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Antimicrobial effect of cinnamon oil, L-lysine, and beta-carotene on multi-drug resistant *Listeria monocytogenes* isolated from milk and dairy products

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ABSTRACT

Despite many achievements in developed countries mainly in health and food sectors, *L. monocytogenes* remains a great challenge in food industries. This study was developed to determine the prevalence and antimicrobial resistance patterns of *L. monocytogenes* in Egyptian dairies. Furthermore, some phytochemicals such as cinnamon oil, L-lysine, and beta-carotene were used as anti*Listeria*l additives in soft cheese as a food substrate. A total of 150 dairy samples (raw milk, farm bulk tank milk, yogurt, Kareish cheese, white soft cheese (Damietta), and ice cream, 25 each) were screened to determine the prevalence of *Listeria* spp. The results revealed that 5, 10, 8, 6, 3, and 2 samples of bulk tank milk, market raw milk, Kariesh cheese, soft cheese, yogurt, and ice cream were contaminated with *Listeria* spp., respectively. The antimicrobial resistance profiling showed that 100% of *L. monocytogenes* (12 isolates) revealed resistance to penicillin G, amoxicillin/clavulanate, piperacillin/tazobactam, cefoxitin, and cefepime. Furthermore, molecular characterization revealed that all *L. monocytogenes* harbored the hylA virulent gene. Application of some phytochemicals such as cinnamon oil and L-lysine significantly ($p<0.05$) reduced *L. monocytogenes* growth in soft cheese artificially contaminated with *L. monocytogenes*, while beta-carotene did not reveal any significant ($p<0.05$) growth inhibition during 28 days of storage. In conclusion, Egyptian dairy products are considered a vehicle for the transmission of *L. monocytogenes*, thus strict hygienic measures should be adopted from farm to fork. In addition, cinnamon oil and L-lysine are considered good candidates for dairy sectors as food preservatives.

Introduction

Food-borne illnesses are mostly associated with microbial contamination of foods with pathogenic bacteria due to unhygienic measures adopted at any stage of food processing from farm to fork. In developing countries such as Egypt where poverty, poor hygienic conditions, and lack of awareness among farmers can be predisposing factors for the spreading of many diseases that affect negatively health and economy (Ulusoy and Chirkena, 2019).

Among foodborne diseases, listeriosis has gained a lot of attention from many researchers. Listeriosis is one of severe foodborne diseases caused by *Listeria monocytogenes* (*L. monocytogenes*). *L. monocytogenes* is a Gram-positive psychrotrophic bacterium widely distributed in the environment and contributes a potent economic and health threat (Akrami-Mohajeri *et al*., 2018). It has the ability to tolerate a vast range of different food-related stressors such as chilling, thermal, pH, and osmosis (Elafify *et al*., 2022b). Listeriosis outbreaks have been recorded previously due to the consumption of contaminated dairy products with a high risk in immune-compromised patients, newborn babies, and pregnant women (El Hag *et al*., 2021; Waak *et al*., 2002). *L. monocytogenes* has been recovered from both raw and ready-to-eat foods (Jamali *et al*., 2013).

Rapidly increasing the occurrence of listeriosis outbreaks might be due to the increased consumption of unpasteurized dairy products (Sarfraz *et al*., 2017). In Egypt, raw milk and unpasteurized dairy products are the main balanced diet for Egyptian families and are considered the main income for many farmers in addition posing a great risk for consumers. For instance, Kareish cheese (acid curd skim-milk cheese) and Damietta cheese are the main types of soft cheeses sold in Egypt, manufactured from unpasteurized milk, and sold in supermarkets without any hygienic conditions. Several pathogens have been recovered from Egyptian dairies (Al-Ashmawy *et al*., 2016; Elafify *et al*., 2022b). Thus continuous screening of harmful zoonotic pathogens as *L. monocytogenes* in dairy products with providing a promising solution for controlling its growth in the foods is still a mandatory issue for food scientists and food sectors.

On the other hand, the application of antibiotics in animal feed as a growth promoter has been associated with economic benefits. However, random usage of antibiotics either as a growth promoter or for animal treatment without proper veterinary supervision led to the emergence of antimicrobial resistance strains (Akrami-Mohajeri *et al*., 2018; Elafify *et al*., 2022b). Recently, antibiotic resistance among *L. monocytogenes* strains isolated from foods has increased and this problem is considered one of the major threats for food and health sectors. In addition, the development of more antimicrobial resistance reflects negatively on animal and human treatment (Olaimat *et al*., 2018). Antibiotic-resistant bacteria are incriminated in 700,000 deaths annually worldwide and the progress depends on how much applied efforts from different sectors across this issue (O'neill, 2014).

Globally, several restrictions have been applied regarding direct addition of chemical preservatives or high thermal treatment for controlling the pathogens growth and for extending the shelf-life of the foods. These traditional methods have some drawbacks because some of chemical additives have a potent health hazard (e,g nitrite and hydrogen-peroxide have carcinogenic effect). Furthermore, high thermal treatments are linked with a destructive effect on the nutritive value of the final products and have a negative effect on the sensory characterizations of the foods (Garcia-Fuentes *et al*., 2015). Thus, there is currently a large interest in the dairy industry to use eco-friendly and natural antimicrobial additives to control the growth of the pathogen in milk and dairy products and to respond to the consumer's demand for natural antimicrobial additives. Recent increases in the demand for phytochemicals have investigated in vivo and in vitro and have revealed antimicrobial activity against foodborne pathogens (Corte *et al*., 2004; Sondi and Salopek-Sondi, 2004; Elafify *et al*., 2020).

According to the previously mentioned facts, this study was undertaken to determine the prevalence, molecular characterization, and antimicrobial profiles of *Listeria* spp., in milk and dairy products retailed in Egypt. Furthermore, this study was extended to evaluate the inhibitory activity of some phytochemicals as cinnamon oil, L-lysine, and beta-caro-

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tene on *L. monocytogenes* contaminated cheese as a food matrix.

Materials and methods

Collection and preparation of samples

One hundred and fifty dairy samples (market raw milk, farm bulk tank milk, yogurt, Kareish cheese, white soft cheese (Damietta), and ice cream, 25 each) were gathered randomly from various shops, markets, grocery stores, and farms in Mansoura city, Dakahlia Province, Egypt, under complete aseptic condition from March 2022 to December 2022. The collected samples were preserved in an insulated ice box till reached the Laboratory of Food Hygiene and Control, Faculty of Veterinary Medicine, Mansoura University, Egypt for investigations.

For preparation of dairy samples, a total of 25 mL or grams of milk and dairy products were weighed and mixed with 225 mL of 0.1% bacteriological peptone (Oxoid, UK) and homogenized well using a laboratory blender then the obtained homogenate was incubated overnight at 37°C for bacteriological examination. Regarding ice cream, the samples were placed in a water bath adjusted previously at 40°C till thawing then the thawed samples were treated as the same procedure.

Isolation and identification of Listeria spp.

Isolation of *Listeria* spp. was done according to Roberts and Greenwood (2008). In brief, 25 mL of overnight incubated homogenate from each dairy product was added separately to 225 mL of *Listeria* enrichment broth base (CM862, Oxoid) with *Listeria* selective enrichment supplement (Nalidixic acid, cycloheximide, and acriflavine hydrochloride) (SR141, Oxoid) then incubated for 48 h at 37°C. After that, a loopful was taken aseptically by a sterile platinum loop and streaked onto Oxford agar plates as a selective medium (CM856, Oxoid) supplemented with *Listeria* selective supplement (SR140, Oxoid), and all plates were incubated for 48 h at 37°C for checking the growing colonies. In general, at least five presumptive olive green colonies surrounded by black zones were considered as *Listeria*. The colonies were picked up again for further purification onto the same selective medium then the purified colonies were preserved in 15% glycerol at −20°C for further examination.

For an initial confirmation of the obtained isolates, morphological and biochemical tests were performed. In brief, the stored colonies were refreshed again into tryptone soy agar (CM0131, Oxoid) supplemented with 0.6% yeast extract with overnight incubation at 37°C. The refreshed pure colonies were microscopically examined and appeared as coccobacilli and non-sporulating bacteria. The colonies were also exposed to some biochemical tests such as oxidase, catalase test, and sugar fermentation tests (D-glucose, L-rhamnose, xylose, and mannitol) in addition to umbrella motility test (Elafify *et al*., 2022b). Furthermore, the CAMP test was performed against S. aureus (Elafify *et al*., 2022b). Finally, the confirmed biochemical isolates were exposed to serological tests using *Listeria* latex agglutination kit (Oxoid, Basingstoke, Hampshire, England). All procedures were carried out according to the manufacturer's instructions (Pagotto *et al*., 2001).

Phenotypic antimicrobial susceptibility testing (Antibiogram)

The confirmed biochemical and serological strains of *L. monocytogenes* were selected for screening their resistance to antimicrobial agents due to the higher pathogenicity of these strains to cause severe hazards in human and animals than other obtained strains (Sarfraz *et al*., 2017). The confirmed strains were exposed to antimicrobial susceptibility testing using agar disk diffusion methods on Mueller-Hinton agar (Elafify *et al*., 2022b). The used antimicrobial agents were; ciprofloxacin (CIP) (10µg); vancomycin (VA) (30µg); gentamycin (GEN) (10µg); amoxicillin/clavulanate (AMC) (20/10µg); imipenem (IPM) (10µg); penicillin (P) (10µg); piperacillin/tazobactam (TPZ) (100/10µg); co-trimoxazole (COT) (25µg); erythromycin (E) (15µg); levofloxacin (LE) (5µg); cefepime (FEP) (30 µg); cefoxitin (CX) (30µg); doxycycline (DO) (30µg); clindamycin (DA) (2µg); and linezolid (LZ) (30µg) were placed onto the surface of agar and fixed well using a sterile forceps. The sensitivity of the isolates to antimicrobial agents was evaluated according to CLSI (2018). Furthermore, Multiple antibiotic resistance (MAR) index was estimated (Elafify *et al*., 2022a) according to the following equation:

MAR index= Number of resistance isolates / total numbers of tested antimicrobials

Molecular characterization of L. monocytogenes

DNA extraction

DNA from two or three overnight cultured bacteria was extracted by using boiling methods at 100°C for 15–20 min and the obtained centrifuged supernatant (DNA) was stored at -80°C for further use (Elafify *et al*., 2022a).

Detection of *hly*A gene as a virulent gene for *L. monocytogenes*

The amplification was performed using the primer sequences *hly*A (F) 5′ GCATCTGCATTCAATAAAGA'3 *hly*A (R) 5'TGTCACTGCATCTCCGTGGT'3 (Deneer and Boychuk, 1991) at a product size 174 bp. The amplification condition was: an initial denaturation at 94°C for 5min followed by 35 cycles each of 94°C/30 sec, 50°C/30 sec, and 72°C/30 sec followed by a final extension for 7 min at 72°C and the PCR products were held at 4°C (Deneer and Boychuk, 1991). Amplified DNA fragments were run on 1% agarose gel electrophoresis (AppliChem, GmbH, Germany) in 1x TBE buffer stained with ethidium bromide and visualized on a UV transilluminator.

Evaluation of the inhibitory effect of cinnamon oil, L-lysine, and beta-carotene on Listeria monocytogenes growth

Bacterial preparations

Firstly, one confirmed and sequencing strain of *L. monocytogenes* strains isolated from cheese (ID,13) was refreshed by culturing the isolates onto sterile TSB (Oxoid, UK) and incubated at 37°C/24 h. Then a loopful was taken from the incubated broth and streaked onto tryptic soy agar (TSA, Oxoid, UK) supplemented with 0.6% yeast extract followed by an incubation at 37°C/24 h. Then a single pure colony was taken and cultured onto TSB and incubated at 37°C/24 h till reached a stationary phase of approximately 10^9 CFU/mL.

Preparation of anti-*Listeria* additives and media

L-lysine (Lys, 98%) was purchased from Sigma-Aldrich Chemical Co. Germany. While, beta-carotene (30% oil suspension) was purchased from DSM Nutritional Products Co, Switzerland. Cinnamon essential oil (pure 100%) was purchased locally from Herbal Mood brand, Obi Consumer Products Ltd, Egypt. All media were used according to the manufacturer's instructions.

An initial screening of the anti-*Listeria*l activities of natural additives using agar well diffusion method

The antimicrobial effects of cinnamon oil, L-lysine, and beta-carotene were evaluated according to Mayo (1998). In brief, *L. monocytogenes* was refreshed using TSB then the overnight turbid broth was cultured onto TSA for 24h at 37°C. A single pure colony was cultured onto TSB and incubated at 37°C/24h. After that, a loopful was taken from the incubated broth (previously adjusted at 10^5–10^6 CFU/mL) by sterile autoclaved

cotton swab and spread well onto the surface of Müeller-Hinton agar (Oxoid). Then, 50µL of each cinnamon oil (100% purity), L-lysine (1gm/mL), and beta-carotene (30% oil suspension) were placed in the wells of Müeller-Hinton. The plates were kept sometimes at room temperature to give a chance for the extracts to diffuse onto the medium then incubated at 37°C/24 h. The inhibition zones at the lowest concentrations were recorded and used in our experiment. Any of these substances did not reveal any inhibition zone was excluded from the subsequent steps.

Determination of Minimal Inhibitory Concentration (MIC)

The MIC for natural antimicrobial agents was evaluated according to the methods established (CLSI, 2018) using 96-well microtiter plates. Each well was filled with 100µL of consecutive dilution of cinnamon oil and L-lysine at a concentration ranging from 500 mg/ml to .977 mg/mL, each. Beta-carotene was not used because it did not reveal any inhibition zone at Müeller-Hinton agar. Then, 100 µL of each bacterial culture broth at a concentration of 1×10^6 CFU/mL in Muller Hinton Broth (MHB) was placed in each well. Furthermore, the control positive and negative was also considered. Tween 80 at 0.1% (v/v) was used to emulsify the cinnamon essential oil to get a colloidal suspension. After good mixing, the plates were incubated at 20h at 37°C, and MIC was determined as the lowest concentration that exhibited no bacterial growth or turbidity (Shohayeb *et al*., 2014).

Preparation and inoculation of soft cheese with natural additives as a food model

Fresh soft cheese (Damietta) was prepared according to Elafify *et al*. (2022a). In brief, fresh cow's milk was collected from a dairy farm located in Dakahlia province, Mansoura, Egypt. The collected milk was pasteurized at 63°C/30 min in a water bath and then left to warm at 45°C. After that, sodium chloride (2%), calcium chloride (0.02 g/L), and *L. monocytogenes* (10^7 CFU/mL) were added to the warm milk. Additionally, the rennet (0.2 g/L), cinnamon oil (1.95 mg/mL), and L-lysine (62.5 mg/mL) were added separately to the inoculated milk. The inoculated milk containing natural additives was incubated at 37°C till complete curd formation. Once curd and coagulation were performed, the curd was separated from whey via autoclaved clean drainage cloth. The obtained cheese was preserved in a clean sterile container at 4°C for bacteriological analysis. In parallel, the control cheese was manufactured with the same manner without the addition of any antimicrobial additives. The samples were taken from both treated and controlled groups each 4 days till 28 days and the viability of *Listeria monocytogenes* was counted via pour plating method. Each experiment in each condition was carried out in triplicate (three independent trials).

Sensory evaluation of treated cheese

The manufactured cheese samples were evaluated for flavor (aroma & taste), color, texture, and overall acceptability via panelists by using nine-point hedonic scale where score of 7 or higher indicates an acceptable sensory quality (Ihekoronye and Ngoddy, 1985). The evaluation of cheese was carried out by 10 trained panelists from the members of the Department of Food Hygiene and Control, Mansoura University. Panelists were requested to rinse their mouths by drinking warm water between each sample.

Statistical Analysis

All data obtained from control and treated were transformed into log CFU/g. One-way ANOVA followed by Dunnett's post hoc test was used to compare results at various treatments. Statistical analyses were carried out using R statistical software (v. 3.5.2, R Foundation for Statistical Computing, Vienna). P value of 0.05 is considered significant.

Results

Prevalence of Listeria spp. in milk and dairy products

The results presented in this study showed that 20%, 40%, 32%, 24%, 12%, and 8% of bulk tank milk, market raw milk, Kareish cheese, white soft cheese, yogurt, and ice cream were contaminated with *Listeria* spp., respectively. *L. monocytogenes* was found at 20%, 12%, 8%, and 8% in market raw milk, Kareish cheese, white soft cheese, and yogurt, respectively, whereas no *L. monocytogenes* was recovered from bulk tank milk and ice cream. All positive isolates of *Listeria* spp. were also biochemically differentiated to determine their species (Table 1).

Antimicrobial resistance and virulence characterization of L. monocytogenes

All *L. monocytogenes* (12 isolates) were screened for the presence of virulent *hly*A gene. The results illustrated that all isolates harbored the *hly*A gene. The antimicrobial resistance profiling revealed that all isolates revealed resistance to penicillin G, piperacillin/tazobactam, amoxicillin/clavulanate, cefoxitin, and cefepime. On the contrary, all the isolates were susceptible to linezolid and clindamycin (Table 2). MAR index for all isolates was evaluated (Table 3).

Antimicrobial activity of natural additives against L. monocytogenes using disc diffusion method

The obtained results revealed that cinnamon oil (purity 100%) inhibited the growth of *L. monocytogenes* with the widest inhibition zone diameter (31mm) followed by L-lysine (1g/ml) with an inhibition diameter of (28mm) while beta-carotene (30% oil suspension) did not reveal any inhibition zone. Cinnamon essential oil showed the lowest MIC value of 1.95mg/ml while L-lysine was 125mg/ml.

Table 1. Prevalence of *Listeria* species in raw milk and dairy products (n=150, 25 each).

Sample	*L. monocytogenes*		*L. innocua*		*L. ivanovii*		*L. seeligeri*		*L. welshimeri*		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Bulk tank milk	0	0	4	16	0	0	0	0	1	4	5	20
Market raw milk	5	20	3	12	0	0	2	8	0	0	10	40
Kareish cheese	3	12	3	12	0	0	2	8	0	0	8	32
White soft cheese	2	8	4	16	0	0	0	0	0	0	6	24
Yogurt	2	8	0	0	1	4	0	0	0	0	3	12
Ice cream	0	0	2	8	0	0	0	0	0	0	2	8
Total	12	8	16	10.7	1	0.7	4	2.7	1	0.7	34	22.7

Antimicrobial activity of cinnamon oil and L-lysine on L. monocytogenes inoculated into white soft cheese

The inhibitory effects of cinnamon oil and L-lysine against *L. monocytogenes* growth in soft cheese were evaluated. The results displayed that cinnamon oil (1.95 mg/ml) had a marked bactericidal effect against *L. monocytogenes* at 4°C, which reduced the count of *L. monocytogenes* by 3.9 log CFU/g after four days with a reduction percentage of 99.99% and after eight days of treatment with cinnamon oil, *L. monocytogenes* count was not detected with a reduction percent 100% and remained constant until the end of the experiment (28 days). In comparison, L-lysine revealed a significant ($p<0.05$) bacteriostatic effect against *L. monocytogenes* with a reduction of 2.1 log CFU/g after 28 days of treatment (99% reduction) (Figure 1). The sensory evaluation was done in our experimental trial via the addition of cinnamon oil and L-lysine onto soft cheese at different concentrations. The results showed that these concentrations 1.95mg/ml and 62.5 mg/ml of cinnamon oil and L-lysine, respectively, were more acceptable in sensory attributes (taste, color, odor, body and texture, and overall acceptability) than other concentrations (Figure 2).

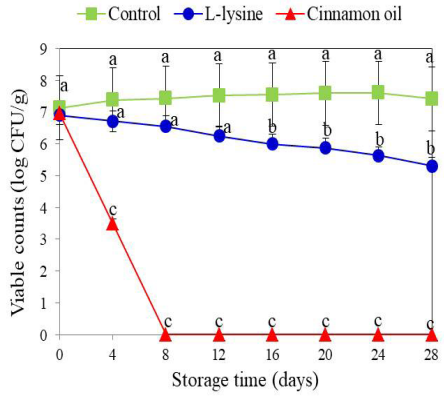

Figure 1. Inhibitory effect of cinnamon oil and L-lysine against *Listeria monocytogenes* artificially inoculated into soft cheese at 4oC and stored for 28 days. Different letters indicate a significant difference at $p<0.05$.

Table 2. Antimicrobial resistance patterns of *L. monocytogenes* isolated from milk and dairy products (n=12 isolates).

Antimicrobial agents	Antimicrobial group	Sensitive (S)		Intermediate (I)		Resistant (R)	
		No.	%	No.	%	No.	%
Penicillin G (P)	Penicillins	-	-	-	-	12	100
Amoxicillin/clavulanate (AMC)	Penicillins	-	-	-	-	12	100
Piperacillin/tazobactam (TPZ)	Penicillins	-	-	-	-	12	100
Cefoxitin (CX)	Cephalosporins (1st generation)	-	-	-	-	12	100
Cefepime (FEP)	Cephalosporins (4th generation)	-	-	-	-	12	100
Vancomycin (VA)	Glycopeptide antibiotics	1	8.33	4	33.33	7	58.33
Erythromycin (E)	Macrolides	8	66.67	-	-	4	33.33
Doxycycline (DO)	Tetracyclines	9	75	1	8.33	2	16.67
Levofloxacin (LE)	Fluoroquinolones	7	58.33	4	33.33	1	8.33
Imipenem (IPM)	carbapenems	5	41.67	6	50	1	8.33
Gentamycin (GEN)	Aminoglycosides	9	75	2	16.67	1	8.33
Ciprofloxacin (CIP)	Fluoroquinolones	11	91.67	-	-	1	8.33
Co-trimoxazole (COT)	Antifolates	11	91.67	-	-	1	8.33
Clindamycin (DA)	Lincosamides	12	100	-	-	-	-
Linezolid (LZ)	Oxazolidinones	12	100	-	-	-	-

Table 3. Virulence and antimicrobial resistance characterization of *L. monocytogenes* isolated from milk and dairy products (n=12).

No.	ID	Sample	Antimicrobial resistance profile	MAR index	Virulent gene (*hlyA)*
1	56	Market raw milk	P, FEP, CX,TPZ,AMC,VA	0.4	+
2	60	Market raw milk	P, FEP, CX,TPZ,AMC,VA, DO	0.47	+
3	75	Market raw milk	P, FEP, CX,TPZ,AMC,VA, LE, E	0.53	+
4	80	Market raw milk	P, FEP, CX,TPZ,AMC, E	0.4	+
5	89	Market raw milk	P, FEP, CX,TPZ,AMC, IMP,GEN	0.47	+
6	13	Kareish cheese	P, FEP, CX,TPZ,AMC,CIP	0.4	+
7	18	Kareish cheese	P, FEP, CX,TPZ,AMC,VA	0.4	+
8	29	Kareish cheese	P, FEP, CX,TPZ,AMC	0.33	+
9	32	White soft cheese	P, FEP, CX,TPZ,AMC,VA	0.4	+
10	38	White soft cheese	P, FEP, CX,TPZ,AMC,VA	0.4	+
11	44	Yogurt	P, FEP, CX,TPZ,AMC,VA, COT, E, DO	0.6	+
12	49	Yogurt	P, FEP, CX,TPZ,AMC, E	0.4	+

P: penicillin G; FEP: cefepime; CX : cefoxitin; TPZ : piperacillin/tazobactam; AMC : amoxicillin/clavulanate; VA : vancomycin; DO: doxycycline; LE: levofloxacin; E: erythromycin; IMP: imipenem; CIP: ciprofloxacin; COT: co-trimoxazole; GEN: gentamycin; DA: clindamycin; LZ: linezolid.

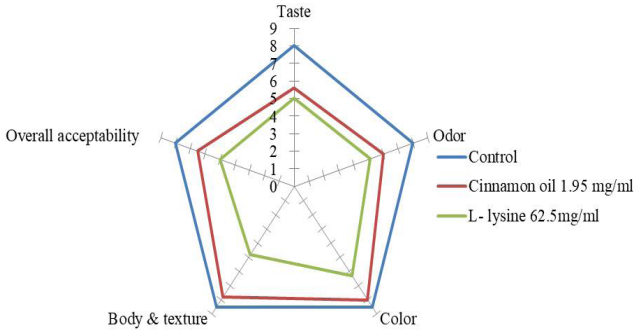

Figure 2. Sensory evaluation of cinnamon oil and L-lysine as natural food additives. Spider chart representing mean score of the evaluated sensory attributed of experimental paneer sample treated with cinnamon oil and L-lysine.

Discussion

Listeriosis is one of the most emerging and zoonotic food-borne illnesses caused by *L. monocytogenes* which results in serious clinical consequences such as meningitis, encephalitis, and abortion (EL-Naenaeey *et al*., 2019). Therefore, controlling such fatal diseases is considered one of the most important challenges in the food and medical sectors (Elafify *et al*., 2022b).

In the current study, the results showed that 20%, 40%, 32%, 24%, 12%, and 8% of bulk tank milk, market raw milk, Kareish cheese, white soft cheese, yogurt, and ice cream were positive for *Listeria* spp., respectively. The percentage of *L. monocytogenes* as an important serotype was 20% in market raw milk, 12% of Kareish cheese, 8% of soft cheese, and 8% of yogurt, whereas no *L. monocytogenes* was detected in examined bulk tank milk and ice cream samples. These findings are in agreement with a previous study reported by Gohar *et al.* (2017), where the prevalence of *Listeria* spp. in sold samples in Faisalabad was 21.3% in raw milk, 12% in Kariesh cheese, and 8% in yogurt while, The prevalence of *L. monocytogenes* was found to be 16%, 12%, and 8% in raw milk, Kariesh cheese, and yogurt, respectively. In Egypt, our results were similar to those obtained by (Alashmawy, 2016), where 10% of market raw milk, 8% of Kariesh cheese, 8% of Talaga cheese, and 4% of Damietta cheese were contaminated with *L. monocytogenes*. In contrast (El-Etriby, 2016) reported that 42% of farm milk, 32% of raw milk, 48% of Kariesh cheese, 64% of Talaga cheese, and 52% of Damietta cheese were contaminated with *Listeria* spp. Furthermore, in Faisalabad, (Zafar *et al*., 2020) stated that 40%, 20%, and 12% of raw milk, cheese, and yogurt were contaminated with *L. monocytogenes*.

The variation in the prevalence of *Listeria* spp. might be due to sample size, geographic location, and methods of sample collection. In addition, the higher prevalence of pathogen contamination could be attributed to the health of dairy animals, the cleanliness of milking containers, milkers, and the hygiene status of dairy facilities (Seyoum *et al*., 2015; Mary and Shrinithivihahshini, 2017; Zafar *et al*., 2020). In addition to the previous reasons, the processing techniques and packaging also contributed critical elements for cheese contamination (Metwally *et al*., 2014).

Nowadays, antibiotic resistance of various pathogenic bacteria is recognized as a global threat to public health due to the widespread of multi-drug-resistant bacteria. This phenomenon emerged due to the random usage of antibiotics for animal treatment or as a growth promotor without proper veterinary supervision and also farmers did not pay attention to the withdrawal period (Akrami-Mohajeri *et al*., 2018; Elafify *et al*., 2022b).

In the current study, the obtained results displayed that all isolates (100%) of *L. monocytogenes* revealed a resistance to penicillin G, piperacillin/tazobactam, amoxicillin/clavulanate, cefoxitin, and cefepime followed by vancomycin 58.33% and these results are similar to that obtained by Mohamed *et al*. (2022) who recorded that all isolates of *L. monocytogenes* were resistant to penicillin G, amoxicillin/clavulanic acid, and 50% of the isolates were resistant to vancomycin. Also, our results agreed with Al-Ashmawy *et al*. (2014) who recorded that 100% of *L. monocytogenes* isolates revealed a resistance against penicillin G and vancomycin (81.5%). In addition, a study conducted by Atabey *et al*. (2021) showed that all *L. monocytogenes* strains isolated from meat and dairy products showed resistance to cefoxitin. On the other hand, all the isolates were susceptible to linezolid and clindamycin (100%, each) followed by Ciprofloxacin and Co-trimoxazole, 91.67% each. These results are matching with Tahoun *et al*. (2017), who reported that 100% of *L. monocytogenes* isolates were susceptible to linezolid. Also, Sala *et al*. (2016) recorded that 100% and 96% of the isolates showed resistance to linezolid and ciprofloxacin, respectively.

The virulence-associated genes of *Listeria monocytogenes* include *inl*B, *prf*A, *hly*A, *Inl*A, and others, but the pathogenicity of *L. monocytogenes* requires a specific virulent factor called listeriolysin O (LLO) which is encoded by *hly*A gene. The presence of LLO reveals the ability of *L. monocytogenes* to invade and spread within host cells (Warke *et al*., 2019). In the present study, the results illustrated that all isolates harbored the *hly*A gene. These results are in agreement with other previous studies (Warke *et al*., 2019; Bouymajane *et al*., 2021; Mohamed *et al*., 2022).

Food preservatives are very essential in the food industry to prevent microbial contamination, deterioration of foods, extend the shelf life of the products, and keep their quality and safety. Recently, consumers have become increasingly aware of natural food preservatives instead of chemical ones due to their negative impact on public health (Lima *et al*., 2021). In the current study, we tested the inhibitory effect of some natural antimicrobial additives, such as cinnamon essential oil, L-lysine, and beta-carotene against *L. monocytogenes*. Essential oils, such as cinnamon oil, are classified as safe natural food additives by the US Food and Drug Administration (Dos Santos *et al*., 2022). Cinnamon oil is one of the potent natural antimicrobial agents due to the presence of cinnamaldehyde which is responsible for rupturing the cell membrane of bacteria, changing the lipid profile causing small ions to flow out, and blocking the enzymes required for the synthesis of amino acids (Sobhy *et al*., 2022). L-lysine has antimicrobial properties due to the presence of positively charged cationic amino acid groups, which are hig*hly* efficient in breaking down bacterial membranes and destroying bacteria (Švedienė *et al*., 2021). Natural pigments such as beta-carotene have antioxidant properties that inhibit pathogenic microorganisms' growth and prolong processed food's shelf life (Alhooei *et al*., 2019).

In the current study, the results displayed that the cinnamon oil inhibited the growth of *L. monocytogenes* with a significant inhibition zone (31mm) and these results were nearly similar to the results of El-Zehery *et al*. (2022) and Nematollahi *et al*. (2020) in which the cinnamon oil revealed a zone of inhibitions (30mm and 26.8mm) against *L. monocytogenes*, respectively. MIC of cinnamon oil was 1.95mg/ml and this result was lower than that reported by Sobhy *et al*. (2022) who detected the MIC of cinnamon oil at 1.56% which is equal to 15.6 mg/ml, on the other hand, our results were higher than the findings of Somrani *et al*. (2020) who determined that the MIC of cinnamon oil at 0.1mg/ml. L-lysine showed an inhibition zone of 28mm at a concentration of 1g/ml, and this concentration was applied according to Švedienė *et al*. (2021). The MIC of L-lysine was 125mg/ml and the same MIC was recorded by Švedienė *et al*. (2021) against S. aureus which is a gram-positive bacteria like *L. monocytogenes*. Beta-carotene (30% oil suspension) did not reveal any inhibition zone and this agrees with the previous study of Alhooei *et al*. (2019) who reported that gram-positive bacteria showed a higher degree of resistance against natural pigments.

The sensory evaluation has been evaluated to examine the effect of natural additives on taste, color, odor, body and texture, and overall acceptability using cheese as a food matrix. The evaluation was conducted on ten panelists who decided that the concentrations 1.95 mg/mL and

62.5 mg/mL for cinnamon oil and L-lysine were more acceptable than the others.

According to the obtained results, cinnamon oil revealed a marked inhibitory effect against *L. monocytogenes* inoculated in soft cheese by 3.9 log CFU/g reduction with a reduction percent of 99.99 after four days, and after eight days, *L. monocytogenes* was not detected with a reduction percent 100% and remained constant until the end of the experiment (28 days). According to the results obtained by Sobhy *et al*. (2022), the count of *L. monocytogenes* is reduced by 5.6 log CFU/g with a reduction percent of 67.5% during the 1st week from the addition of cinnamon oil to Tallaga cheese inoculated with *L. monocytogenes*. Also the results of Mortazavi and Aliakbarlu (2019), the cinnamon-treated low and high fat milk content contaminated with *L. monocytogenes* showed significant results with a reduction of 2.5 and 3 log, respectively, CFU/g during sex days of storage. Moreover, Boulares *et al*. (2023) recorded a reduction in *L. monocytogenes* count by 5.07 log CFU/g in meat samples during 12 days of storage.

Additionally, L-lysine revealed a bacteriostatic effect against *L. monocytogenes* in soft cheese, and *L. monocytogenes* counts were reduced by 2.1 after 28 days of treatment with a reduction percentage of 99%.

Therefore, further studies are still required to determine the accurate application mechanism of cinnamon oil and L-lysine against different zoonotic foodborne pathogens under different food-related stressors.

Conclusion

Multidrug resistant *Listeria monocytogenes* isolated from Egyptian dairies is of particular concern because it contributes a potential health concern for consumers especially pregnant women, the elderly, and immunocompromised patients. In addition, this pathogen contributes a risk for economy. Therefore, increasing the awareness for improving the quality and avoiding health hazards are required. In addition, L- lysine and cinnamon oil showed a significant inhibition in *L. monocytogenes* growth in soft cheese indicating that they are a good candidate for dairy industry as food preservatives.

Conflict of interest

The authors declare that they have no conflict of interest.

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