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RESEARCH ARTICLE

Prevalence of *Listeria* Species in Dairy Cows and Pregnant Women with Reference to Virulotyping of *Listeria monocytogenes* in Egypt

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Abstract

This study was conducted to determine the prevalence of *Listeria* spp. in milk and feces of dairy cows as well as stool of pregnant women in Sharkia and Dakahlia Governorates, Egypt. Three hundred and fifty samples including; normal raw milk (n=200), mastitis milk (n=50), feces of dairy cattle (n=50) and stool of pregnant women (n=50) were randomly collected. *Listeria* spp. were isolated on Oxford agar and then subjected to biochemical and molecular identification. The overall isolation rate of *Listeria* spp. was 16%. The prevalence rates of *Listeria monocytogenes* were 8%, 4%, 2% and 4% in feces of dairy cows, normal milk, mastitis milk and stool of pregnant women, respectively. The prevalence of *L. ivanovii* and *L. welshimeri* in milk of dairy cows were 6% and 4%, respectively. *L. monocytogenes* isolates were molecularly confirmed to the species level by amplification of *16S rRNA* gene. The distribution of internalin A (*inl*A) and internalin B (*inl*B) was determined in 15 identified *L. monocytogenes* isolates. The overall prevalence of *inl*A gene and *inl*B gene in *L. monocytogenes* isolates from different sources were 80% and 40%, respectively. Two *L. monocytogenes* isolates from normal raw milk and feces of dairy cows didn't harbor both *inl*A and *inl*B genes. *Listeria* spp. isolated from normal milk, feces, mastitis milk and stool of pregnant women, potentially can cause human illness and abortion in pregnant women. Internalin (A&B) genes are considered the best indicator for virulence determination of *L. monocytogenes* isolated from different sources.

Keywords: *Listeria monocytogenes*, *16S rRNA* gene, Internalin A&B, Milk, Pregnant women.

Introduction

Listeria species are ubiquitous bacteria, widely spread in the nature such as soil, mud, plant, decaying vegetation, raw and treated sewage, fecal material, water, food processing facilities [1].The genus *Listeria* comprises of 6 species including; *Listeria monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. grayi*. These organisms are short Gram-positive bacilli, facultative anaerobe, non-capsulated and non-spore former. The main two pathogenic *Listeria* spp. for humans and animals are *L. monocytogenes* and *L. ivanovii* in spite of occasional human cases reported by *L. ivanovii* infection [2]**.** The ubiquitous nature of *L. monocytogenes* enables its entrance and proliferation into human food chain. *Listeria* may be a common contaminant in the dairy farms [3].

Raw milk and animals' manure have been considered as vital sources for *Listeria* in the dairy environment. The oral- fecal cycle resulted in persistence of such pathogen in the farm [4].

Diseases attributed to food borne pathogens represent a worldwide hazard to public health. Listeriosis is one of most important zoonotic disease with fatality rates up to20- 30% [5]. Listeriosis is a foodborne disease transmitted through the consumption of raw or insufficiently cooked food. *L. monocytogenes* is the main etiological agent frequently incriminated in such illness. Food recognized as common vehicle for Listeria infection

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include milk, dairy products, raw vegetables, poultry, seafood, meat and meat products [1]. The individuals at high risk for listeriosis are pregnant women, neonates, older age and individuals with poor immunity. Wide ranges of infections were reported in human including; gastroenteritis, fever, diarrhea, muscle aches, nausea, vomiting and fatigue; however, severe forms may result in encephalitis, meningitis and abortion in pregnant women [6].

Sporadic cases and epidemic outbreaks of listeriosis in humans and large number of animal species were recorded. Latent listeriosis in pregnant women resulted in spontaneous abortions, placental necrosis, intrauterine deaths and fetal malformations. The prevalence rate of listeriosis in general population is 0.7 in 100000 while the incidence rate is 12 in 100000 in pregnant women (that is a 17-fold increase) [6-9].

Mastitis is a multi-etiological disease that induces major economic losses in the dairy industry all over the world. *Listeria monocytogenes* could infect the udder from localization after systemic infection or from outside penetration of the teat [10]. Moreover, *L. monocytogenes* might infect one or all of quarters and the bacteria are excreted in milk causing a potential risk to the public health. Listeric mastitis is difficult to treat and many cases of Listeric mastitis go unobserved or unnoticed [11].

PCR assays are one of the preferable ways for detection and confirmation of such pathogen. Lastly, researchers have been attracted toward molecular techniques relying on 16S rRNA sequences as a reliable method for identification and differentiation of wide ranges of bacteria at the genus level across almost all phyla and also at species and sub species levels. It is large enough (1,500 bp) for genetic information [12,13].

Internalins have an essential role in pathogenicity of *L. monocytogenes* and its success as facultative intracellular pathogen. Internalin genes (*inl*A & *inl*B) are a panel of *Listeria* virulence-specific genes that encoded by proteins in inner bacterial surface which involved in *L. monocytogenes* passage through epithelial cells of the host as well as through the intestinal barriers for the successive stages of infection [14]. Internalin A binds to the host cell receptor E-cadherin which is required for the entry into human epithelial cells such as enterocytic Caco-2 cells and crossing the intestinal barrier to host blood stream [15]. Besides, internalin B is an invasion surface protein on *L. monocytogenes* which could play a great role in the entrance into multiple host cells as hepatocytes, endothelial and epithelial cells [16]. Differential expression levels of *inl*A and *inl*B may have an important role in the virulence capacities of *L. monocytogenes* strains [17].

This study was aimed to investigate the prevalence of *Listeria* species in milk, feces of dairy cows as well as stool of pregnant women in two Governorates (Sharkia and Dakahlia) in Egypt, and also to determine the prevalence of some virulence genes (*inl*A and *inl*B) in molecularly identified isolates of *L. monocytogenes.*

Materials and Methods

Sample collection

A total of 350 samples including; normal milk (n=200), mastitis milk (n=50), feces of dairy cows (n=50) and stool of pregnant women (n=50) were randomly collected from different dairy farms and hospitals in Sharkia and Dakahlia Governorates, Egypt during the period from January 2018 to January 2019. The collected samples were transferred in an ice box immediately to the Microbiology and Zoonoses Departments laboratories, Faculty of Veterinary Medicine, Zagazig University, Egypt until isolation and identification.

Milk samples were randomly screened for mastitis by using California Mastitis Test (CMT). Fifty milk samples were mastitis positive and involved in this study [18].

Isolation and identification of Listeria spp.

Twenty-five milliliter of milk samples either normal raw milk or mastitis milk was pre-enriched in 225 mL of Tryptone soya broth (TSB) (Oxoid, CMO129, UK). Twenty-five grams either from feces of dairy cows or stool of pregnant women were also pre-enriched in 225 mL of Tryptone soya broth (TSB) (Oxoid, CMO129, UK). All previous samples were incubated at 37°C for18-24h. Ten milliliter of an initial suspension was placed into a sterile tube contained 90 ml of Listeria enrichment broth base (LEB) (Oxoid, CM0862,UK) with Listeria enrichment selective supplement (Oxoid, SR0141,UK) and incubated for 2-7days at 30°C for enrichment

of Listeria spp. Finally, a loopful of incubated enrichment broth was streaked onto petri dish containing Listeria selective agar (Oxford formulation) (Oxoid, CM0856, UK) with Listeria selective supplement (Oxoid, SR0140, UK) and placed in an incubator at 37℃ for 24-48 h. Presumptive gray colonies surrounded by black zone were purified by subculture on Tryptic soy agar yeast extract (TSAye) and incubated at 37°C for18- 24 h [19].

Identification of Listeria spp.

For identification of isolates to species level, typical colonies were subjected to the following tests (Gram staining, catalase, hemolysis on blood agar, oxidase, motility, and fermentation of Rhamnose, Xylose and Mannitol sugars) [20, 21].

Molecular characterization of isolated L. monocytogenes

Genomic DNA from biochemically identified *L. monocytogenes* strains was extracted by using QIAamp DNA Mini Kit Catalogue no.51304 (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's guidelines. Extracted DNA was subjected to PCR using primers targeting 1200 bp of the *16S rRNA* specific for *L. monocytogenes*. The oligonucleotide primers *16S rRNA* was carried out as previously described [22] in Table 1

Table 1: Oligonucleotide primers used for amplification of *16S rRNA* gene, *inl*A and *inl*B genes in isolates of *L. monocytogenes* from dairy cows and pregnant women.

Gene	Primer sequence (5'→3')	Expected product size (bp)	References
16S rRNA [1]	*16S rRNA-Forward*	GGA CCG GGG CTA ATA CCG AAT GAT AA	1200
	16S rRNA-Reverse	TTC ATG TAG GCG AGT TGC AGC CTA	22
***inl*A** [2]	*inl*A- *Forward*	ACG AGT AAC GGG ACA AAT GC	800
	*inl*A-*Reverse*	CCC GAC AGT GGT GCT AGA TT	23
***inl*B** [3]	*inl*B- *Forward*	CTGGAAAGTTTGTATTTGGGAAA	343
	*inl*B- *Reverse*	TTTCATAATCGCCATCATCACT	24

[1]The *16S rRNA* primers were designed for species-specific recognition and the [2]*inlA* and [3] *inlB* primers for virulence determination of *L. monocytogenes*.

All PCR reactions were carried out in a final total volume of 25µl (12.5 µl of Emerald Amp GT PCR master mix (2x premix) (Takara, Code No. RR310Akit, Japan), 1 µl of 20 pmol of forward primer, 1 µl of 20 pmol of reverse primer, 5.5 µl PCR grade water and 5µl Template DNA). The cycling conditions for *16S rRNA* PCR involved a primary denaturation of DNA at 94°C for 5 min followed by 35 cycles of secondary denaturation at 94°C for 30 sec, annealing at 60°Cfor 1 min, extension at 72°C for 1 min, then final extension at 72°C for 12 min. PCR products stained with ethidium bromide (Sigma, US) were electrophoresed on 1.5 % agarose gel at 1.5volts\cm for 30 min in Tris borate EDTA (TBE) buffer. The amplified DNA products were seen using UV transilluminator. A positive control isolate of *L. monocytogenes* was kindly provided from Biotechnology unit, Reference laboratory for Veterinary Quality Control on Poultry Production, Animal Research Institute, Dokki, Giza, Egypt.

Molecular detection of internalin genes in L. monocytogenes isolates

Isolates identified as *L. monocytogenes* were further subjected to screening for the presence of some virulence genes (*inl*A & *inl*B). In Table 1, the primers sequences for amplification of internalin (A & B) genes were designed as previously described [23, 24], respectively. The amplification conditions were 35 cycles of primary denaturation for 5 min at 94ºC, secondary denaturation for 30 sec at 94ºC, annealing for 40 sec at 55ºC, extension for 50 sec at 72ºC and final extension at 72ºC for 10 min.

Statistical analysis

The data was analyzed using SPSS version 22 for Windows (IBM corp, 2013, Armonk, NY) to compute PV values for Pearson chi-square.

Results

Out of 350 examined samples, 56 (16%) were contaminated with *Listeria* spp. by using culture techniques, and biochemical tests. Among the 56 isolated *Listeria* spp.; 38 (19%), 6 (12%), 8 (16%) and 4 (8%) were recovered from normal raw milk, mastitis milk, feces of dairy cows and stool of pregnant women, respectively. The *Listeria* spp. isolates from different sources included *L. monocytogenes* (n = 15, 4.28%), *L. ivanovii* (n = 18, 5%), *L. innocua* (n = 5, 1%), *L. grayi* (n = 9, 2.5%) and *L. welshimeri* (n = 9, 2.5%).

The prevalence rates of *Listeria monocytogenes* were 8%, 4%, 2% and 4% in feces of dairy cows, normal milk, mastitis milk and stool of pregnant women, respectively (Table 2). *L. ivanovii* was isolated from normal raw milk and mastitis milk (6%, each). The prevalence rates of *L. ivanovii were* 4% and 2% in feces of dairy cow samples and stool of pregnant women samples, respectively. *L. innocua* was only isolated from raw milk and mastitis milk samples (2%, each). *L. grayi* was isolated from 4%, 3% and 2% of feces of dairy cows, normal raw milk and mastitis milk samples, respectively. The prevalence rates of *L. welshimeri* were 4% and 2% in normal raw milk and stool of pregnant women, respectively. *L. welshimeri* was not isolated from mastitis milk and feces of dairy cow (Table 2).

Table 2: Prevalence of *Listeria* spp. in normal milk, mastitis milk, feces of dairy cows and stool of pregnant women at Sharkia and Dakahlia Governorates, Egypt from January 2018 to January 2019.

Source of Samples	No. of examined samples	No. of positive samples	*L. monocytogene*	*L. ivanovii*	*L. innocua*	*L. grayi*	*L. welshimeri*
Milk of dairy cows	200	38(19%)	8(4%)	12(6%)	4(2%)	6(3%)	8(4%)
Mastitis milk	50	6(12%)	1(2%)	3(6%)	1(2%)	1(2%)	0(0%)
Feces of dairy cows	50	8(16%)	4(8%)	2(4%)	0(0%)	2(4%)	0(0%)
Stool of pregnant women	50	4(8%)	2(4%)	1(2%)	0(0%)	0(0%)	1(2%)
Total	350	56(16%)	15(4.28%)	18(5%)	5(1%)	9(2.5%)	9(2.5%)
X^2				4.31^{NS}			

NS: Non specific

The statistical analysis revealed that there was no significant differences between the of *L. monocytogenes* isolates recovered from different sources (P>0.05) as shown in Table 2.

All the 15 biochemically characterized *L. monocytogenes* isolates were confirmed by PCR assay, using the species- spesific*16S rRNA* gene (100%). *L. monocytogenes* strains revealed an amplified product of 1200 bp as illustrated in Figure 1.

PCR amplification of *inl*A and *inl*B genes revealed their presence with correct size 800 bp and 343 bp, respectively (Figure 2). *Inl*A gene was molecularly detected in 12 *L. monocytogenes* isolates (80%); (7/8) of normal raw milk, (1/1) of mastitis milk, (3/4) of feces of dairy cows and (1/2) of stool of pregnant women. However, *inl*B gene was observed in only 6 *L. monocytogenes* isolates (40%); (3/8) of normal raw milk, (1/1) of mastitis milk, (1/4) of feces of dairy cows and (1/2) of stool of pregnant women (Table 3).

Table 3: Distribution of genus species specific *16S rRNA* and internalin (*inl*A, *inl*B) genes in isolates of *L. monocytogenes* from normal milk, mastitis milk, feces of dairy cows and stool of pregnant women at Sharkia and Dakahlia Governorates, Egypt from January 2018 to January 2019.

Source of samples	[1]No. of positive samples	[2]*16S rRNA*	*inl*A	*inl*B
Milk of dairy cows	8	8/8	7/8	3/8
Mastitis milk	1	1/1	1/1	1/1
Feces of dairy cows	4	4/4	3/4	1/4
Stool of pregnant women	2	2/2	1/2	1/2
Total	15	15/15(100%)	12/15(80%)	6/15(40%)

[1]Biochemically suspected isolates of *L. monocytogenes*.
[2]The *L. monocytogenes* isolates were molecularly confirmed by PCR targeting species specific *16S rRNA* gene.

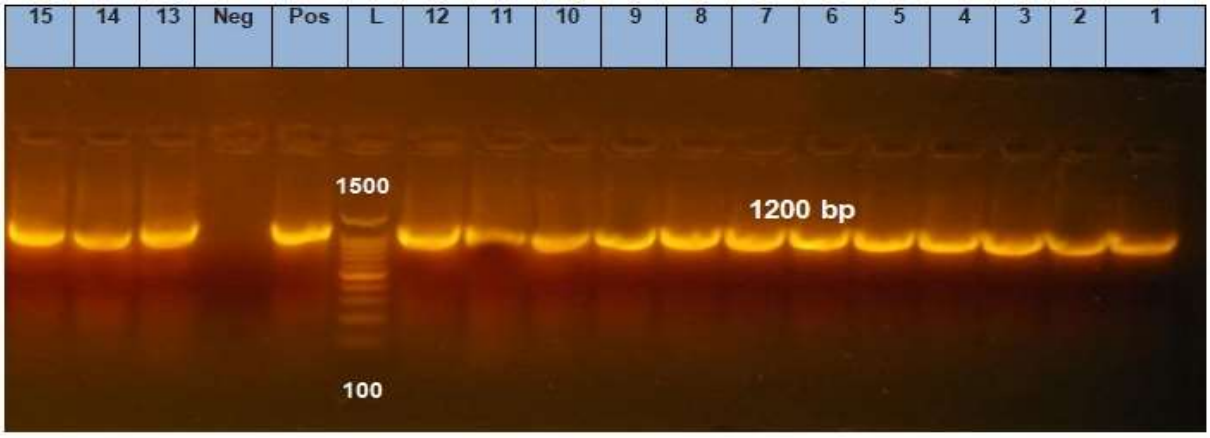

Figure 1: Agarose gel electrophoresis of *16S rRNA* gene in *L. monocytogenes* isolates from normal milk, mastitis milk, feces of cows and stool of pregnant women collected from Sharkia and Dakahlia Governorates, Egypt during the period from January 2018 to January 2019. Ladder (L): 100-1500 bp; Neg.: Negative control; Pos.: Positive control; Lanes 1 - 15: positive *L. monocytogenes* isolates for *16S rRNA* gene (1200bp).

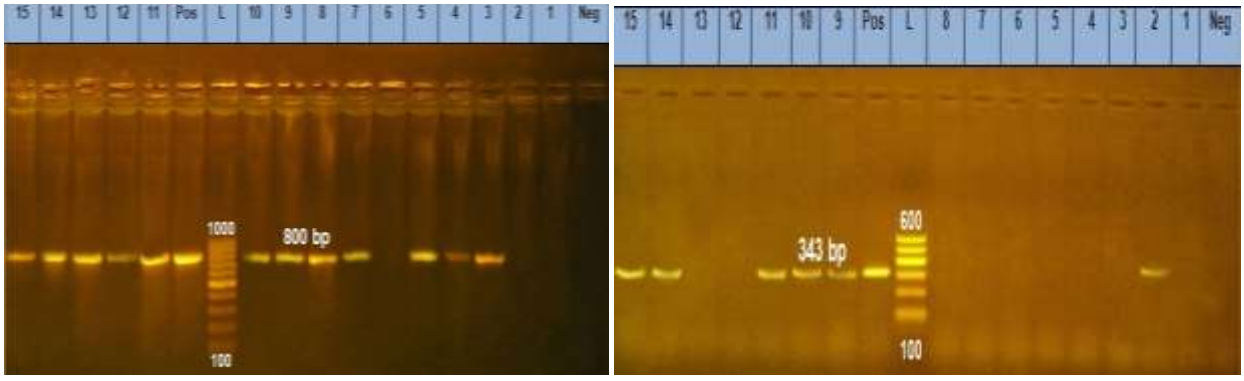

Figure 2 (A): Agarose gel electrophoresis of *inl*A gene amplification of *L. monocytogenes* isolates from Sharkia and Dakahlia Governorates, Egypt during the period from January 2018 to January 2019. Ladder (L): 100 -1000 bp; Pos.: Positive control; Neg.: Negative control; Lanes (1, 2, 6): negative *L. monocytogenes* for *inl*A gene; Lanes (3-5 & 7-15): positive *L. monocytogenes* for *inl*A gene (800 bp). (B): Agarose gel electrophoresis of *inl*B gene amplification of *L. monocytogenes* isolates from different sources from Sharkia and Dakahlia Governorates, Egypt during the period from January 2018 to January 2019. Ladder (L):100 - 600bp; Pos.: Positive control; Neg.: Negative control; Lanes (2, 9, 10, 11, 14, 15): positive *L. monocytogenes* for *inl*B gene; Lanes (1, 3, 4, 5, 6, 7, 8, 12, 13): negative *L. monocytogenes* for *inl*B gene (343 bp).

Discussion

Listeria monocytogenes is currently considered as the third major pathogen frequently transmitted from food. The high prevalence of *Listeria* spp. in milk, feces and dairy products has a great hazard on public health and dairy industry [25]. Milk and dairy products are implicated in most outbreaks of listeriosis all over the world. The first report of listeriosis case in USA was resulted from consumption of pasteurized milk in 1983 [26].

In the current study, the higher isolation rate of *Listeria* spp. was detected in milk of dairy cows (19%) followed by feces of dairy cows (16%), mastitis milk (12%) and then stool of pregnant women (8%). Therefore, higher prevalence of *Listeria* spp. in milk samples in this study might be attributed to insufficient hygienic practice in dairy farms together with the poor hygiene of surrounding environments**.** Dairy farms are an important reservoir of *Listeria*s pp., so, contamination of milk by such pathogens may occurs through direct contact with contaminated sources in the dairy farm environment as the milking machines, the piping system, milking utensils, teat cups and milk handlers which are

considered as critical points of milk contamination at the farm. So, cleanliness of dairy farms' environment seems to be an important risk factor associated with milk contamination by *L. monocytogenes* as previously supported by Hassan *et al*. [27], Oliver *et al*. [28] and Vilar *et al.* [29].

Regarding the overall prevalence of *Listeria* spp. in milk samples (19%), similar result (16.7%) was reported in Egypt by Abdel-Aal [30]. However, lower percentages of 13.3% and 6% were respectively recorded in milk samples of dairy cows in Giza by Reda *et al*. [31] and in Dakahlia Governorate by Abd El Aal and Atta [32]. These differences in the prevalence of *Listeria* spp. could be attributed to sample size and locality [33,34]. The prevalence of *L. ivanovii* and *L. welshimeri* from milk of dairy cows were 6% and 4%, respectively in this study. In Turkey, nearly similar finding for *L. ivanovii (*5.4%) and *L. welshimeri* (4.5%) isolates from milk and milk products were detected by Sanlibaba *et al*. [35]. On the other hand, lower prevalence of *L. ivanovii* (1.4%) and *L. welshimeri* (0.7%) in previous study were detected from cow milk samples by El- Sherbini *et al.* [36] in Sharkia Governorate**.**

The prevalence rate of *L. monocytogenes* in milk of dairy cows was 4%in this study. This finding was concurred with previous result detected in India [37]. Also, nearly similar report **(**5.4%) for isolates of *L. monocytogenes* from milk was recorded in Iran [38]**.** On the contrary, lower prevalence of *L. monocytogenes* was detected in cow milk samples (2%) by Khedr *et al.* [39] in Egypt, and (1.1%) by Almeida *et al.* [40] in Portugal**.** However, higher prevalence of *L. monocytogenes* (8.3%) from cow milk samples was cited by El-Marnissi *et al*. [41] in Morocco, and (6%) by Abd El-Tawab *et al*. [42] in Egypt.

Milk is a proper medium for growth of *Listeria* spp. that account for the occurrence of food poisoning. Differences in the prevalence rate of *L. monocytogenes* in previous studies might be due to several factors affecting the level of *Listeria* contamination in raw milk as health status of dairy cattle, methods of detection, environmental conditions of dairy farms and hygienic managements of dairy environments and dairy farmers [39,43].

L. monocytogenes was detected in 2% of mastitis milk samples of dairy cows. This result was in agreement with the finding of Yadav *et al.* [11] who isolated *L. monocytogenes* with a percentage of 3.5 in Gujarat state, India. While higher percentage (6%) was recorded by Sunitha *et al.* [44] in Kerala, India. This may suggest that *L. monocytogenes*, *L. ivanovii* and *L. welshimeri* are considered as predisposing causes of mastitis in dairy cows. Little literatures are available about the prevalence of *Listeria* spp. in mastitis milk. This was the first report for isolation of *L. monocytogenes, L ivanovii* and *L. welshimeri* from mastitis milk in Egypt.

The overall prevalence of *Listeria* spp. was 16% in feces of dairy cows. Nearly similar result of 15.6% was reported in Egypt [45]. In contrary, lower percentages of 3.3% and 9.6% were reported in Egypt [46] and Finland [47], respectively. While, higher prevalence rate of *Listeria* spp. (68%) was previously reported [48]. However, Ahmed [49] didn't recover any *Listeria* spp. from feces of dairy cattle in Egypt.

The prevalence rate of *L. monocytogenes* in feces of dairy cows was 8% in the current study. Nearly similar percentage of 7.5% for *L. monocytogenes* in feces of dairy cows was previously reported in New York State [50]. However, lower isolation rates of 6% in Sweden [51] and 1.5% in Turkey [52] were documented.

This finding could be explained on the fact that milk could be contaminated by feces of infected farm animals or asymptomatic carriers. Also, feces is considered as an important source of raw milk contamination by *L. monocytogenes* or using these fecal samples as fertilizers which facilitate contamination of vegetables, plants and crops with *L. monocytogenes* and creating a hazard on dairy cows as well as milk consumers [4].

In this study, *Listeria* spp. was isolated from stool of pregnant women with prevalence of 8%. *L. monocytogenes* was isolated from stool of pregnant women with a percentage of 4% (2 out of 50).

Lower percentage (2%) was detected in stool of pregnant women in Scotland [53]. In Egypt, Abuhatab [45] recovered *Listeria* spp. in 11.1% from feces of aborted women.

Other studies revealed that isolation rate of *Listeria* spp. was 7.14 % from stool of children [54] in Assiut city**.** *L. monocytogenes* was recovered from stool samples of dairy handlers with a percentage of 5 in Mansoura city [55]. In India, fecal samples, vaginal swabs, placental bits, blood and urine samples of aborted women showed isolation rate (3.3%) of *L. monocytogenes* by Kaur *et al*. [8].

The higher prevalence of *L. monocytogenes* in this study could be attributed to decline in cell-mediated immunity in pregnant women which is the most predisposing factor for *L. monocytogenes* infection as previously advocated by Pouillot *et al.* [56].

In this study, 15 isolates of *L. monocytogenes* from different sources were molecularly identified to the species level using PCR amplification of *16S rRNA* gene. Similarly *L. monocytogenes* from different raw milk samples based on *16S rRNA* gene was recorded in Egypt [39].

In this study, *inl*A and *inl*B were reported in 80% and 40% of isolated *L. monocytogenes*, respectively. In previous studies, Khedr *et al.* [39] detected *inl*A and *inl*B genes in 100% and 75% of examined *L. monocytogenes* isolates, respectively. However, Coroneo *et al.* [57] recorded *inl*A and *inl*B genes in 60% and 47% of *L. monocytogenes* strains recovered from Ricotta salata cheese, respectively.

The differences in the distribution of virulence internalin genes could be attributed to the source of samples or *L. monocytogenes* serotype or mutations in these genes resulted in reduction in invasion or virulence in animal model studies or tissue culture, assuring the significant role of such genes in the pathogenesis and virulence of *L. monocytogenes* [57-59]. It was noticed that 12 isolates of *L. monocytogenes* carry *inl*A gene and 6 isolates carry *inl*B gene that indicates virulent strains of *L. monocytogenes* and creates hazard in dairy farms as well as milk handlers, milk consumers and pregnant women.

Conclusion

Prevalence of *L. monocytogenes* in raw milk could constitute a public health hazard for milk handlers, workers in dairy farms and milk consumers. The molecular detection of *inl*A and *inl*B in isolates of *L. monocytogenes* are considered as virulent determinants for such zoonotic pathogen. Therefore, to control the risk of milk and dairy products contamination with *L. monocytogenes*, it is important to emphasize on the hygiene measures in dairy farms in addition to screening of *Listeria* spp.in dairy farms, milk of dairy cows, milk handlers.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments:

Staff members of Biotechnology Unit, Reference laboratory for Veterinary Quality Control on Poultry Production, Animal Research Institute, Doki, Giza, Egypt.

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الملخص العربي

انتشار انواع الليستريا في الابقار الحلوب والنساء الحوامل مع الاشاره الي تصنيف الانواع ذات الضراوة من الليستريا مونوسيتوجينز في مصر

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أجريت هذه الدراسة للتعرف علي نسب العزل لانواع من ميكروب الليستيريا المعزولة من اللبن الطبيعي ولبن التهاب الضرع وروث الأبقار الحلوب وبراز النساء الحوامل. ثلاثمائة وخمسون عينة اشتملت علي ٢٠٠ عينه من اللبن الطبيعي,٥٠عينه من لبن التهاب الضرع,٥٠ عينه من روث الابقار الحلوب و ٥٠ عينه من براز النساء الحوامل من محافظتي الشرقية والدقهلية - مصر. انواع من الليستيريا تم تنميتهاعلى أكسفور داجار وتم إجراء التصنيف البايو كيميائي لها. كان معدل العزل الكلي لأنواع الليستريا ١٦٪ . معدل تواجد الليستريا مونوسيتوجينز كان ٨٪ من روث الابقار الحلوب ,٤٪ من لبن الابقار الحلوب ,٢٪ من لبن التهاب الضرع و٤٪ من برازالنساء الحوامل. كانت نسبة انتشار الليستريا ايفانوفي والليستريا ويلشيميري في لبن الابقارالحلوب ٦٪ و٤٪على التوالي. تم تاكيد عزلات الليستريا المونوسيتوجينز باستخدام التصنيف الجزيئى لجين*16SrRNA*.ثم تم دراسه نسب توزيع جينات *inl*B و*inl*A في عدد ١٥ عزله من الليستريا مونوسيتوجينيز. تم تحديد المعدل الكلي للجينات (*inl*A و*inl*B) بنسب ٨٠٪ و٤٠٪ من عزلات الليستريا مونوسيتوجينيز المعزوله من مصادر مختلفه علي التوالي. عزلتان من الليستيريا مونوسيتوجينيزالمعزوله من اللبن وروث الابقار الحلوب لا تمتلك كل من *inl*A and *inl*B. تحت ظروف الدراسه الحاليه نجد ان انواع من الليستريا تنتشرفي اللبن وروث الابقار ولبن التهاب الضرع وبراز النساء الحوامل,والتي تسبب امراض للإنسان واجهاض للنساء الحوامل .تعتبرالجينات Internalin (A & B) من افضل محددات الضراوه لليستريا مونوسيتوجينز المعزوله من مصادر مختلفه.