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VIRULENCE GENES OF *LISTERIA MONOCYTOGENES* ISOLATED FROM SOME READY-TO-EAT CHICKEN MEALS

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Received: 30 September 2018; **Accepted:** 28 October 2018

ABSTRACT

This study was conducted in Assiut, Egypt, to investigate the prevalence of *Listeria monocytogenes* in a total of 75 ready-to-eat (RTE) cooked chicken meals collected from different restaurants. All isolates were further examined for the virulence marker gene and antibiotic resistance genes. *L. monocytogenes* were isolated from 4(5.3%) of the samples analyzed, including 2(8%) of chicken shawerma, 1(4%) of chicken burger and 1(4%) of chicken breast fillet. All the recovered *L. monocytogenes* organisms were confirmed by PCR assay for the presence of *16S rRNA* gene and all of the tested isolates harboured this gene, among which 100% were revealed to incode *inlA* and *inlB* virulence genes. Whereas, all four (100%) isolates of *L. monocytogenes* were found to harbor *mefA* gene (macrolides resistance gene) and *Aad6* gene (aminoglycosides resistance gene). While, *Kan* gene (Kanamycin resistance gene), *tetM* gene (tetracycline resistance gene) and *Cat* gene (chloramphenicol resistance gene) couldn't be detected in any examined strains. These results signify the importance of sustained surveillance of *L. monocytogenes* in cooked chicken meat to minimize the risk of contamination and protecting consumers against outbreaks.

Key words: *L. monocytogenes*, virulence genes, RTE cooked chicken meals.

INTRODUCTION

Listeria monocytogenes has been recognized as an important opportunistic human pathogen since 1929 and as food borne pathogen since 1981 (Jeyasekaran *et al*., 1996). Ready-to-eat (RTE) meat products represent high risk to the consumers because they are usually cooked during manufacture and consumed without further heating, so cross contamination with food borne pathogens during the processing cannot be overcome (Goulet *et al*., 2008). The extended distribution throughout the food processing environment and asymptomatic human carriers (Wagner *et al*., 2005) and the psychrotrophic character of Listeria species appear to be the main causes of the prevalence in different kinds of refrigerated RTE meat products and contamination could occur either pre- or post-processing (Lianou and Sofos, 2007). Of the 20 RTE food categories evaluated by the Food and Drug Administration and the Food Safety and Inspection Service, deli meats were classified in the very high risk category to be the principal potential source of *L. monocytogenes* (FAO/WHO, 2004). In general, consumption of food contaminated with *L. monocytogenes* may cause listeriosis which may result in serious human illness with symptoms of septicemia, meningitis, encephalitis and gastroenteritis particularly in children, the elderly and immunosuppressed individuals. It may also cause miscarriage in pregnant women (Blum-Menezes *et al*., 2013). *L. monocytogenes* had the second highest fatality rate (20%) and the highest hospitalization rate (90%) in virulence (Swaminathan, 2001). Multistate outbreaks of food borne listeriosis were recorded (Gottlieb *et al*., 2006).

In study conducted by Gusman *et al.* (2014), the prevalence of *L. monocytogenes* in examined samples of RTE foods was 1.97%, and the count of *L. monocytogenes* in all positive samples exceeded the limit of 100 colony forming units (CFUs) per gram. According to the data reported by the (EFSA) European Food Safety Authority (2015), prevalence rate of *L. monocytogenes* in RTE foods was 4.4%.

Multiple key virulence factors such as internalin (inlA), listeriolysin (hlyA), phosphatidylinositol phospholipase C (plcA), actin polymerization protein (actA) and invasive associated protein (iap) are important in *L. monocytogenes* pathogenesis (Furrer *et al.*, 1991 and Portnoy *et al.*, 1992). Therefore, detection of just one virulence associated gene by PCR is not always sufficient to identify *L. monocytogenes* (Nishibori *et al.*, 1995). In addition, it is plausible that some *L. monocytogenes* strain may lack one or more virulence determinants because of spontaneous mutations (Cooray *et al.*, 1994).

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L. monocytogenes is usually susceptible to a wide range of antibiotics, but in 1988 a multidrug-resistant strain was found in France (Poyart-Salmeron, 1990). Since then other strains resistant to one or more antibiotics have been recovered from food, the environment and from sporadic cases of human listeriosis (Conter *et al.,* 2009). The occurrence of antibiotic resistance complicates therapy and lengthens convalescence from illness. Antibiotic use in clinical medicine (appropriate and otherwise) has contributed to the emergence of multidrug-resistant strains, but another contributor has been the use of antibiotics in animal feed as growth promoters (Harakeh *et al.,* 2009). In 2013, based on the proposals issued by the European Food Safety Authority, the EU put forward and discussed with the member states a new legislation on the harmonized monitoring of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator bacteria in food-producing animals and food (EFSA, 2015), but there are relatively few epidemiological studies, and thus, only limited information on antibiotic resistance prevalence and spread concerning *Listeria* spp. Considering the high mortality rate of listeriosis in vulnerable populations, it is important to insure the effectiveness of antimicrobials and monitor the emergence of antimicrobial-resistant *Listeria* strains (Gómez *et al.,* 2014).

The increase of RTE food consumption due to changes in the lifestyle and the ability of *L. monocytogenes* to attach to different surfaces forming biofilms and consequently its persistence in food environment necessitate periodically repeated surveys for determining the prevalence and the distribution of some virulence genes in *L. monocytogenes* isolated from RTE food and to evaluate the resistance genotype of isolated *L. monocytogenes* strains to selected antibiotics used for treating listeriosis.

MATERIALS AND METHODS

Collection of samples:
A total of 75samples of cooked RTE chicken meat (25 for each shawerma, burger and breast fillet) were collected during the period from October to December 2017 from different restaurants in Assiut province. The samples were collected hygienically in sterile plastic bags and transported to the laboratory in icebox within 2 to 4 h.

Isolation and identification of *L. monocytogenes*:
Listeria monocytogenes was isolated from RTE chicken meat samples following the procedure recommended by the International Organization for Standardization (ISO11290 -1, 2017). Briefly, a 25 g meat sample was aseptically homogenized in 225 ml pre-enrichment half-Fraser broth (CM0895, Oxoid Ltd) supplemented with half-Fraser supplement (SR0166E, Oxoid Ltd) in Stomacher bags (Seward Ltd, West Sussex, UK) for 30 s using a Stomacher circulator (Easy Mix, AES Laboratoire, Bruz, France), followed by incubation at 30ºC for 24 h. Then 0.1 ml half-Fraser broth was added to 10 ml Fraser broth containing Fraser supplement and incubated at 37ºC for 48 h. At the end of incubation, a loopful of Fraser broth was streaked on chromogenic Listeria agar (ALOA) supplemented with Brilliance Listeria Differential Supplement (SR0228E, Oxoid Ltd) and incubated at 35ºC for 24 to 48 h. *L. monocytogenes* appear as green–blue colonies surrounded by an opaque halo. For biochemical identification of *L. monocytogenes*, five suspect colonies from each plate were streaked on TSA (M290, Oxoid Ltd) supplemented by (0.6%) yeast extract (LP0021) and incubated at 37ºC for 18–24 h.

Biochemical confirmation of *L. monocytogenes*:
Suspected colonies were verified by Gram staining, catalase, oxidase, haemolysis and CAMP tests, motility, Methyl Red-Voges Proskauer (MR-VP) reactions, nitrate reduction and the production of acids from rhamnose, xylose and mannitol for the identification as described by ISO11290 -1 (2017).

PCR assay for identification of *16S rRNA*, virulence genes and resistance genes of *L. monocytogenes*:-
The isolated *L. monocytogenes* strains were sent to the Reference laboratory for veterinary Quality Control of poultry production in Animal Health Research Institute, Dokki, Giza, Egypt, for identification of *16S rRNA*, virulence genes and resistance genes of *L. monocytogenes* as follow:

DNA extraction:
DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 20 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer:
Primers used were supplied from Metabion (Germany) are listed in table (1).

PCR amplification:
Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products:
The products of PCR were separated by electrophoresis on 1% agarose gel (Applichem,

Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. Gelpilot100 bp and 100 bp plus Ladders (Qiagen, Germany, GmbH) and generuler 100 bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table 1: Primers sequences, target genes, amplicon sizes and cycling conditions used in PCR assayes for *L. monocytogenes*.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
16S rRNA	ggACCgggg CTA ATA CCg AAT gAT AA TTC ATgTAggCgAgTTgC AgC CTA	1200	94˚C 5 min.	94˚C 30 sec.	60˚C 50 sec.	72˚C 1 min.	72˚C 12 min.	Kumar *et al.*, 2015
plcA	ACA AGC TGC ACC TGT TGC AG TGA CAG CGT GTG TAG TAG CA	1484	94˚C 5 min.	94˚C 30 sec.	60˚C 50 sec.	72˚C 1 min.	72˚C 12 min.	Soni *et al.*, 2014
iap	CTG CTT GAG CGT TCA TGT CTC ATC CCC C CAT GGG TTT CAC TCT CCT TCT AC	131	94˚C 5 min.	94˚C 30 sec.	60˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	
prfA	TCT-CCG-AGC-AAC-CTC-GGA-ACC TGG-ATT-GAC-AAA-ATG-GAA-CA	1052	94˚C 5 min.	94˚C 30 sec.	50˚C 50 sec.	72˚C 1 min.	72˚C 10 min.	Dickinson *et al*., 1995
inlA	ACG AGT AAC GGG ACA AAT GC CCC GAC AGT GGT GCT AGA TT	800	94˚C 5 min.	94˚C 30 sec.	55˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	Liu *et al.*, 2007
inlB	CTGGAAAGTTTGT ATTTGGGAAA TTTCATAATCGCC ATCATCACT	343	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	
hly	GCA-TCT-GCA-TTC-AAT-AAA-GA TGT-CAC-TGC-ATC-TCC-GTG-GT	174	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	Deneer and Boychuk, 1991
Aad6	AGAAGATGTAAT AATATAG CTGTAATCACTGT TCCCGCCT	978	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	Morvan *et al*., 2010
Cat	GAACAGGAATTA ATAGTGAG GGTAACCATCACA TAC	384	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	
mefA	AGTATCATTAATC ACTAGTGC TTCTTCTGGTACT AAAAGTGG	345	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	
tetM	GTGGACAAAGGT ACAACGAG CGGTAAAGTTCGT CACACAC	405	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	
Kan	GTGTTTATGGCTC TCTTGGTC CCGTGTCGTTCTG TCCACTCC	621	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	Frana *et al*., 2001

RESULTS

Table 2: Isolation rate of *Listeria monocytogenes* from some ready-to-eat chicken samples.

Type of samples	No. of examined samples	Positive samples	
		No.	%
Chicken Shawerma	25	2	8
Chicken Burger	25	1	4
Chicken breast fillet	25	1	4
Total	75	4	5.3

Table 3: PCR results of the specific gene and different virulence genes of isolated *Listeria monocytogenes*

No. of isolated *listeria monocytogenes*	*listeria monocytogenes* specific gene *16S rRNA* gene	*listeria monocytogenes* virulence genes					
		inlA	*inlB*	*hly*	*iap*	*plcA*	*prfA*
1	+	+	+	-	-	-	-
2	+	+	+	-	-	-	-
3	+	+	+	-	-	-	-
4	+	+	+	-	-	-	-
Positive %	**100**	**100**	**100**	**0**	**0**	**0**	**0**

inlA gene (internalin A gene)
inlB gene (internalin B gene)
hly gene (listeriolysin O gene)
iap gene (invasion- associated protein)
plcA gene (Phospholipase gene)
prfA gene (Pleiotropic regulatory factor)

Table 4: PCR results of resistance genes of isolated *Listeria monocytogenes*

No. of isolated *listeria monocytogenes*	*listeria monocytogenes* resistance genes				
	mefA	*Kan*	*Aad6*	*tetM*	*Cat*
1	+	-	+	-	-
2	+	-	+	-	-
3	+	-	+	-	-
4	+	-	+	-	-
Positive %	**100**	**0**	**100**	**0**	**0**

mefA gene (macrolides resistance gene)
Kan gene (Kanamycin resistance gene)
Aad6 gene (aminoglycosides resistance gene)
tetM gene (tetracycline resistance gene)
Cat gene (chloramphenicol resistance gene)

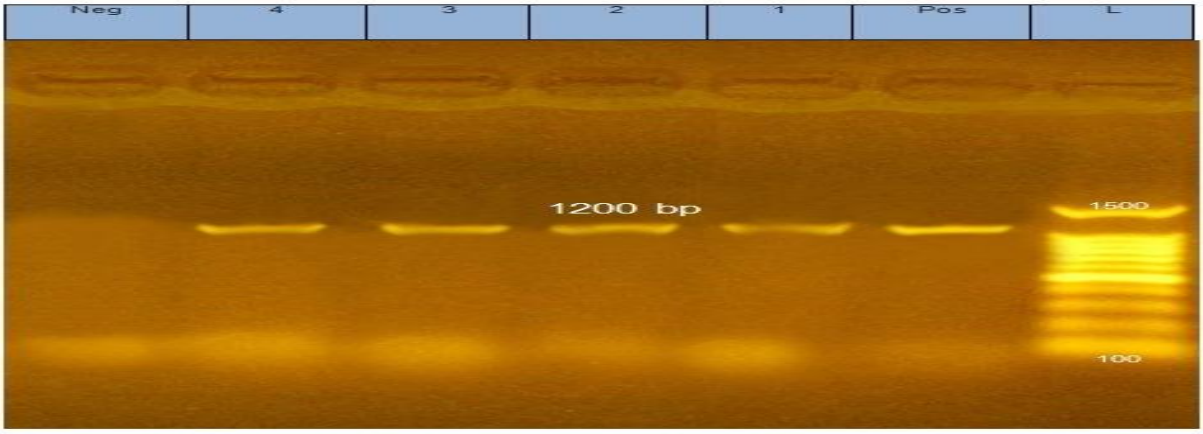

Figure 1: Agarose gel electrophoresis of PCR of *16S rRNA* gene (1200bp) in isolated *Listeria monocytogenes.*
Lane L : 100 -1500bp ladder as molecular size DNA marker.
Lane Pos: Control positive *Listeria monocytogenes* for *16S rRNA* gene.
Lane Neg.: Control negative *Listeria monocytogenes* for *16S rRNA* gene.
Lanes: 1—4 are positive *Listeria monocytogenes* for *16S rRNA* gene.

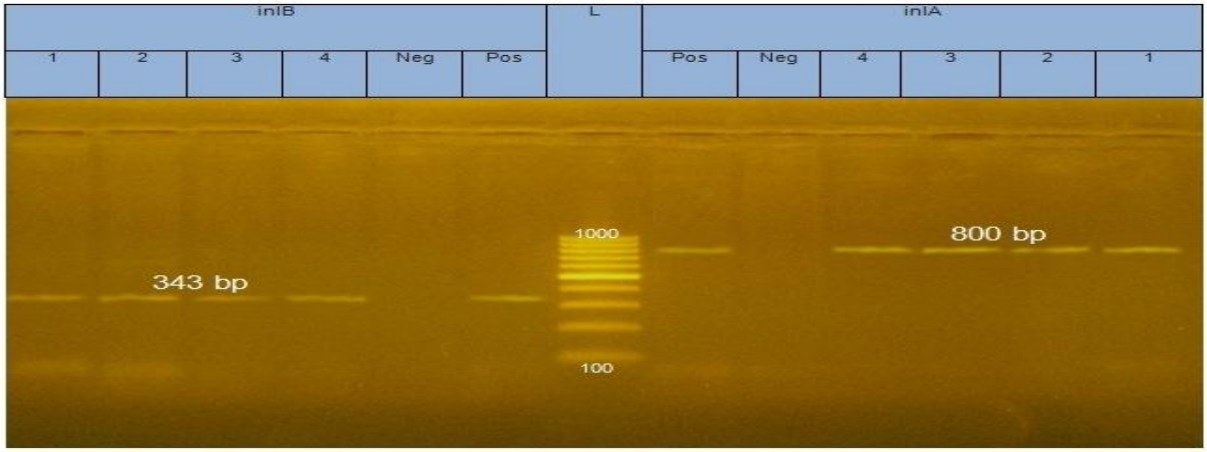

Figure 2: Agarose gel electrophoresis of PCR of *inlB* gene (343bp) and *inlA gene* (800bp) in isolated *Listeria monocytogenes.*
Lane L : 100 -1000bp ladder as molecular size DNA marker.
Lane Pos: Control positive *Listeria monocytogenes* for *inlB* gene and *inlA gene.*
Lane Neg.: Control negative *Listeria monocytogenes* for *inlB* gene and *inlA gene.*
Lanes: 1—4 are positive *Listeria monocytogenes* for *inlB* gene and *inlA gene.*

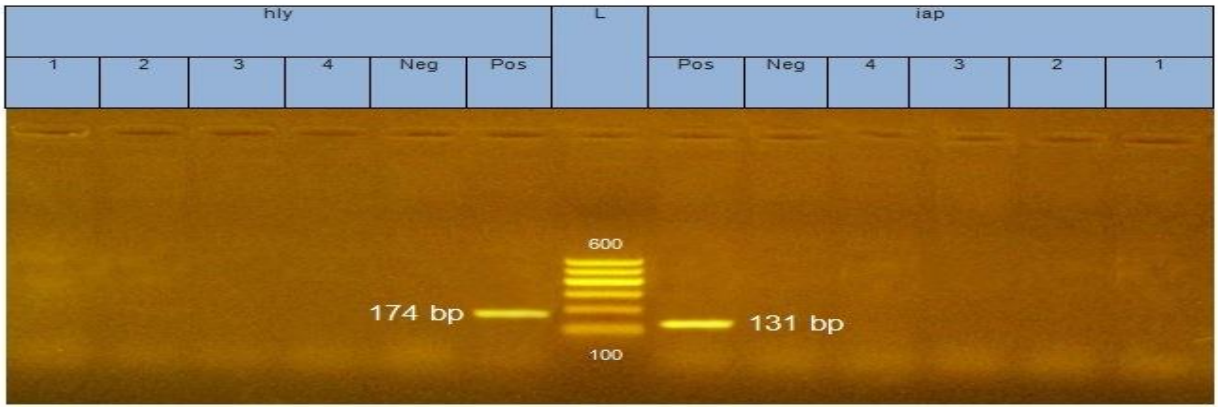

Figure 3: Agarose gel electrophoresis of PCR of *hly* gene (174bp) and *iap gene* (131bp) in isolated *Listeria monocytogenes.*
Lane L : 100 -600bp ladder as molecular size DNA marker.
Lane Pos: Control positive *Listeria monocytogenes* for *hly* gene and *iap gene.*
Lane Neg.: Control negative *Listeria monocytogenes* for *hly* gene and *iap gene*
Lanes: 1—4 are negative *Listeria monocytogenes* for *hly* gene and *iap gene.*

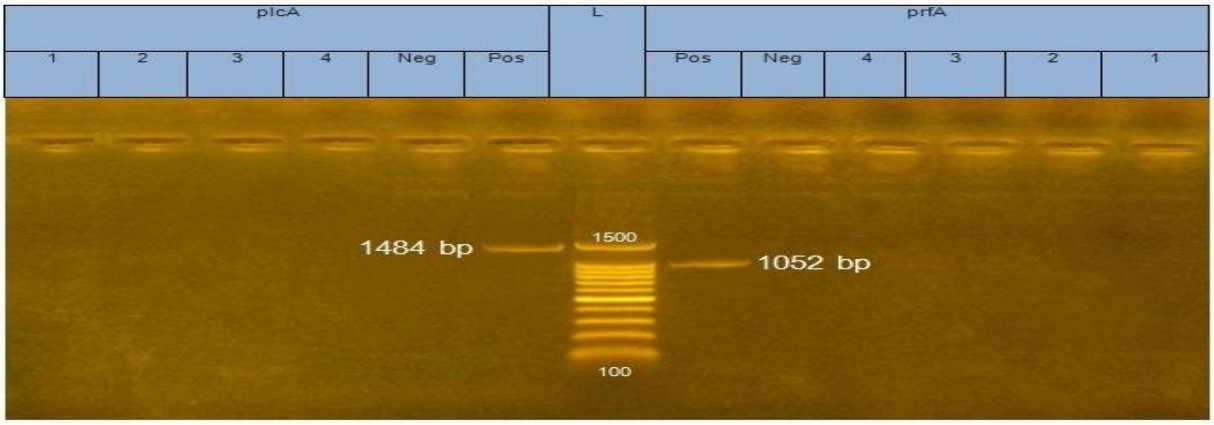

Figure 4: Agarose gel electrophoresis of PCR of *plcA* gene (1484bp) and *prfA gene* (1052bp) in isolated *Listeria monocytogenes*.
Lane L : 100 -1500bp ladder as molecular size DNA marker.
Lane Pos: Control positive *Listeria monocytogenes* for *plcA* gene and *prfA gene.*
Lane Neg.: Control negative *Listeria monocytogenes* for *plcA* gene and *prfA gene.*
Lanes: 1—4 are negative *Listeria monocytogenes* for *plcA* gene and *prfA gene.*

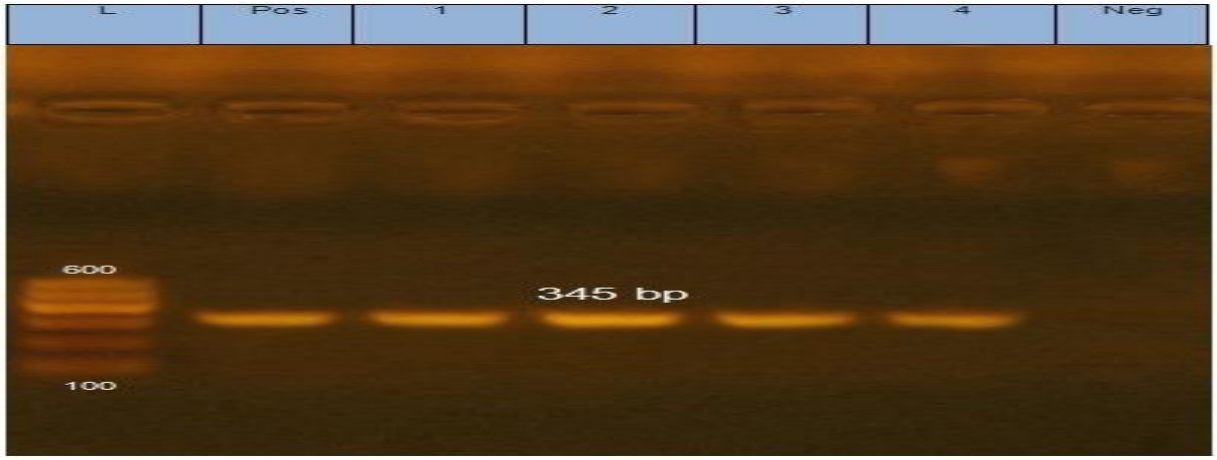

Figure 5: Agarose gel electrophoresis of PCR of *mefA* gene (345bp) in isolated *Listeria monocytogenes*.
Lane L : 100 -600bp ladder as molecular size DNA marker.
Lane Pos: Control positive *Listeria monocytogenes* for *mefA* gene.
Lane Neg.: Control negative *Listeria monocytogenes* for *mefA* gene.
Lanes: 1—4 are positive *Listeria monocytogenes* for *mefA* gene.

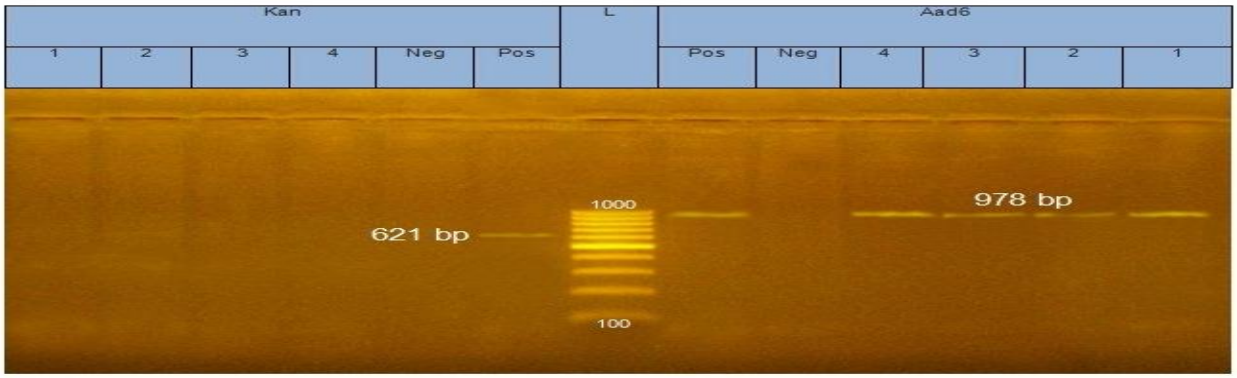

Figure 6: Agarose gel electrophoresis of PCR of *Kan* gene (621bp) and *Aad6gene* (978bp) in isolated *Listeria monocytogenes*.
Lane L : 100 -1000bp ladder as molecular size DNA marker.
Lane Pos: Control positive *Listeria monocytogenes* for *Kan* gene and *Aad6 gene.*
Lane Neg.: Control negative *Listeria monocytogenes* for *Kan* gene and *Aad6 gene.*
Lanes: Left 1—4 are negative *Listeria monocytogenes* for *Kan* gene & Right 1-4 are positive *Listeria monocytogenes* for *Aad6 gene.*

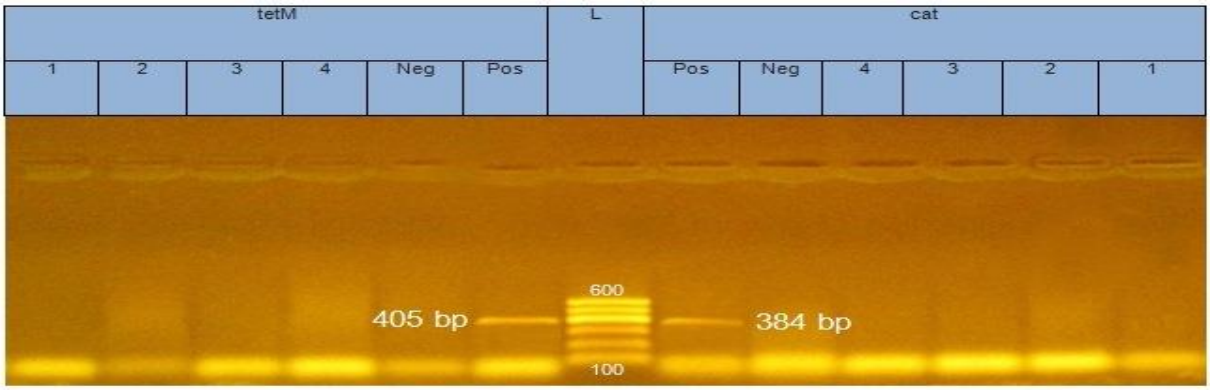

Figure 7: Agarose gel electrophoresis of PCR of *tetM* gene (405bp) and *Cat gene* (384bp) in isolated *Listeria monocytogenes.*

Lane L : 100 -600bp ladder as molecular size DNA marker.
Lane Pos: Control positive *Listeria monocytogenes* for *tetM* gene and *Cat gene.*
Lane Neg.: Control negative *Listeria monocytogenes* for *tetM* gene and *Cat gene.*
Lanes: 1—4 are negative *Listeria monocytogenes* for *tetM* gene and *Cat gene.*

DISCUSSION

L. monocytogenes has been recognized as one of the most serious emerging bacterial diseases during the last two decades that is transmitted through the consumption of contaminated foods (Nørrung, 2000). Results of the current study revealed that from the total of 75 ready-to-eat chicken samples 4 (5.3%) were found to be contaminated with *L. monocytogenes* (Table 2). Our findings are consistent with the results obtained by Cabedo *et al.* (2008) who found *L. monocytogenes* in ready-to-eat foods at a range of 6.2% to 20.0%, depending on the food products.

Interestingly, chicken shawerma samples showed higher isolation rates for *L. monocytogenes* (8%). It is noteworthy that the high isolation rate of *L. monocytogenes* from chicken shawerma underscored the potential role that may be played by this product to convey *L. monocytogenes* to the human gut. However, a lower rate of contamination was obtained by Abd-El-Malek (2017) who detected *L. monocytogenes* in 1(2.9%) of chicken shawerma collected from different restaurants in Assiut city. Comparatively, Alsheikh *et al.* (2013) recorded slightly lower isolation rate (4%) of *L. monocytogenes* in chicken shawerma samples purchased from restaurants in Khartoum state Sudan. Also, *L. monocytogenes* was isolated from 12 (4%) of 301 chicken shawerma samples in Jordan (Osaili *et al.*, 2014). On the other hand, higher records were reported by several investigators as Moustafa El-Shenawy *et al.* (2011) who found *L. monocytogenes* in 3 (12.5%) of 24 samples of street-vended RTE shawerma in Alexandria city. Moreover, In Amman, Jordan, Osaili *et al.* (2011) isolated *L.monocytogenes* from shawerma with percent 13.3% of samples. The contamination by *L. monocytogenes* in RTE meat primarily occurs during slicing and packaging after cooking. In addition, cross-contamination between raw materials, equipments, utensils, humans could contribute to the spread of *L. monocytogenes* in food processing plants (Jemmi and Stephen, 2006).

Additionally, one chicken burger sample yielded *L. monocytogenes* (4%). The contamination of RTE foods could be due to many factors. One of the possible factors is via cross-contamination after the foods were cooked. We could not rule out the possibility that the cooking process is not sufficient to inactive these tough bacteria. In a study done by Wong *et al.* (2011), *L. monocytogenes* was not detected after six minutes of cooking chicken burger patties, but it was detected after four minutes of cooking. Therefore, efficient cooking of burgers is very important to prevent food-borne illness from burgers that may be contaminated with *L. monocytogenes*.

With regard to RTE cooked chicken breast fillet, it is evident from the data presented in Tables 2 that one sample with an incidence of 4% were contaminated with *L. monocytogenes*. In contrast, higher prevalence rate (24%) was recorded by Abd-El-Malek (2017). On the contrary, in a related study performed by Diaz-Lopez *et al.* (2011), the presence of *L. monocytogenes* from grilled chicken was not detected by culture or PCR.

The detection of *L. monocytogenes* by molecular methods is very specific. 4 *L. monocytogenes* strains isolated from RTE cooked chicken samples by conventional methods were similarly confirmed as *L. monocytogenes* by PCR (Table 3) &Figure (1).

Results illustrated in Table 3 and Figure 2 revealed that all four (100%) isolates of *L. monocytogenes*

were found to harbor *InlA* and *inlB* genes. *InlA* and *inlB* genes are associated with internalization of *L. monocytogenes* into the host cells (Bierne and Cossart, 2002; Orsi *et al*., 2007). The presence of internalin genes (*inlA* and *inlB*) in *our L. monocytogenes* isolates indicates the potential health hazard should such contaminated RTE foods were consumed by immune-compromised individuals. *hlyA* gene was not detected in all *L. monocytogenes* isolates in the current study. The absence of *hlyA* may be explained by the occurrence of a specific evolutionary event that resulted in alteration of the profile of genes responsible for pathogenesis. A similar observation was made by Ndahi *et al.* (2014), in which of 12 *L. monocytogenes* isolates from raw and processed meat products, only one harbored the *hlyA* gene. Also *iap* gene, *plcA* gene and *prfA* gene were not detected in all *L. monocytogenes* isolates in the current study.

The data outlined in Table 4, Figure 5, Figure 6 and Figure 7 illustrated that all four (100%) isolates of *L. monocytogenes* were found to harbor *mefA* gene (macrolides resistance gene) and *Aad6* gene (aminoglycosides resistance gene). While, *Kan* gene (Kanamycin resistance gene), *tetM* gene (tetracycline resistance gene) and *Cat* gene (chloramphenicol resistance gene) couldn't detected in any examined strains. Regarding to the result of *tetM* gene which cannot be detected in the present study was disagreed the results reported by Charpentier and Courvalin, (1999), Bertsch *et al*. (2014) and Escolar *et al*.(2017) who found this gene in 100% of tetracycline- resistant strains. On the other hand our results was agreed the results that was recorded by Terzi *et al*. (2015) who cited that Listeria Species have no resistance to Tetracycline phenotypically. Erythromycin is a member of Macrolides antibiotic groups, Macrolide resistance gene (*mefA* gene) which detected in the present study also detected by Granier *et al*. (2011) who found two genes for resistance for Erythromycin *erm(B*) and *erm(C)* in food and the environment in France. Moreover, this result was coincides with Anas *et al*. (2015) who reported that presence of resistance to erythromycin against *Listeria monocytogenes* isolated from raw and processed meat products phenotypically in Jordan.

Listeriosis in humans is frequently transmitted via food products; consequently antibiotic-resistant *L. monocytogenes* isolates can have important public health consequences, especially in developing countries where there is widespread and often uncontrolled use of antibiotics. Since antibiotic resistance in *L. monocytogenes* is mainly due to acquisition of mobile elements such as plasmids and conjugative transposons (Charpentier and Courvalin, 1999), it is realistic to anticipate increased observations of bacterial antibiotic resistance in the future.

CONCLUSION

This study represents the presence of potentially pathogenic *L. monocytogenes* in various RTE cooked chicken meat purchased from different restaurants in Assiut province, by the PCR technique using primers targeting six virulence genes. Among the genes detected by the primers used, internalin genes (*inlA* and *inlB*) was most frequently found in isolates. All *L. monocytogenes* isolates were found to harbor *mefA* gene (macrolides resistance gene) and *Aad6* gene (aminoglycosides resistance gene). The data from this study should serve as motivation to develop guidelines in order to ensure the overall safety of raw and processed chicken meat products.

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جينات الضراوة لميكروب الليستيريا مونوسيتوجينز المعزول من بعض وجبات الدجاج الجاهزه للاستهلاك

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أجريت هذه الدراسة على ٧٥ عينه من وجبات الدجاج الجاهزه للاكل بواقع خمس وعشرين عينة من كل من (شاورمه- برجر- بانيه) الدجاج والمجمعة من بعض المطاعم في مدينة أسيوط وذلك لمعرفة مدى تواجد ميكروب الليستيريا مونوسيتوجينز فى هذه العينات ، والكشف على جينات الضراوه فى العترات المعزولة . وقد أسفرت النتائج عن تواجد ميكروب الليستيريا مونوسيتوجينز بنسبة ٨% , ٤% و٤% فى عينات شاورمه الدجاج , برجر الدجاج وبانيه الدجاج على التوالى. وقد تم تأكيد عترات الليستيريا مونوسيتوجينزالمعزوله بإستخدام تقنية تفاعل إنزيم البلمرة المتسلسل للكشف عن وجود جين *16S rRNA* وتبين وجوده فى جميع العترات المعزوله فى حين انه تم الكشف عن وجود جينات *inlA* و***i****nlB* فى جميع العترات المعزوله. بينما تم وجود جينات *mefA* (macrolides resistance) و*Aad6*(aminoglycosides resistance) فى جميع العترات المعزوله. وأوصت الدراسة بضرورة تطبيق الاشتراطات الصحية الجيده أثناء عمليات اعداد وتجهيز وجبات الدجاج لتقليل تلوثها بميكروب الليستيريا مونوسيتوجينز.