5&6) small scale ice cream, (7-9) kariesh cheese and (10) domiati cheese;

lanes (11-13): *L. innocua* isolates; (11) raw milk and (12&13) small scale ice cream;

lanes (14-17): *L. welshimeri* isolates; (14-16) raw milk and (17) small scale ice cream;

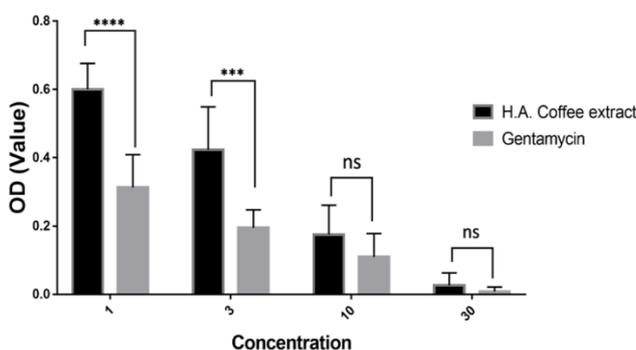
lanes (18-27): *L. grayii* isolates; (18&19) raw milk, (20&21) small scale ice cream.

Regarding molecular confirmation of *L. monocytogenes* by PCR method it was found that 8 out of 10 strains were positive to 16S rRNA species-specific gene amplification of *L. monocytogenes* that was isolated from the examined samples by conventional PCR Fig. 2.

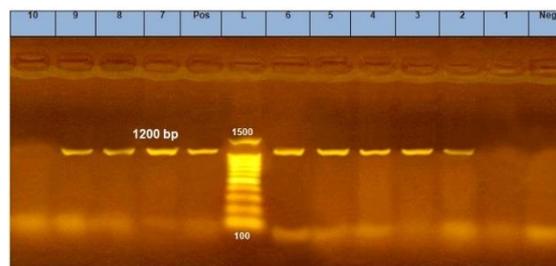
Concerning the antibacterial effect of both gentamicin and H. A. coffee extract on the isolated *L. monocytogenes* Table 5 reported that the effect of gentamicin and H. A. coffee extract against *L. monocytogenes* strain at concentrations (0, 1, 3, 10 and 30 µg/mL) was estimated by measuring of O.D. values at 620 nm after

**Table 5. Comparison of mean optical density (O.D.) value for *L. monocytogenes* between H. A. coffee extract & gentamicin at 1, 3, 10 and 30 µg/ml concentrations using One-way ANOVA Test followed by Tukey’s multiple comparison test.**

Concentration	Mean ±SE		P value
	H. A. Coffee Extract	Gentamicin	
1 µg/mL	0.60±0.08	0.31±0.10	****
3 µg/mL	0.42±0.13	0.19±0.05	***
10 µg/mL	0.18±0.09	0.11±0.07	ns
30 µg/mL	0.03±0.04	0.01±0.01	ns



incubation with the mean values 0.44±0.05, 0.31±0.10, 0.19±0.05, 0.11±0.07 and 0.01±0.01, respectively for gentamicin and 0.69±0.03, 0.60±0.08, 0.42±0.13, 0.18±0.09 and 0.03±0.04, respectively for H. A. coffee extract with non-significant difference at 10 and 30 µg/mL concentrations between H. A. coffee extract and gentamicin as showed in Fig. 3.



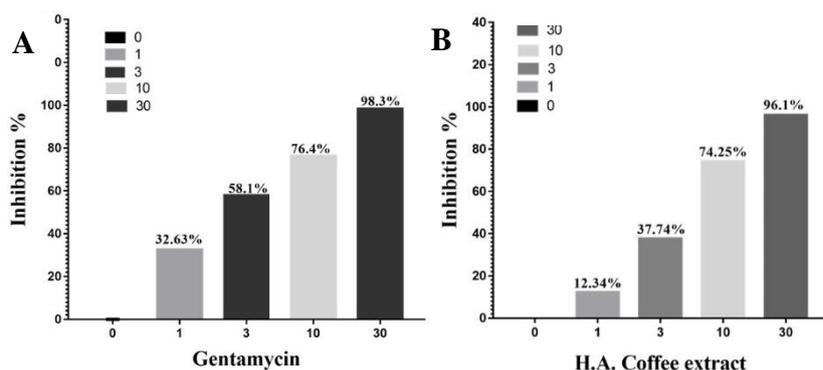
**Fig. 2. Agarose gel electrophoresis of 16S rRNA species-specific gene amplification of *L. monocytogenes* isolates from the examined samples.** Lane 1-10, eight out of ten strains were positive result of 16S rRNA gene detection in *L. monocytogenes* isolates Lane (2-9), while Lane 1 and 10 were negative. (1-4) raw milk, (5&6) small scale ice cream, (7-9) kareish cheese and (10) domiati cheese. Lane Pos. positive control strain. Lane Neg. Negative control.

**Fig. 3. Effect of concentrations of H. A. Coffee extract compared with the effect of Gentamicin on bacterial growth of *L. monocytogenes*;** Values represent means ± SD of each concentration; \*\*\*p < 0.001, \*\*\*\*p < 0.0001 and (ns) means non-significant difference, significant differences are related to the control.

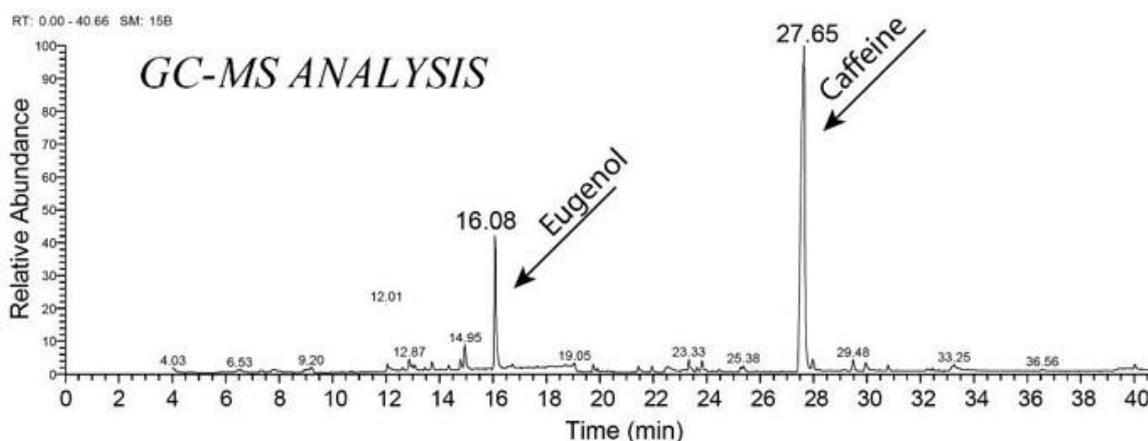
The inhibitory effect of different concentrations of gentamicin and H. A. coffee extract on growth of *L. monocytogenes* were demonstrated in Fig. 4A. where at (0, 1, 3, 10 and 30  $\mu\text{g/ml}$ ) gave 0%, 32.63%, 58.1%, 76.4% and 98.3% of bacterial growth inhibition, respectively and Fig. 4B. where at (0, 1, 3, 10 and 30  $\mu\text{g/ml}$ ) gave 0%, 12.34%, 37.74%, 74.25%

and 96.1 % of bacterial growth inhibition, respectively.

As illustrated in Fig. 5. The GC-MS spectrum confirmed the presence of various components with different retention times, but this investigation by GC-MS revealed that there are two mainly bioactive components in H. A. coffee extract with high percentage of peak area scientifically known as Eugenol and Caffeine.



**Fig. 4.** Effect of gentamicin (A) & H. A. coffee extract (B) at the concentrations of (0, 1, 3, 10 and 30  $\mu\text{g/ml}$ ) on growth of *L. monocytogenes*.



**Fig. 5.** Phytoconstituents detected in the H. A. coffee extract using GC-MS analysis and chemical structure of two phytochemicals identified based on the retention time and peak area. namely (1) Eugenol (16.08 RT and 13.16 % peak area) and (2) Caffeine (27.65 RT and 59.31% peak area).

## Discussion

*Genus Listeria* is ubiquitously in habitat isolated from the surroundings (Liu, 2008) and therefore, can quickly enter the food chain (Leite et al., 2006) hence, it may contaminate milk directly through clinical and subclinical mastitis, feces of

animals and listeria related miscarriage or indirectly via milking equipments in dairy farm, lack of hygiene and ingestion of low quality silage (Husu, 2010).

In the current study, raw milk exhibited the highest prevalence rate of 20% for

*Listeria spp.* as recorded in Table 3. Nearly similar result (19%) was recorded by EL-Naenaeey et al., (2019), while, it was higher as compared to those observed by Saleh et al. (2021) (13.33%). Higher incidence rates (36%) were investigated by Youssef et al. (2020). Variation in the incidence of *Listeria spp.* in this study and other studies in Egypt or elsewhere may be attributed to differences in milk sampling, detection methodologies, animals' husbandry and hygienic milking conditions.

The detection and isolation of *Listeria spp.* from food products is challenging due to the concurrence presence of other organisms within the food product (Nayak et al., 2015).

On the species level, it was found that *L. monocytogenes* was the most frequent 4(8%) followed by *L. welshimeri* 3(6%), *L. grayi* 2(4%) and *L. innocua* 1(2%) of the examined samples Table 3 and Fig. 1. Although other *Listeria spp.* are not pathogenic, their presence indicates an unsatisfactory process at risk of contamination with *L. monocytogenes* (Greenwood et al., 1991).

According to results of PCR presented in Fig. 2., it is apparent that 6% of raw milk was contaminated with *L. monocytogenes*. This result is nearly similar to Kalorey et al., (2008) (5.1%).

The another hand, lower incidences of *L. monocytogenes* were reported by EL-Naenaeey et al., (2019) (4%) and Hanna, (2021) (2%), while Aygun and Pehlivanlar, (2006) who failed to isolate *L. monocytogenes* from examined raw milk samples. This result has not concurred with Youssef et al., (2020) (14%) where higher incidence of *L. monocytogenes* was recorded.

Interestingly, the Failure to detect *L. monocytogenes* from pasteurized milk and

flavored milk samples in our findings Table 3 may be attributed to application of efficient thermal treatment and avoiding post heat treatment contamination. This was in accordance with studies performed by Sarker and Ahmed (2015). As opposed to our study, Saleh et al. (2021) detected *Listeria spp.* in pasteurized milk 12%.

Concerning, the examined ice cream samples, out of 100 large and small scale ice cream only 7 samples (14%) were suspected to have *Listeria spp.* in small scale ice cream samples and failed to detect *Listeria spp.* in large scale ice cream as illustrated in Table 4, this result was agreed with Saleh et al., (2021).

Higher results of *Listeria spp.* (20%) were reported by EL-Shinaway et al., (2017a), whereas lower results (8%) were reported by Youssef et al. (2020). In contrast, other studies performed by Kevenk and Gulel, (2016) failed to detect *Listeria spp.* in the examined ice cream samples.

The cultural and biochemical confirmation result of *Listeria spp.* in small scale ice cream showed that 2 (4%) were *L. monocytogenes*, *L. innocua* and *L. grayi* for each and 1 (2%) was for *L. welshimeri* Table 4. Whereas, the PCR confirmation result gave nearly similar result to biochemically confirmed strain except only one *L. grayi* isolate was negative as shown in Fig. 1. There are multifactorial causes sustain the microbial survival and give its chance for growth in ice cream, including the presence of nourished constituents, aging step, low-quality ingredients, its neutral pH, the use of polluted water supplies and prolonged defreezed time (EL-Shinaway et al., 2017a).

According to the result presented in Fig. 2., *L. monocytogenes* were confirmed in 4% of examined ice cream samples by

PCR. These findings agreed with El-Sharef et al., (2015) (4%). Higher incidence was reported by Windrantz and Arias, (2000) (12.30%).

Referring to cheese results, out of 50 kareish cheese samples, 8 (16%) samples yielded growth of *Listeria spp.* Table 4 and molecularly confirmed as shown in Fig. 1. Nearly similar results (13.3%) were reported by El Sayed et al., (2011). Higher rate of contamination (33.2%) was obtained by Arslan and Ozdemir, (2008), while EL-Shinaway et al., (2017b) reported low rate of contamination (12.5%). Contrary to the postulated results, *Listeria spp.* couldn't be isolated from examined home-made cheese samples in studies performed by Jalali and Abedi, (2008).

On the species level, *L. grayii* was the most prevalent species isolated from kareish cheese (10%) followed by *L. monocytogenes* (6%) Table 4, this result was agreed with (El-Shinaway et al., 2017a) who reported that *L. grayii* was the main *Listeria spp.* isolated from kareish cheese.

The present results revealed that 3 (6%) of 50 kareish cheese samples were PCR confirmed *L. monocytogenes* exceeding other tested dairy products and similar to raw milk samples Fig. 2. may be due to the use of unpasteurized milk, unhygienic handling, non-salted whey is used, and highwater content (EL-Shinaway et al., 2017b). This result finding go hand in hand with the findings of Abd El Tawab et al., (2015). Meanwhile, they disagreed with AL-Ashmawy et al. (2014) who mentioned the absence of *L. monocytogenes*. On the other hands, in domiati cheese, a lower non-complying level (4%) was showed in compared to results of other products in this study Table 4. *L. monocytogenes* could not be detected by PCR in domiati cheese may be attributed to its chemical properties described as the high salty level, low pH

and highwater content enhancing the species compatible effectively against *L. monocytogenes* strains (Zamani-Zadeh et al., 2011). The similar postulated results were recorded by Ramos and Costa, (2003). Unlike to our results, Hanna, (2021) detected *L. monocytogenes* in 6%.

This study declared that yoghurt samples were free from *Listeria spp.* Table 4. Our result matches with EL-Shinaway et al., (2017b). On the contrary, Gohar et al., (2017) and Youssef et al., (2020) found *Listeria spp.* in yoghurt as 5% and 6%, respectively. Notably, the fortunate of the absence of *Listeria spp.* in yoghurt is due to a low pH, high titratable acidity, the antagonistic activity of either bacteriocins produced by the lactic acid bacteria or starter action against harmful bacteria and heat treatment application.

With respect to the European economic Communities standards (1992) as well as Egyptian Standards (2000 & 2005) which notified that milk and dairy products should be free from *L. monocytogenes*. According these findings, both national and international standards are not fulfilled. So, there is a real threat for public health.

Currently, in this study, we suggested use of plant extracts in food industry is required as food preservatives, since *L. monocytogenes* show high multiple antibiotics resistance (Mpondo et al., 2021).

Recently, use of natural antimicrobial in food industry is preferred especially when compared with chemical additives caused health risks (Martínez-Tomé et al., 2011). Several studies were performed on coffee and proved its antimicrobial activity on a vast range of bacteria, Gram-positive and Gram-negative (Mueller et al., 2011).

Thus, in the current work, A unique approach for measuring the antibacterial activity of hydroalcoholic coffee extract

using a microplate in ELISA reader is provided. Using ELISA reader, turbidity at OD620 was measured to quantify bacterial growth. Given the above findings in Table 5, it was stated that the decrease in O.D. values of treated bacterial broth with extract than bacterial broth extracts free with increase the concentration of H. A. coffee extract indicates the influence of coffee extract is increased with the elevation of concentration. Thus, referring to the mean value of O.D. is  $0.03 \pm 0.04$  at  $30 \mu\text{g/ml}$  concentration can be expressed as a bactericidal concentration towards *L. monocytogenes* where the O.D. value is the same at both 0 and 24 h.

As expressed by the following equation: Inhibition % =  $[(\text{O.D. c} - \text{O.D. t}) / \text{O.D. c}] * 100$  can be calculated, where O.D. c is the absorbance for the wells with the blank (control) and O.D. t is that for the tested complex with extract or antibiotic samples. As illustrated in Fig. 4 among different extract concentrations, the most effective concentration was  $30 \mu\text{g/mL}$  which was not exhibit *listeria* growth up to 96.1 % followed by 10 with inhibition percentage of 74.25%. While, the listeria growth inhibition % after exposure to gentamicin at 10 and  $30 \mu\text{g/mL}$  were 76,4% and 98,3%, respectively.

As demonstrated in Fig. 5. GC-MS revealed that there are two mainly bioactive components in H. A. coffee extract with high percentage of peak area scientifically known as Eugenol and Caffeine. Some supported studies to these findings, demonstrated the remarkable antibacterial role of Caffeine as a significant bioactive component in a coffee (Mueller et al., 2011 and Chakraborty et al., 2020). As well, eugenol, some studies confirmed its anti-listerial activity (Gill and Holley, 2004).

In the current research, hydroalcoholic coffee extract showed good results for *L.*

*monocytogenes* growth inhibition in vitro. So, this study confirmed the efficacy of H. A. coffee extract as natural antimicrobial. In future, calling for the possibility of utilizing it in dairy products as a preservative.

## Conclusion

In conclusion, this study indicates that raw milk and some traditional dairy derivatives marketed in Qena may be considered a threat to consumers since there is a risk of infection with *L. monocytogenes*. So, they should be categorized as RTE food in which the growth of *L. monocytogenes* can take place. Whereas, in pasteurized dairy products, *L. monocytogenes* could not be detected. Hence, we suggest that continuous surveillance is needed. Gentamicin showed an inhibitory activity against *L. monocytogenes*. Besides, this study highlights on the use of plant extract which may be considered a promising aspect as food preservation method recently. H. A. Coffee extract might contribute to food industry as a natural anti-listerial preservative, as confirmed by the proposed method in this study.

## Conflict of interest statement

The authors declare that they have no conflict of interest.

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