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Research Article

Virulence Potential of *Listeria monocytogenes* Recovered from Ice cream and Aborted Women Samples in Sohag city, Egypt

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Abstract | The circulation of virulence strains of *L. monocytogenes* in food products increased the burden of transmission of these pathogens to human especially those with impaired immunity such as pregnant women leading to abortion. This study detected the existence of *L. monocytogenes* and some of its virulence genes in ice cream sold in different food premises in Sohag city and aborted women admitted to Sohag Governmental Hospitals, as well as exhibit the risk factors related to aborted women infection. The samples including 200 ice cream samples from which; 100 produced in small scale and 100 in large scale, and from 95 aborted women; vaginal swabs, stool, and urine were collected from each woman. The bacteriological examination and PCR were used for *L. monocytogenes* detection, and the virulence genes such as *hlyA*, *Iap*, *PrfA*, and *InlA* were examined by PCR, while multiplex PCR was used for the detection of biofilm genes like *LuxS* and *flaA* which have a great role in surfaces contamination in dairy processing plants. Data from aborted women was collected through standard form. 11 (5.5%) ice cream samples and 9 (9.5%) aborted women were positive for *L. monocytogenes*. Ice cream that produced in small scale exhibit a higher microbial load than that produced in large scale with a percentage of 8% and 3%, respectively. The significant factors that increase *L. monocytogenes* infection in aborted women were residence, the medical history, ice cream consumption, and contact with animals. Among the 6 examined genes in *L. monocytogenes* isolates; *hylA* gene constitutes the highest percent in both ice cream and aborted women isolates. Control measures should be applied through increased knowledge of population about listeriosis infection and using rapid diagnostic methods for effective treatment, as well as strict hygienic measures at all levels of food production chain is important for producing safe food products.

Keywords | *L. monocytogenes*, Virulence genes, Biofilm genes, Aborted women, Ice cream

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INTRODUCTION

Listeria monocytogenes is a gram-positive bacterial pathogen which is considered as a ubiquitous in nature and can be isolated from the environmental sources, including water, soil, sewage, vegetables, and food (Soni et al., 2015). The main route of infection by *L. monocytogenes* is through ingestion of contaminated food, such as unpasteurized milk, milk products and raw unwashed vegetables (Rawool et al., 2016).

L. monocytogenes causing several outbreaks after the consumption of milk and dairy products especially ice cream (Shamloo et al., 2019). Ice cream is considered one of the most dairy products that predominates interest of popu-

lations (Abd El Fatah et al., 2015), it provides a suitable environment for *L. monocytogenes* growth due to its high nutritional value, low temperature, neutral pH value and long storage periods (Molla et al., 2004). *L. monocytogenes* can experience the cold conditions in dairy processing plants causing cold adaption of these bacteria which promote its survival in frozen dairy products such as ice cream (El shinaway et al., 2017). It has the ability to survive for 36 months in ice cream at storage temperature of −20°C without significant decrease in its number (Salazer et al., 2020).

L. monocytogenes can pose serious complications to human especially during pregnancy and neonatal infection due to its intracellular localization in the placenta, besides decreasing the cell-mediated immunity in pregnant women and in neonatal period causing fetal infections or miscarriage (Heidarzadeh et al., 2018). Pregnant women are more exposed to listeriosis than non- pregnant women by 18-times (Awofisayo et al., 2015). The World Health Organization reported that pregnancy related listeriosis represents nearly 43% of all listeria infections (Wadhwa Desai and Smith, 2017).

The detection of *L. monocytogenes* in food and environmental sources is a serious step toward prevention and control of *L. monocytogenes* outbreaks. The traditional culture method and PCR methods were used for isolation and identification of *L. monocytogenes* (Chen et al., 2017).

The virulent strains of *L. monocytogenes* are responsible for major illness and several deaths in human and animals (Pournajaf et al., 2016). *L. monocytogenes* pathogenesis is enhanced by a group of virulence genes (Thorat et al., 2019). Internalins encoded by *InlA* and *InlB* are surface proteins mediating the bacteria attachment and entry inside the host cells. Listeriolysin O (*hylA*) is cholesterol dependant toxin which facilitates the escape of bacteria from the phagocytic vacuoles and replication in the cytosol. *ActA* is also a surface protein that controls the motility of bacteria in the cytoplasm by polymerization of actin, and *Iap* is invasion associated protein. The *prfA* gene is considered the regulatory factor for several virulence genes such as *InlA, hylA, Iap*, and *actA* genes (Zahirnia et al., 2019).

The ability of biofilm formation is an important virulence determinant for *L. monocytogenes* pathogenicity and survival on surfaces in the food processing environment causing a great concern about food safety because it acts as a source of contamination (Colagiorgi et al., 2017). The *flaA* and *LuxS* genes play an important role in biofilm formation and surface attachment of *L. monocytogenes*. The flagellar gene (*flaA*) produces flagella which enhance the attachment of bacteria to biotic and abiotic surfaces (Warke et al., 2017). The *LuxS* gene encodes Sribosylhomocysteinase which act as a precursor of autoinducer AI-2 molecule which regulate the biofilm formation (Bonsaglia et al., 2013).

The aim of this work was to determine the occurrence of *L. monocytogenes* in different kinds of ice cream produced in Sohag city, Egypt and aborted women samples, and spotlight on the factors associated with aborted women infection. Also, detection of different virulence genes has a role in human public health and biofilm formation in the isolated strains.

MATERIAL AND METHODS

Study design

Milk product (ice cream) and aborted women samples were examined for the existence of *L. monocytogenes* using bacteriological culture examination followed by PCR, based on specific gene detection for *L. monocytogenes*. All isolates of ice cream and aborted women were examined for the presence of some virulence genes using PCR and multiplex PCR. Data from patients were collected to detect the factors associated with their infection.

Collection of data and samples

Two hundred ice cream samples were purchased from diversity food premises in Sohag city; 100 samples were from small scale producers and 100 samples were from 2 different brands of large scale producers (50 for each). The human samples were collected from patients admitted to Sohag Governmental hospitals; the population under study includes 95 aborted women. 285 samples were collected from the aborted women (three samples from each woman; vaginal swab, stool and urine). Data including age, residence, knowledge about *L. monocytogenes,* abortion history, trimester, medical history, ice cream consumption, and contact with animals were collected from women through a standard form.

Ethical consideration

This approval was obtained from local ethical committee of Sohag University. Participation of patients and samples collection was done after their consent or their guardian consent.

Bacteriological examination of samples

Isolation and identification of *L. monocytogenes* from ice cream and aborted women samples was implemented according to Arslan and Özdemir, (2020).

Molecular identification of *L. monocytogenes*

DNA was extracted from the suspected isolates of ice cream and aborted women using QIAamp DNA, Qiagen. PCR was applied for detection of *16S rRNA* gene specific for *L. monocytogenes* with primer sequence F: GGA CCG

Table 1: Incidence of *L. monocytogenes* in ice-cream samples

	Ice cream (n=200)							
	Positive samples		Large scale (n=100)				Small scale (n=100)	
			Brand I		Brand II			
	No	%	No	%	No	%	No	%
L. monocytogenes	11	5.5	3	3	0	0	8	8

Table 2: Incidence of *L. monocytogenes* in aborted women samples

Positive samples	Aborted women (n=95)							
	Positive women		Source of samples*					
			Vaginal swabs		Stool		Urine	
	No	%	No	%	No	%	No	%
L. monocytogenes	9	9.5	7	7.4	2	2.1	2	2.1

*One women has *L. monocytogenes* in all samples

Table 3: Characteristics and factors related to aborted women infected with *L. monocytogenes*

Risk factors	Aborted women n=95				*p* value
	Examined women		women with *L. monocytogenes*		
	No.	%	No.	%	
Age [b]					0.137
18-25	14	14.7	2	2.1	
26-30	51	53.7	0	0	
31-35	16	16.8	2	2.1	
>35	14	14.7	5	5.3	
Residence [a]					
Rural	34	35.8	7	7.4	0.01
urban	61	64.2	2	2.1	
Knowledge about *L. monocytogenes* [b]					0.293
Good	3	3.2	1	1.1	
Fair	11	11.6	1	1.1	
Poor	81	85.3	7	7.4	
Abortion history [b]					0.451
1st time	26	27.4	3	3.2	
2nd time	48	50.5	5	5.3	
3rd time	21	22.1	1	1.1	
Trimester [b]					0.619
First	27	28.4	3	3.2	
Second	40	42.1	4	4.2	
Third	28	29.5	2	2.1	
Medical history * [a]	21	22.1	7	7.4	0.01
Renal diseases	7	7.4	3	3.2	
Liver diseases	5	5.3	0	0	
Heart diseases	4	4.2	1	1.1	
Diabetes mellitus (DM)	9	9.5	2	2.1	
Bronchial asthma	5	5.3	1	1.1	
Hypertension	7	7.4	2	2.1	
Exposure to infection					

Ice cream consumption [a]						0.05
Usually	29	30.5	6	6.3		
Sometimes	49	51.6	2	2.1		
No	17	17.9	1	1.1		
Contact with animals [a]						
Yes	27	28.4	6	6.3		0.01
No	68	71.6	3	3.2		

*patients have more than one disease; [a] significant factors. [b] non significant factors

Table 4: Frequency distribution of virulence genes in *L. monocytogenes* isolated from ice cream and aborted women samples

***L. monocytogenes* isolates**	**Virulence genes**											
	hylA		***Iap***		***prfA***		***InlA***		***LuxS***		***flaA***	
Ice cream isolates	No	%	No	%	No	%	No	%	No	%	No	**%**
Small scale (n=8)	8	100	3	37.5	3	37.5	7	87.5	8	100	7	87.5
Large scale (n=3)	2	66.7	0	0	2	66.7	2	66.7	1	33.3	1	33.3
Total (n=11)	10	90.9	3	27.3	5	45.5	9	81.8	9	81.8	8	72.7
Women isolates												
Vaginal swabs (n=7)	7	100	4	57.1	5	71.4	7	100	6	85.7	4	57.1
Stool samples (n=2)	2	100	0	0	1	50	1	50	2	100	1	50
Urine samples (n=2)	1	50	1	50	2	100	0	0	1	50	2	100
Total (n=11)	10	90.9	5	45.5	8	72.7	8	72.7	9	81.8	7	63.6

GGG CTA ATA CCG AAT GAT AA and R: TTC ATG TAG GCG AGT TGC AGC CTA with 1200 bp according to Kumar et al. (2015) using thermal cycler from Applied biosystem. Some modifications in PCR cycle conditions were performed as following; 5 min at 94°C, followed by 35 cycles at 94°C for 45s, 60°C for 1 min, and 72°C for 1 min, and a final extension for 10 min at 72°C. The product of PCR was examined in 1% agarose gel electrophoresis with ethidium bromide and photographed by light transilluminator from Biometra (Germany).

Detection of virulence genes by PCR

The *L. monocytogenes* strains of both ice cream and aborted women were examined for the presence of some virulence genes according to Aziz and Mohamed, (2020) with modifications in the cycling conditions. These virulence genes were including *hlyA* gene with specific primer sequence F: GCA-TCT-GCA-TTC-AAT-AAA-GA and R: TGT-CAC-TGC-ATC-TCC-GTG-GT with 174 bp and cycling condition: primary denaturation for 4 min at 95°C, 35 cycles at 95°C for 30 sec, followed by 60°C for 30 sec, 72°C for 1 min and final extension for 5 min at 72°C. The *Iap* gene with primer sequence F: CTG CTT GAG CGT TCA TGT CTC ATC CCC C and R: CAT GGG TTT CAC TCT CCT TCT AC with 131 bp and cycling condition: 5 min at 94°C, 35 cycles at 94°C for 30 sec, followed by 55°C for 30sec, 72°C for 1 min, and 72°C for 10 min. The *prfA* gene with primer sequence F: TCT-CCG-AGC-AAC-CTC-GGA-ACC and R: TGG-ATT-GAC-AAA-ATG-GAA-CA with 1052 bp and cycling condition: 95°C for 5 min, 30 cycles for 30 sec at 55°C, followed by 72°C for 2 min, and final extension at 72°C for 5 min. *InlA* gene with primer sequence F: ACG AGT AAC GGG ACA AAT GC and R: CCC GAC AGT GGT GCT AGA TT with 800 bp and cycling condition: 2 min at 94°C, 30 cycles for 20 sec at 94°C, followed by 55°C for 30 sec, 72°C for 50 sec, and final extension for 5 min at 72°C.

Detection of virulence genes by multiplex PCR

LuxS and *flaA* genes were detected by multiplex PCR according to Thorat et al. (2019) with some modifications in the cycling condition using *LuxS* primer sequence F: GGA AATGCCAGCGCTACACTCTTT and R: ATTGCATGCAGGAACTTC TGT CGC with 208 bp and *flaA* gene sequence F: GCGCAAGAACGTTTAG-CATCTGGT and R: TTGAGT AGCAGCACCTG-TAGCAGT with 363 bp. The PCR cycling condition was initial denatruation for 5 min at 95°C, followed by 35 cycles at 94°C for 15 sec, 1 min at 56°C and 45 sec at 72°C and final extension at 72°C for 10 min.

Statistical analysis

SPSS 14 (SPSS, USA) was used to detect the relation between *L. monocytogenes* infection and women characteristics.

RESULTS

L. monocytogenes was investigated using *16S rRNA* gene in ice cream and aborted women samples (Figure 1). Out of 200 ice cream samples, 11 (5.5%) were harbor *L. monocytogenes*. Ice cream sold in small scale producers represented a higher infection rate (8%) than large scale producers (3%) which were positive only for one brand (Table 1). Among 95 aborted women; 9 (9.5%) women were positive for *L.monocytogenes*. Eleven isolates were recovered from the different 9 aborted women samples, only one aborted woman was carried *L. monocytogenes* in all body samples and the vaginal swabs were represented the highest load of infection in 7 samples, followed by 2 in stool samples and 2 in urine samples (Table 2).

The aborted women characteristics in Table 3 showed that most of positive *L. monocytogenes* cases were detected in women aged >35 years in a percentage of 35.3 %, followed by women aged 18-25 and 31-35 with infection rate of 2.1% , while women aged 26-30 years gave negative results. Women live in rural communities (7.4%) represented higher infection rate than those live in urban area (2.1%). The majority of the infected women had poor knowledge about *L. monocytogenes*. Aborted women for second time and those in second trimester of pregnancy reported the highest infection rate in a percentage of 5.3% and 4.2%, respectively. Women have suffered from chronic diseases such as renal, liver, and heart diseases; diabetes mellitus, bronchial asthma, and hypertension were more susceptible to *L. monocytogenes* infection with a percentage of 7.4%. Based on the exposure to infection; women eating ice-cream (6.3%), and women in contact with animals (6.3%) showed high susceptibility to the infection. Regarding factors associated with *L. monocytogenes* infection; residence, the medical history of aborted women, consumption of ice cream, and contact with animals significantly associated with infection, while age, range of knowledge about *L. monocytogenes*, abortion history, and trimester reported no significant relation to infection.

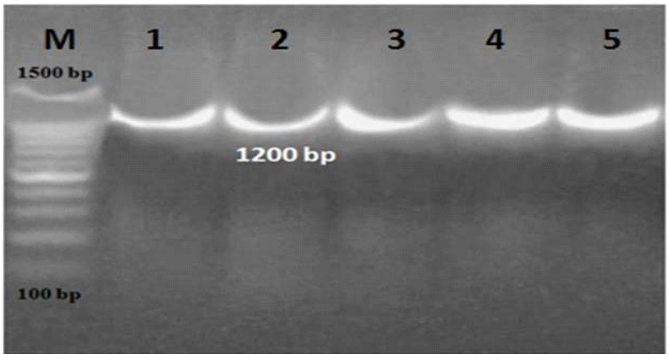

Figure 1: PCR results for *16S rRNA* gene in ice cream and aborted women samples. M: marker, Lane 1-5: positive for *L. monocytogenes*

Referring to the *L. monocytogenes* virulence genes; PCR and multiplex PCR were used for detection of 6 genes (*hylA, Iap, prfA, InlA, LuxS* and *flaA*) in ice cream and aborted women isolates, and found that *hylA* gene was present with the highest percent among all the examined genes. Three (27.3%) of 11 ice cream isolates and 2 (22.2%) out of 9 aborted women isolates were positive for all the examined virulence genes (Table 4, Figure 2, 3).

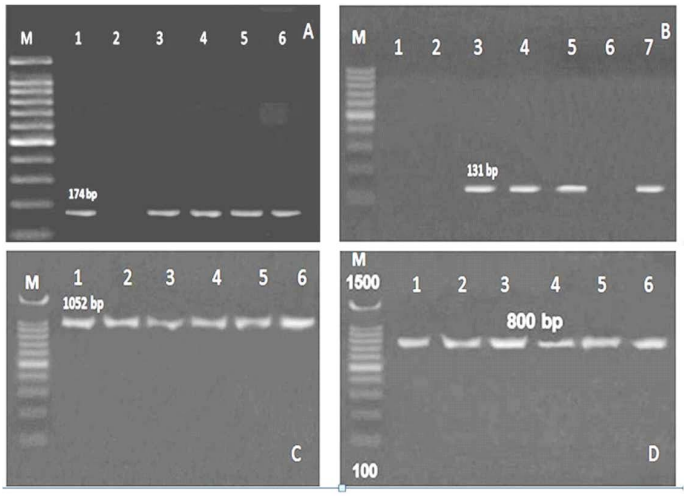

Figure 2: PCR results for virulence genes detected in *L. monocytogenes* isolated from ice cream and aborted women samples **A**: M: marker, Lane 1,3,4,5 and 6: positive for *hylA* gene, Lane 2: negative, **B**: Lane 1, 2 and 6: negative for *Iap* gene, Lane 3, 4,5 and 7: positive, **C**:Lane 1-6: positive for *prfA* gene, **D**: Lane 1-6: positive for *InlA* gene

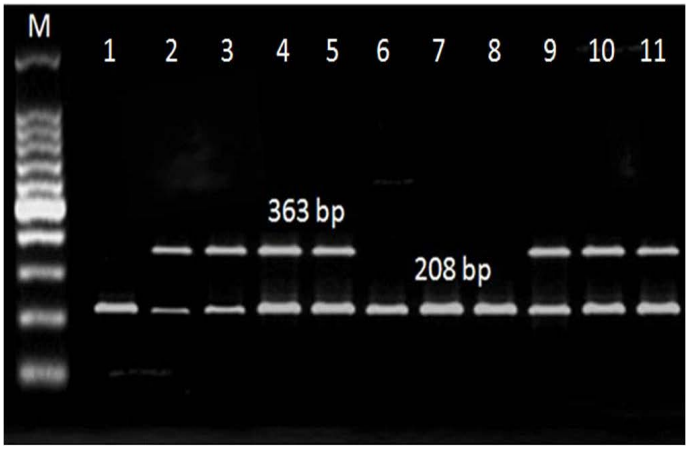

Figure 3: Multiplex PCR results for *LuxS* and *flaA* genes detected in *L. monocytogenes* isolated from ice cream and aborted women samples. M: marker, at 208 bp: Lane 1-11: positive for *LuxS* gene. At 363 bp: Lane 1,6,7, and 8: negative for *flaA* gene, Lane 2,3,4,5,9,10, and 11: positive.

DISCUSSION

Listeriosis infection is associated with contaminated food consumption such as ready to eat meat products, unpasteurized milk, seafood and ice cream. The contamination of food may occur during food processing stages such as

preparation, packaging, handling, transportation, and storage (Garner and Kathariou, 2016). Ice cream is a popular and delicious food where milk should be pasteurized before freezing (Yousef et al., 2020).

L. monocytogenes was detected by PCR using *16S rRNA* gene in ice cream samples with a percentage of 5.5% (Table 1). Nearly similar results were reported by Adil et al. (2017) who detected *L. monocytogenes* in 6% of ice cream samples, while high incidence (12.3%) was detected by Windrantz and Arias (2000) and low incidence (2%) was obtained by Youssef et al. (2020). In the contrary, Abrahão et al. (2008) cannot detect *L. monocytogenes* in ice cream samples. Two types of ice cream were examined; the first one produced in a large scale which manufactured from pasteurized milk and sold packaged in different markets and groceries with several brands, and we studied two brands only. The second one produced in a small scale with unpasteurized milk and sold unpackaged in different shops. *L. monocytogenes* was detected in 8% of small scale samples, and in 3% of large scale samples in brand I, while brand II reported negative results. Increased infection in small scale ice cream may be attributed to the source of milk which used in production and other raw materials, also it exposed to several sources of contamination during processing, transportation and handling from food handlers, contaminated equipment and environment. The presence of *L. monocytogenes* in one brand of the large scale ice cream may be resulted from post pasteurization contamination of milk in the factory. Therefore, the major points for the control of pathogens are pasteurization of milk, freezing, and hardening steps, but the latter 2 not decrease the level of *L. monocytogenes* (Abrahão et al., 2008). As a result of the post pasteurization contamination of ice cream; *L. monocytogenes* can survive in ice cream due to its neutral pH which enables its growth. Also, it can grow at chilled temperatures at 4, 8, 12 and 16°C and it survive at a static freezing temperature at -5, -15, -23, and -33°C without any decrease in the bacterial numbers up to 90 days (Gougouli et al., 2008).

Although *L. monocytogenes* is uncommon microorganism to cause diseases for the general population, it capable of inducing a wide range of illness in pregnant women, neonates, elderly people, and immunocompromised individuals (Mehmood et al., 2017). Pregnant women are more susceptible to infection nearly 18 times larger than the general population due to the natural immunosuppression of pregnancy (Mateus et al., 2013).

L. monocytogenes was detected in 9 (9.5%) women of 95 aborted women. Eleven isolates were recovered from 9 patients, out of these, 7 of vaginal swabs, 2 urine samples and 2 of stool samples (Table 2). Different results were mentioned by Eslami et al. (2014) and Al-Mayahi et al. (2020) who detected *L. monocytogenes* in 16.7% and 4.8% of aborted women, respectively. The variation in results may be related to women immunity, medical history, food habit, geographic region, contact with infected animals, and rang of knowledge about disease transmission. As shown in Table 3, out of 9 infected women with *L. monocytogenes*; 5 (5.3%) aged >35 years, 7 (7.4%) live in rural areas, 7 (7.4%) represented poor knowledge about listeriosis, 7 (7.4%) had chronic diseases, 4 (4.2%) aborted in the second trimester, and 5(5.3%) aborted for the second time. Our results revealed that aborted women have *L. monocytogenes* exhibit influenza like symptoms such as myalgia, headache, fever below 39°C, and diarrhoea. The unexplained fever and non-specific symptoms of listeriosis during pregnancy make it difficult to be diagnosed which leads to abortion, especially in the second trimester (Pourkaveh et al., 2016).

The risk factors significantly associated with women infection with *L. monocytogenes* include residence ($p< 0.01$), medical history ($p< 0.01$), consumption of ice cream ($p< 0.05$), and contact with animals ($p< 0.01$) (Table 3). This significance may be attributed to the decreased immunity of aborted women due to chronic diseases beside pregnancy which makes these women more susceptible to infection from different sources such as, contaminated food, infected farm animals, untreated water and soil, especially in rural communities in which women more exposed to infection in agriculture work, and home activities including rearing of farm animals and birds. The food habits such as eating unpackaged food, and under cooked food, and low knowledge about diseases transmission may increase the chance of disease occurrence in the community. Therefore, increase the awareness of people about disease source, mode of transmission, severity, and the preventive measures may change their behaviour toward positive practice and reduce the infection with diseases.

Infection with *L. monocytogenes* depends on the immunity of the infected subjects, infection dose and the type of virulence genes in the bacterial strain. However, the susceptibility for infection was increased in case of immunodeficiency people (Wang et al., 2021). Once *L. monocytogenes* invade the human body by oral administration, it reaches to the small intestine mucosa, and then invades other organs through the circulation and lymph nodes. Different proteins were secreted by *L. monocytogenes* to attack the host cells such as internalins. After entrance inside the cell, it releases phospholipases and listeriolysins to dissolve the membrane of the phagocytic vacuoles to survive in the cells. When *L. monocytigenes* reach the cytoplasm, it proliferates and enhances the development of actin filaments, and then penetrates the cytoplasm into the plasma membrane and intercellular diffusion to infect the neighbouring cells. The intercellular circulation allows *L. monocytogenes* to escape

from human T cell immunity and move from one cell to another and infect other tissues and organs especially placenta tissue in case of pregnancy (Charlier et al., 2014).

L. monocytogenes can form biofilm which allows bacteria to resist the environmental stress such as varied temperature, dehydration, and treatment with sanitizing and antimicrobial agents. Formation of biofilm on surfaces during food processing considered an important factor of *L. monocytogenes* survival (Barbosa et al., 2013). Detection of biofilm genes such as *LuxS* and *flaA* are a determinant genes of *L. monocytogenes* pathogenicity (Warke et al., 2017).

PCR and multiplex PCR were used to detect some virulence genes in *L. monocytogenes* isolates using specific primers. The isolated strains of *L. monocytogenes* from ice cream and aborted women harbour some virulence genes such *as hylA, Iap, prfA, InlA, LuxS*, and *flaA* (Table 4). Consistent with results that reported by Throat et al. (2019) who detected *plcA, hlyA, actA, prfA, inlC, inlJ, luxS* and *flaA* genes in *L. monocytogenes* isolated from different sources as human, food, animals, and mosquitoes. Listeriolysin O (*LLO*) is considered the most virulence factor of *L. monocytogenes* which encoded by *hylA* gene and present only in the virulence strains (AL-Ashmawy et al., 2014). The most prominent gene in both women and ice cream isolates of *L. monocytogenes* was *hylA* gene which indicated that theses isolates is virulent strains.

Ice cream isolates of *L. monocytogenes* harbour *hylA* gene with a percentage of 90.9%, followed by *LuxS* and *InlA* with a rate of 81.8%, followed by *flaA* (72.7), *prfA* (45.5%), and *Iap* (57.1). Consistent with Abd El Tawab et al. (2015) who detected *InlA, InlB, hlyA* and *prfA* genes in *L. monocytogenes* isolated from ice cream samples. Presence of pathogenic strains of *L. monocytogenes* in ice cream samples revealed that freezing temperature not affects on its survival and pathogenicity. We cannot depend on only storage of dairy products at refrigeration or freezing temperatures to control *L. monocytogenes* (Bucur et al., 2018). Therefore in dairy industry; using safe materials and following hygienic measures in all steps of manufacturing, packaging, transportation and handling is important to produce safe products.

Aborted women isolates of *L. monocytogenes* have *hylA* gene with a percentage of 90.9%, followed by *LuxS* gene (81.8%), *prfA* and *InlA* (72.7%), *flaA* (63.6%), and *Iap* (45.5%). These results goes parallel with Kaur et al. (2007) who detected *prfA, hylA*,and *Iap* genes in *L. monocytogenes* isolates of women with spontaneous abortion, and Shaker and Hassanien (2015) who detected *hylA*, and *InlA* genes. Presence of these genes in the isolated strains increases *L. monocytogenes* attachment, invasion, development and survival inside the human cells which enhance its pathogenicity especially in immunodeficiency patients and it is a good indicator of the virulence level of *L. monocytogenes* (Liu et al., 2007).

CONCLUSION

Pathogenic strains of *L. monocytogenes* were detected in ice cream although it stored at freezing temperature which pose a great threat to consumers. Contamination of large scale and small scale produced ice cream indicated that surveillance and control measures should be applied at all food processing stages. Detection of *L. monocytogenes* and the risk factors associated with infection in aborted women is important for effective treatment and control of listeriosis infection. Detection of virulence genes in the isolated strains is an important indication for its pathogenicity and survival in the surfaces through biofilm formation.

CONFLICT OF INTEREST

None.

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AUTHORS CONTRIBUTION

AAH and EMS contributed equally in designing the study, samples and data collection, literature search, laboratory work, data analysis, writing and preparation of the manuscript. Both authors approved the final manuscript.

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