



Prevalence and molecular studies on *Listeria monocytogenes* isolated from chickens in El-Gharbia Governorate

Ashraf A. Abd El-Tawab¹; Seham N. Homouda²; Shimaa E. El-Haw³

¹Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Benha University, 13736 Moshtohar, Benha, Egypt; ²Animal Health Research Institute "Tanta branch"; ³ Veterinarian.

ABSTRACT

Listeria monocytogenes is the causative agent of listeriosis and food-borne disease that can lead to meningitis and bacteremia. *L. monocytogenes* is the most pathogenic species in the genus *Listeria*. A total of 400 random samples of muscles, liver, spleen and kidneys (100 samples each) were taken from 100 diseased chickens. All samples were obtained from different poultry farms and markets in El-Gharbia Governorate to estimate the prevalence of *Listeria monocytogenes* in chicken. The bacteriological examination of the samples resulted; 53(13.25%) isolates as 11, 21, 8 and 13 from muscles, liver, spleen and kidneys respectively. The antimicrobial sensitivity test showed that the isolated *L. monocytogenes* were sensitive to sulfamethoxate-trimethoprim (91.6%) followed by ampicillin, gentamycin, vancomycin and chloromphenicol (83.3%) followed by ciprofloxacin (66.6%) followed by erythromycin and tetracycline (50.0%), while the isolated strains were completely resistant to cephalothin. All *L. monocytogenes* strains were virulent where all of them were positive to CAMP test and Anton's test. The PCR results for six studied strains of isolated *L. monocytogenes* showed that all genes (*16S rRNA*; *inlA*; *prfA* and *hlyA*) were detected (100.0%) while *plcA* gene was not detected.

Keywords: *Listeria monocytogenes*, chicken, PCR, virulence genes.

(BVMJ, Vol. 34, No. 1:430 - 442, 2018)

1. INTRODUCTION

Listeria monocytogenes is the causative agent of listeriosis, a food-borne disease that can lead to meningitis and bacteremia (Mackiw *et al.*, 2016).

Listeria spp. are considered as an important cause of zoonoses infecting many types of animals such as domestic pets, avian species, rodents, livestock, fish, amphibians and arthropods. The approximate fatality rate is 30% that may

increase up to 75% in high risk groups, such as neonates, pregnant women and immune-compromised adults (Jalali and Abedi, 2007).

The genus *Listeria* presently composed of 17 different species: *L. monocytogenes*, *L. seeligeri*, *L. ivanovii*, *L. welshimeri*, *L. marthii*, *L. innocua*, *L. grayi*, *L. fleischmannii*, *L. floridensis*, *L. aquatica*, *L. newyorkensis*, *L. cornellensis*, *L. rocourtiae*, *L. weihenstephanensis*, *L.*

grandensis, *L. riparia* and *L. booriae* (Orsi and Wiedmann, 2016).

Morphologically, *L. monocytogenes* is a gram-positive small, ranging from 1 to 2 µm in length and 0.5 µm in diameter, rod-like shape bacteria with rounded ends. Cells are usually in single units but can be clustered in short chains (3-5 or more) arranged in a V or Y disposition as well as in palisades. It is considered motile showing tumbling motility due to the production of peritrichous flagella when grown below 30 °C, due to flagellin being produced and assembled in flagella at cell surface. If grown at 37 °C, flagellin production is reduced to residual (Ryser and Marth, 2007).

L. monocytogenes are classified into 13 different serotypes based on somatic (O) and flagellar (H) antigens. 1/2a, 1/2b, 1/2c, and 4b are the four main pathogenic serotypes from which 1/2a, 1/2b and 4b are responsible for 98% of human Listeriosis (Jacquet *et al.*, 2002).

L. monocytogenes usually shed in all the secretions and excretions of the infected birds. Disease is transmitted through ingestion of contaminated feed, water, litter and soil. Infection can also follow inhalation or wound contamination. In birds, generally, the infection is subclinical and incubation period is not reported (Kurazono *et al.*, 2003).

Signs of Listeriosis infection in birds, if seen, are suggestive of septicemia and may include depression and listlessness, emaciation, diarrhea and per acute/sudden death can occur at times (Akanbi *et al.*, 2008).

Listeria is sensitive to a wide range of antibiotics: ampicillin, amoxicillin, tetracycline, chloramphenicol, -lactam antibiotics together with an aminoglycoside, trimethoprim and sulphamethoxazole are recommended (Altuntas *et al.*, 2012).

Further identification of the pathogenic *L. monocytogenes* a set of genes are responsible for the virulence activity of *L. monocytogenes* must be detected (Shen *et al.*, 2013). The virulence genes of *L. monocytogenes* are *inlA*, *inlB*, *hlyA*, *prfA*, *plcA*, *plcB*, *mpl* and *actA* (Karthikeyan *et al.*, 2015). *inlA* is a gene that responsible for the survival and invasion of *L. monocytogenes* of intestinal epithelium (Liu *et al.* 2007) While *inlB* responsible for hepatocyte colonization (Kirkan *et al.*, 2005) and the well-recognized virulence gene is *hlyA* which encodes the Listeriolysin O (Gouws and liedemann 2005) and finally the regulatory gene for those virulent genes *prfA* which encodes the promoter protein for *inlA*, *inlB* and *hlyA* (Scorti *et al.*, 2007).

2. MATERIAL AND METHODS

2.1. Collection of the samples:

A total of 400 random samples of muscles, liver, spleen and kidneys (100 samples each) were taken from 100 diseased chickens during November 2016 to April 2017. All samples were obtained from different poultry farms and markets in El-Gharbia Governorate. The examined samples were collected separately under hygienic measure as possible and transferred directly in ice box to the laboratory (Animal health research laboratory in Tanta) for bacteriological examination.

Prevalence and molecular studies on *Listeria monocytogenes*
isolated from chickens in El-Gharbia Governorate

2.2. *Bacteriological examination:*

Listeria monocytogenes was isolated from the examined samples according to 11290-1:1996/Amd.1:2004 (ISO, 2005).

Approximately 25 grams of samples were added to 225 ml half-Fraser broth (Oxoid) in a 500ml flask and mixed well by shaking. The enrichment broth was incubated at 30°C for 24 hours. Then, 1ml from the half-Fraser broth was transferred into 9 ml of Fraser broth (Oxoid) and incubated at 37°C for 48 hours.

Loopful of incubated Fraser broth was streaked onto PALCAM agar media then the plates were incubated at 37±1°C for 48 hours and examined after 24±3 hours and the positive one was further streaked onto Oxford agar and ALOA agar plates then the plates were incubated at 37±1°C for 48 hours and examined after 24±3 hours. The *Listeria* like colonies were picked and streaked onto Tryptic Soy agar with 0.6 % yeast extract (TSA, YA) then, incubated at 35°C for 48 hours, the isolates were morphologically identified by Gram' stain according to Quinn *et al.*, (2011) and biochemical tests (Catalase reaction, Oxidase test, Nitrate reduction test, Urease test, Methyl red test (MR), Vages-proskauer test (VP), Carbohydrate fermentation test, Esculin test Gelatin hydrolysis test and Motility tests) were applied according to Markey *et al.*, (2013).

2.3. *Virulence tests:*

2.3.1. *Hemolytic activity:*

All isolates were cultured onto 5% sheep blood agar to determine their hemolytic activity. In addition to, they were

subjected to CAMP test (Christie-Atkins-Munoh-Peterson), (McKellar, 1994) by streaking of *Staphylococcus aureus* strains in single straight lines in parallel on sheep blood agar plates, the isolated *Listeria* strains streaked perpendicularly, with quite touching (1-2mm). After incubation for 24-48 hours at 35°C, a positive reaction consists of an enhanced zone of β-hemolysis.

2.3.2. *The biological characters:*

Anton's test (Quinn *et al.*, 2002) by instillation 2-3 drops of *Listeria* suspension into the conjunctiva of rabbits.

2.4. *In-Vitro anti-microbial sensitivity test (CLSI, 2014):*

All *L. monocytogenes* isolates were tested for their antimicrobial resistance/susceptibility pattern by disc diffusion technique according to (CLSI, 2014). By using antimicrobial discs as fluoroquinolones represented by ciprofloxacin; β -lactam represented by ampicillin; cephalosporins represented by cephalothin; aminoglycosides represented by gentamycin; tetracycline represented by tetracycline; phenicols represented by chloromphenicol; sulphonamides and trimethoprim represented by sulfamethoxate-trimethoprim; macrolides represented by erythromycin; glycopeptide represented by vancomycin (Oxoid).

2.5.2.5. *Genotypic detection of isolated L. monocytogenes and some virulence genes:*

PCR using five sets of primers (Table 1) was used for genotypic detection of *L. monocytogenes* strains and their virulence

genes. These genes were *16SrRNA* gene; internalin A (*inlA*); Positive regulatory factor (*prfA*); Listeriolysin O, haemolysin (*hlyA*) and Phospholipase A (*plcA*). It was applied on six random isolates of *L. monocytogenes*, following QIA amp® DNA

Mini Kit instructions (Catalogue no. M501DP100), Emerald Amp GTPCR master mix (Takara) with Code No. RR310A and 1.5 % agarose gel electrophoreses (Sambrook *et al.*, 1989).

Table (1): Oligonucleotide primers sequences

Primer	Sequence	Amplified product	Reference
<i>16S rRNA</i>	F- GGA CCG GGG CTA ATA CCG AAT GAT AA R- TTC ATG TAG GCG AGT TGC AGC CTA	1200 bp	Kumar <i>et al.</i> , 2015
<i>prfA</i>	F- TCT-CCG-AGC-AAC-CTC-GGA-ACC R- TGG-ATT-GAC-AAA-ATG-GAA-CA	1052 bp	Dickinson <i>et al.</i> , 1995
<i>inlA</i>	F- ACG AGT AAC GGG ACA AAT GC R- CCC GAC AGT GGT GCT AGA TT	800 bp	Liu <i>et al.</i> , 2007
<i>hlyA</i>	F- GCA-TCT-GCA-TTC-AAT-AAA-GA R- TGT-CAC-TGC-ATC-TCC-GTG-GT	174 bp	Deneer and Boychuk, 1991
<i>plcA</i>	F- ACA AGC TGC ACC TGT TGC AG R- TGA CAG CGT GTG TAG TAG CA	1484 bp	Soni <i>et al.</i> , 2014

3. RESULTS

A total of 53 (13.25%) isolates of *Listeria monocytogenes* were recovered from 400 samples includes 11(2.75%) isolates from muscles, 21(5.25%) from liver, 8 (2%) from spleen and 13 (3.25%) from kidneys (Table 2).

The isolated colonies grow well and showed gray green colonies with black depressed button center and black hollow surrounded them on PALCAM agar, black colonies with dimpled centers on Oxford agar and green-blue colonies surrounded by an opaque halo on ALOA agar.

They were Gram - positive bacilli or coccobacilli; were motile at room temperature on Semisolid trypticase soy

agar with yeast extract and showing the characteristics umbrella growth and the templing motility.

The Biochemical reactions showed that all strains were catalase (+); oxidase (-) and produce acid with dextrose, L-rhamnose but not with mannitol, D-xylose and sucrose.

The results of virulence tests proved that, all isolated *L. monocytogenes* strains were CAMP test positive with zone of β -hemolysis at the junction of tested strains and *S. aureus* strains. In addition, all of them produced purulent conjunctivitis within 24-48 hours followed by keratitis in all rabbits (Anton's test positive).

The results of antimicrobial sensitivity tests for the isolated *L. monocytogenes*

Prevalence and molecular studies on *Listeria monocytogenes*
isolated from chickens in El-Gharbia Governorate

(Table 3) showed that the isolated *L. monocytogenes* (n=12) were sensitive to sulfamethoxate-trimethoprim (91.6%) followed by ampicillin, gentamycin, vancomycin and chloromphenicol (83.3%) followed by ciprofloxacin (66.6%) followed by erythromycin and tetracycline (50.0%), while the isolated strains were completely resistant to cephalothin.

The PCR results for *L. monocytogenes* showed that, all genes (*16SrRNA*; *inlA*; *inlB*; *hlyA* and *prfA*) were detected in all six

studied strains (100.0%) while *plcA* gene was not detected. i.e., all six studied strains were *L. monocytogenes* and were virulent strains.

The (*16SrRNA*; *inlA*; *prfA*; *hlyA*) were amplified at 1200, 800, 1052, 174 bp respectively for the six isolates of *L. monocytogenes* (figures 1-4) but *plcA* gene was not detected (100%) (Figure 5).

Table (2): Prevalence of *Listeria monocytogenes* isolated from different organs in chicken samples

Samples	Number of samples	Number of positive samples	Positive percentage %
Muscles	100	11	2.75
Liver	100	21	5.25
Spleen	100	8	2
Kidneys	100	13	3.25
Total	400	53	13.25

Table (3): Results of antimicrobial sensitivity test for isolated *Listeria monocytogenes* strains (n=12)

Antimicrobial Family	Antimicrobial disc	Sensitive Isolates		Intermediate Isolates		Resistant Isolates	
		Number	%	Number	%	Number	%
Fluoroquinolones	Ciprofloxacin(CIP)	8	66.6	4	33.3	0	0
β-lactam	Ampicillin(AMP)	10	83.3	1	8.3	1	8.3
Cephalosporins	Cephalothin(KF)	0	0	0	0	12	100
Aminoglycosides	Gentamycin (CN)	10	83.3	1	8.3	1	8.3
Tetracycline	Tetracycline (TE)	6	50	0	0	6	50
Phenicols	Chloromphenicol C))	10	83.3	0	0	2	16.6
Sulphonamides and trimethoprim	Sulfamethoxate-Trimethoprim SXT))	11	91.6	0	0	1	8.3
Macrolides	Erythromycin (E)	6	50	2	16.6	4	33.3
Glycopeptide	Vancomycin (VA)	10	83.3	1	8.3	1	8.3

% According to the total no of *L. monocytogenes* isolates

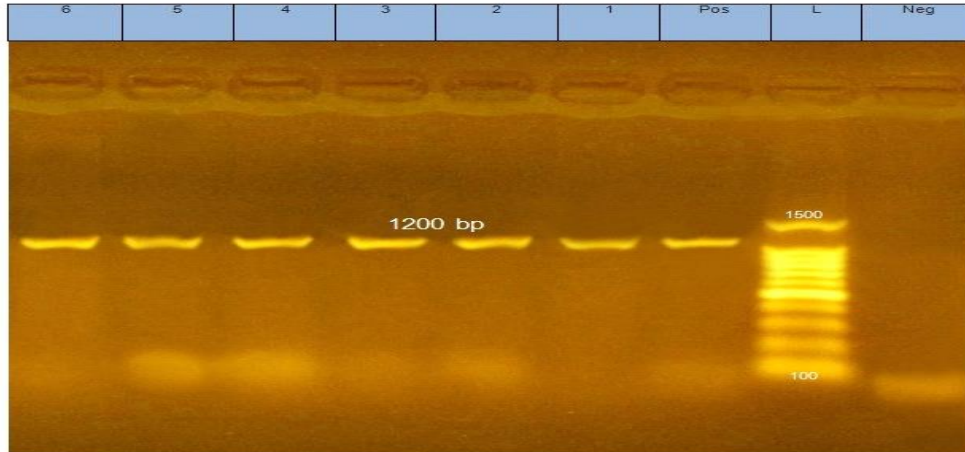


Figure (1): Agarose gel electrophoresis of *16SrRNA* genes. Lane L: 100-1500bp Ladder. Neg.: Negative control. Pos.: Positive control (at 1200bp). Lanes from 1 to 6: *L. monocytogenes (16S rRNA)* gene positive at 1200bp.

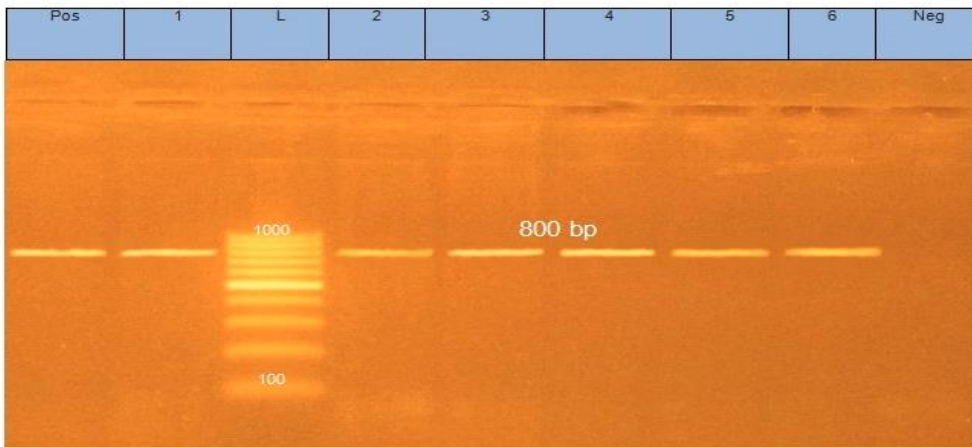


Figure (2): Agarose gel electrophoresis of internalin A (*inlA*) genes. Lane L: 100-1000bp Ladder. Neg.: Negative control. Pos.: Positive control (at 800bp). Lanes from 1 to 6: *L. monocytogenes(inlA)* gene positive at 800bp.

Prevalence and molecular studies on *Listeria monocytogenes*
isolated from chickens in El-Gharbia Governorate

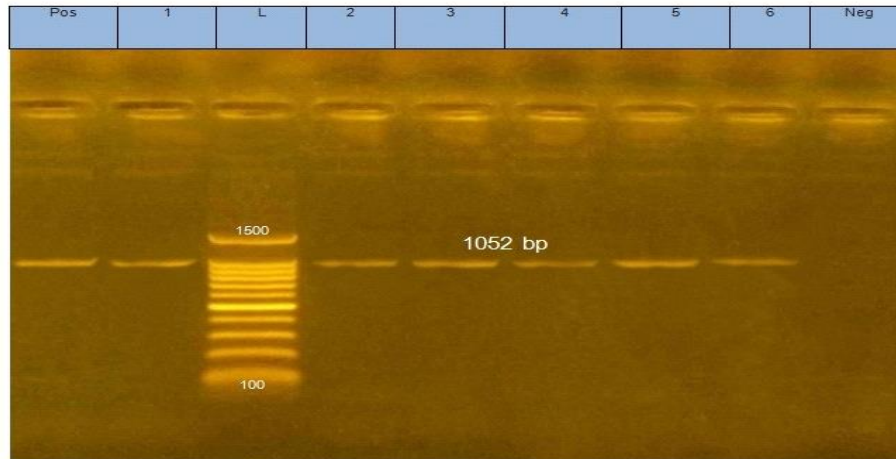


Figure (3): Agarose gel electrophoresis of Positive regulatory factor (*prfA*) genes. Lane L: 100-1500bp Ladder. Neg.: Negative control. Pos.: Positive control (at 1052bp). Lanes from 1 to 6: *L. monocytogenes* (*prfA*) gene positive at 1052bp.

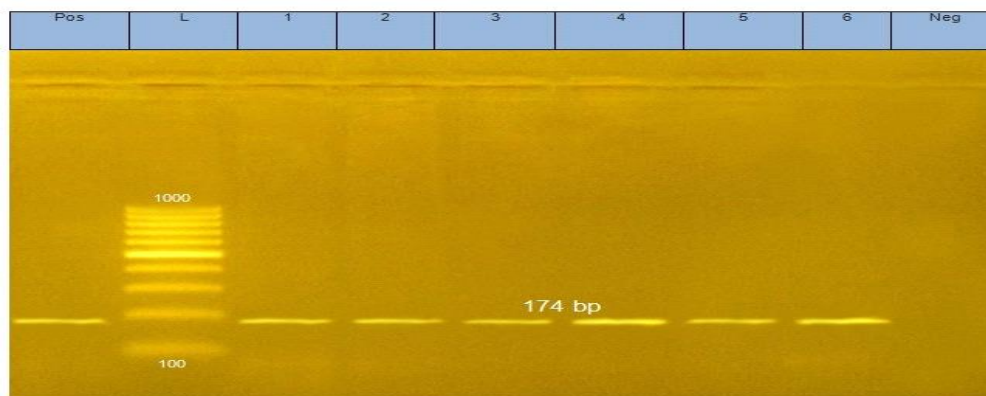


Figure (4): Agarose gel electrophoresis of Listeriolysin O, haemolysin (*hylA*) genes. Lane L: 100-1000 bp Ladder. Neg.: Negative control. Pos.: Positive control (at 174 bp). Lanes from 1 to 6: *L. monocytogenes* (*hylA*) gene positive at 174bp.

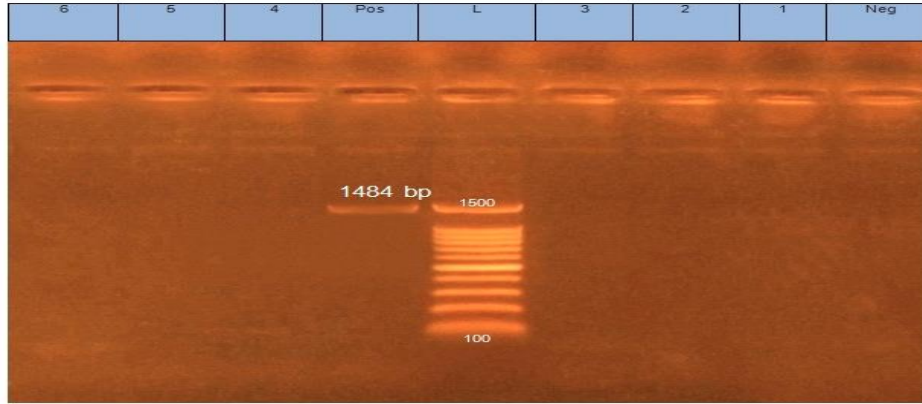


Figure (5): Agarose gel electrophoresis of Phospholipase A (*plcA*) genes. Lane L: 100-1500bp Ladder. Neg.: Negative control. Pos.: Positive control (at 1484bp). Lanes from 1 to 6: *L. monocytogenes* (*plcA*) gene negative.

4. DISCUSSION

In the present study a total of 400 examined chicken samples, 53 isolates (13.25%) of *Listeria monocytogenes* were isolated (Table 2). This is nearly similar to Osaili *et al.*, (2011), Swetha *et al.*, (2013), Saludes *et al.*, (2015) and AL-Jobori *et al.*, (2016) who isolated *L. monocytogenes* from chicken by (18%), (16%), (19%) and (11%) respectively.

These results in El-Gharbia Governorate (13.25%) are higher than recorded by Ali and Shalaby (2002) in Giza city 3.3%, Farghaly (2011) in Cairo Governorate 3.6% and Dahshan *et al.*, (2016) in Sharkia Governorate in Egypt who did not detected *L. monocytogenes* from chicken samples.

However, higher prevalence rate of *L. monocytogenes* isolated from chicken were recorded by Minamia *et al.*, (2010), Pesavento *et al.*, (2010), Kuan *et al.*, (2013) and Zeinali *et al.*, (2017) who isolated *L.*

monocytogenes from chicken by (36%), (24.5%), (26.39%) and (18%), respectively.

The colonial appearance of the recovered isolates in this study grow well and showed: small 2-3 mm in diameter; gray green colonies in color with black depressed button center and black hollow surrounded them (esculin hydrolysis) on PALCAM agar that was similar to the previously recorded studied by (Osman *et al.*, (2014) and Abd El-Tawab *et al.*, (2015). Black colonies with dimpled centers on Oxford agar that was similar to the previously recorded studied by (Magalhães *et al.*, (2014) and Abd El-Tawab *et al.*, (2015). Green-blue (due to presence of β -glucosidase) regular round colonies surround by an opaque halo (due to the activity of phospholipase involved in pathogenic *Listeria*) on ALOA agar that was similar to the previously recorded studied by (Jeyaletchumi *et al.*, (2010a) and Abd El-Tawab *et al.*, (2015).

The results of biochemical identification showed characteristic

Prevalence and molecular studies on *Listeria monocytogenes*
isolated from chickens in El-Gharbia Governorate

identical biochemical reaction to be *Listeria* species that was similar to the previously recorded studied by (Schmid *et al.*, (2005); Todar, (2009) and Abd El-Tawab *et al.*, (2015).

All isolated *Listeria* were motile at room temperature and showing the characteristics umbrella growth and the templing motility that was similar to the previously recorded studied by (Ryser and Marth, (2007); Bhunia, (2008); Todar, (2009) and Abd El-Tawab *et al.*, (2015).

The result of virulence tests for the isolated *Listeria monocytogenes* strains appeared that, all *L. monocytogenes* strains were virulent strains, where all of them were positive to CAMP test and showed narrow zone of β -hemolysis on sheep blood agar. Also, all *L. monocytogenes* isolates were positive for Anton's test. That was similar to the previously recorded studied by (Maarouf *et al.*, (2007); Todar, (2009); Fentahun and Fresebehat, (2012) and Abd El-Tawab *et al.*, (2015).

The results of antimicrobial sensitivity tests for the isolated *L. monocytogenes* (Table 3) showed that the isolated *L. monocytogenes* were sensitive to Sulfamethoxate-Trimethoprim (91.6%) followed by ampicillin, gentamycin, vancomycin and chloromphenicol (83.3%) followed by ciprofloxacin (66.6%) followed by erythromycin and tetracycline (50.0%), while the isolated strains were completely resistant to cephalothin. Nearly similar results were recorded by Conter *et al.*, (2009); Granier *et al.*, (2011) and Altuntas *et al.*, (2012).

The PCR technique can identify the pathogenic *L. monocytogenes* based on the fact that virulence varies not only among different species but also among strains of the same species. Thus, numerous studies have been conducted to identify virulence factors of isolated *L. monocytogenes* strains (Shen *et al.*, 2013; Khan *et al.*, 2014 and Ciolacu *et al.*, 2015).

So, the present study was directed mainly to genotypic detection of *L. monocytogenes* strains and virulence genes that may play a role in virulence of *L. monocytogenes* by using one of the recent developments molecular biological techniques (PCR). These genes were 16 *rRNA* gene; internalin A (*inlA*); Positive regulatory factor (*prfA*); Listeriolysin O, haemolysin (*hlyA*) and Phospholipase A (*plcA*).

The PCR results for six studied strains of *L. monocytogenes* isolates showed that, the genes (*16rRNA*, *inlA*, *hlyA* and *prfA*) were detected (100.0%). i.e., all six studied strains were *L. monocytogenes* and all of them were virulent strains. Similar results were decided by Shen *et al.*, (2013); Abd El-Tawab *et al.*, (2015) and Ciolacu *et al.*, (2015); as well as they reported that, PCR save time for diagnosis hence allowing a rapid identification of *L. monocytogenes* with high sensitivity and specificity.

While the result of PCR for amplification of Phospholipase A (*plcA*) gene in *L. monocytogenes* (Figure 5) showed that, it was not detected in all six studied strains of *L. monocytogenes* isolates in the current study.

Finally, from results of the present work it could be concluded that, *Listeria monocytogenes* are serious pathogens could be contaminated chickens in El-Gharbia Governorates, Egypt.

5. REFERENCES

- Abd El-Tawab, A.A.; Ahmed, A.A.M.; Zeinab, A.M., 2015. Bacteriological and Molecular studies of *Listeria* species in milk and milk products at El-Kaliobia Governorate. Benha Veterinary Medical Journal, Vol. 29 (2):170-181.
- Akanbi, O.B.; Breithaupt, A.; Polster, U.; Alter, T.; Quandt, A.; Bracke, A.; Teifke, J.P., 2008. Systemic Listeriosis in caged canaries (*Serinus canarius*). Avian Pathol., 37: 329-332.
- Ali, F.H.M.; Shalaby, O.F.H.B., 2002. Incidence of *Listeria* and *Yersinia* species among slaughtered poultry and rabbit with special reference to its zoonotic importance. Vet. Med. J. Giza, 50 (4):571-579.
- AL-Jobori, K.M.; Hasan, M.L.M.; Nader, M.I., 2016. Detection of *E. coli*, *Salmonella spp.* and *Listeria monocytogenes* in Retail Chicken Meat and Chicken Giblets Samples Using Multiplex PCR in Baghdad City. Int. J. Curr. Microbiol. App. Sci, 5(9): 290-301.
- Altuntas, E.; Kocan, D.; Cosansu, S.; Ayhan, K.; Juneja, V.; Materon, L., 2012. Antibiotic and Bacteriocin sensitivity of *L. monocytogenes* strains isolated from different foods. Food and Nutrioin Sciences, 3(3): 363-368.
- Bhunja, A.K., 2008. Food-borne Microbial Pathogens: Mechanisms and Pathogenesis. Springer, New York, ISBN-13: 9780387745367, pp: 165-182.
- Ciolacu, L.; Nicolau, A.I.; Wagner, M.; Rychli, K., 2015. *Listeria monocytogenes* isolated from food samples from a Romanian black market show distinct virulence profiles. Int. J. Food Microbiology, 209:44-51.
- Clinical Laboratory Standards Institute (CLSI), 2014. Performance Standards for Antimicrobial Susceptibility Testing; twenty-fourth informational supplement. M100-S24. Clinical and Laboratory Standard Institute for antimicrobial susceptibility testing standards M100-S24, 34(1), 1-226.
- Conter, M.; Paludi, D.; Zanardi, E.; Ghidini, S.; Vergara, A.; Ianieri, A., 2009. Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. International Journal of Food Microbiology, 128, 497-500.
- Dahshan, H.; Merwad, A.M.A.; Mohamed, T.S., 2016. *Listeria* Species in Broiler Poultry Farms: Potential Public Health Hazards. J. Microbiol. Biotechnol. 26(9), 1551–1556.
- Deneer, H.G.; Boychuk, I., 1991. Species-Specific Detection of *Listeria monocytogenes* by DNA Amplification. Applied and Environmental Microbiology, Feb. 1991, p. 606-609.
- Dickinson, J.H.; Kroll, R.G.; Grant, K.A., 1995. The direct application of the

Prevalence and molecular studies on *Listeria monocytogenes*
isolated from chickens in El-Gharbia Governorate

- polymerase chain reaction to DNA extracted from foods. Letters in Applied Microbiology 1995, 20, 212-216.
- Farghaly, T.I.T., 2011. Studies on *Listeria* infection in chickens. Master in Veterinary Medical Science (Bacteriology, Immunology and Mycology), Faculty of Veterinary Medicine Cairo University.
- Fentahun, T.; Fresebehat, A., 2012. A review of Listeriosis in Small Ruminants. Advances in Biological Research, 6(6): 202-209.
- Gouws, P.A.; Liedemann, I., 2005. Evaluation of Diagnostic PCR for the Detection of *Listeria monocytogenes* in Food Products. Food Technol. Biotechnol., 43(2): 201-205.
- Granier, S.A.; Moubareck, C.; Lemire, A.; Roussel, S.; Dao, T.T.; Courvalin, P.; Brisabois, A., 2011. Antimicrobial resistance of *Listeria monocytogenes* isolated from food and the environment in France over a 10-year period. Appl. Environ. Microbiol., (77): 2788-2790.
- ISO, 2005. Microbiology of food and animal feeding stuffs-Horizontal method for the detection and enumeration of *Listeria monocytogenes*-part 1: Detection method. International Standard ISO 11290-1. Amendment 1. Geneva. Switzerland.
- Jacquet, C.; Gouin, E.; Jeannel, D.; Cossart, P.; Rocourt, J., 2002. Expression of *ActA*, *Ami*, *InlB* and listeriolysin O in *Listeria monocytogenes* of human and food origin. Applied and Environmental Microbiology 68: 616-622.
- Jalali, M.; Abedi, D., 2007. Prevalence of *Listeria species* in food products in Isfahan, Iran. Int. J. Food Microbiol., 122: 336-340.
- Jeyaletchumi, P.; Tunung, R.; Margaret, S.P.; Son, P.; Farinazleen, M.G.; Cheah, Y.K., 2010a. Detection of *Listeria monocytogenes* in foods. Int. Food Res. J. 17, 1-11.
- Karthikeyan, R.; Gunasekaran, P.; Rajendhran, J. 2015. Molecular Serotyping and Pathogenic Potential of *Listeria Monocytogenes* Isolated from Milk and Milk Products in Tamil Nadu, India. Foodborne Pathog. Dis. 12(6) 522-8.
- Khan, J.A.; Rathore, R.S.; Khan, S.; Ahmad, I., 2014. In vitro detection of pathogenic *Listeria monocytogenes* from food sources by conventional, molecular and cell culture method. Braz. J. Microbiol., 44(3): 751-759.
- Kirkani, S.; Ergun, O.G.; Osman, K., 2005. Detection of *Listeria monocytogenes* by using PCR in helix pomatia. Turk. J. Vet. Anim. Sci.30 (2005) 375-380.
- Kuan, C.H.; Goh, S.G.; Loo, Y.Y.; Chang, W.S.; Lye, Y.L.; Puspanadan, S.; Tang, J.Y.H.; Nakaguchi, Y.; Nishibuchi, M.; Mahyudin, N.A.; Radu, S. 2013. Prevalence and quantification of *Listeria monocytogenes* in chicken offal at the retail level in Malaysia. Poult. Sci., 92(6), 1664-1669.

- Kumar, A.; Grover, S.; Batish, V.K., 2015. Exploring specific primers targeted against different genes for a multiplex PCR for detection of *Listeria monocytogenes*. 3 Biotech (2015) 5:261–269.
- Kurazono, M.; Nakamura, K.; Yamada, M.; Yonemaru, T.; Sakoda, T., 2003. Pathology of Listerial encephalitis in chickens in Japan. Avian Dis., 47(4): 1496 - 1502.
- Liu, D.; Lawrence, M.L.; Austin, F.W.; Ainsworth, A.J., 2007. A Multiplex PCR for species and virulence-specific determination of *Listeria monocytogenes*. Journal of Microbiology Methods (71): 133-140.
- Maarouf, A.A.; El-Bealawy, M.A.; Moubarak, M.G., 2007. Some studies of Listeriosis in sheep at Kaliobia governate. J. Egypt. Vet. Med. Assoc. 67(4): 27-40.
- Mackiw, E.; Modzelewska, M.; Maka, L.; Scieczynska, H.; Pawlowska, K.; Postupolski, J.; Korsak, D., 2016. “Antimicrobial resistance profiles of *Listeria monocytogenes* isolated from ready to eat product in Poland in 2007-2011. Food Control, 59: 7-11.
- Magalhães, R.; Mena, C.; Ferreira, V.; Almeida, G.; Silva, J.; Teixeira, P., 2014. Traditional methods for isolation of *Listeria monocytogenes*, in *Listeria monocytogenes: Methods and Protocols*, eds K. Jordan, E.M. Fox, and M. Wagner (New York, NY: Springer Science), 15–30.
- Markey, B.K.; Leonard, F.C.; Archambault, M.; Cullinane, A.; Maguire, D., 2013. Clinical Veterinary Microbiology. Second edition. MOSBY. Elsevier Ltd. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto.
- Mckellar, R.C., 1994. Use of the CAMP test for identification of *Listeria monocytogenes*. Appl. Environ. Microbiol., 60 (12): 4219-4225.
- Minamia, A.; Chaicumpab, W.; Chongsanguanc, M.; Samosornsukd, S.; Monden, S.; Takeshi, K.; Makino, S.; Kawamoto, K., 2010. Prevalence of foodborne pathogens in open markets and supermarkets in Thailand. Food Control., 21(3): 221-226.
- Orsi, R.H.; Wiedmann, M., 2016. Characteristics and distribution of *Listeria* spp., including *Listeria* species newly described since 2009. Applied Microbial Biotechnology, pp. 5273-5287.
- Osaili, T.M.; Alaboudi, A.R.; Nesiari, E.A., 2011. “Prevalence of *Listeria* spp. and antibiotic susceptibility of *Listeria monocytogenes* isolated from raw chicken and ready-to-eat chicken products in Jordan. Food Control, 22 (3-4): 586-590.
- Osman, K.M.; Samir, A.; Orabi, A.; Zolnikov, T.R., 2014. Confirmed low prevalence of *Listeria monocytogenes* in she-camel milk delivers a safe, alternative milk for human consumption. *Acta Trop.* 130, 1–6.
- Pesavento, G.; Ducci, B.; Nieri, D.; Comodo, N.; Lo Nostro, A., 2010.

Prevalence and molecular studies on *Listeria monocytogenes*
isolated from chickens in El-Gharbia Governorate

- “Prevalence and antibiotic susceptibility of *Listeria* spp. isolated from raw meat and retail foods. *Food Control* 21, 708-713.
- Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donnelly, W.J.C.; Leonard, F.C.; Maguire, D., 2002. *Veterinary microbiology and microbial disease*. Iowa State Univ. Press, Blackwell Science Ltd, 84-96.
- Quinn, P.J.; Markey, B.K.; Leonard, F.C.; Hartigan, P.; Fanning, S.; Fitz Patrick, E., 2011. *Veterinary microbiology and microbial disease*, John Wiley and Sons.
- Ryser, E.T.; Marth E.H. 2007. *Listeria, Listeriosis and Food Safety*. Taylor and Francis; Boca Raton, FL: 2007.
- Saludes, M.; Troncoso, M.; Figueroa, G. 2015. Presence of *Listeria monocytogenes* in Chilean food matrices. *Food Control*, 50, 331-335.
- Sambrook, J., Fritsch, E.F.; Maniatis, T.; 1989. *Molecular cloning. A laboratory manual*. Vol. 1, Cold spring Harbor Laboratorty press, New York. 3 pages.
- Schmid, M.W.; Ng, E.Y.; Lampidis, R.; Emmerth, M.; Walcher, M.; Kreft, J.; Goebel, W.; Wagner, M.; Schleifer, K.H., 2005. Evolutionary history of the genus *Listeria* and its virulence genes. *Syst. Appl. Microbiol.*, 28(1), 1-18.
- Scotti, M.; Monzó, H.J.; Lacharme-Lora, L.; Lewis, D.A.; Vázquez-Boland, J.A., 2007. The PrfA virulence regulon. *Microbes Infect.*; 9(10):1196-207.
- Shen, J.; Rump, L.; Zhang, Y.; Chen, Y.; Wang, X.; Meng, J., 2013. Molecular subtyping and virulence genes analysis of *Listeria monocytogenes* isolates from food. *Food Microbiology*, (35): 58-64.
- Soni, D.K.; Singh, M.; Singh, D.V.; Dubey, S.K., 2014. Virulence and genotypic characterization of *Listeria monocytogenes* isolated from vegetable and soil samples. *BMC Microbiology*, 14:241.
- Swetha, C.S., Madhava Rao, T.; Krishnalah, N., 2013. Detection of *Listeria monocytogenes* in chicken, pork and fish by PCR technique. *ISOPOL XVIII*: 116.
- Todar, K., 2009. "Listeria monocytogenes". *Todar's Online Textbook of Bacteriology*. <http://textbookofbacteriology.net/Listeria.html>.
- Zeinali, T.; Jamshidi, A.; Bassami, M.; Rad, M., 2017. Isolation and identification of *Listeria* spp. in chicken carcasses marketed in northeast of Iran. *International Food Research Journal* 24(2): 881-887.