Prevalence of *Listeria* among poultry caracasses

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Abstract:

A total of 200 fresh chicken samples comprising thigh , breast muscles, gizzrads and liver samples (50 for each), were randomly collected from different localities and poultry shops of different sanitation levels at Mansoura city, Dakahlia, Egypt to evaluate its status and determine the prevalence of *Listeria* species which may be existed. The results declared that *Listeria* spp. was isolated from 42 (21%) of all samples. 9 (18%) , 10 (20%) , 8 (16%) , 15 (30%)from thigh, breast , gizzard and liver samples respectively .The serological results revealed that *L. monocytogenes* in the chicken samples was 2 (4%) , 4 (8%) , 1 (2%) , 3 (6%) from thigh, breast , gizzard and liver samples respectively, while *L.welshemeri* was 16 (2 for breast muscle and 3 for each of thigh muscle , gizzard and liver samples), *L.innocua* was 2 (7 for liver samples and 3 for each of thigh muscle , gizzard and breast samples) . *L.muryi* was 5 (2 for liver samples and 1 for each of thigh muscle , gizzard and breast samples.Further identification of *L. monocytogenes* was applied by using PCR technique.

Key words: chicken meat, *L.monocytogenes,* virulence gene,

1. Introduction:

Microbiological safety and quality of broiler meat are equally important to producers, retailers and slaughterhouses (**Lindblad et al., 2006**). L. monocytogenes is particularly significant for cold-stored, ready-to-eat foods as it is frequently found in the environment and can grow at refrigerated temperatures. In spite of cleaning and disinfection after the chickens had been taken to the poultry slaughterhouse, microbial contamination from the intestinal contents occurred in the broiler houses in 16.9% of the cases (**Dijkstra, 1978**). Isolation of L.monocytogenes from chicken meat was recorded previously by many investigators (**Keeratipibul and Lekroengsin, 2009; El-Shabacy Rasha, 2008; Lekroengsin et al., 2007; Akpolat et al., 2004 and Miettinen et al., 2001**).. There are six species of genus *Listeria* including *L. monocytogenes, L. grayi, L. innocua, L. ivanovii, L. seeligeri* and *L.welshimeri***(Graves *et al*., 2010).**Infection with *L. monocytogenes* shows low prevalence but high mortality due to septicemia, meningitis, meningoencephalitis in immunocompromised individuals, newborns, the elderly and abortion in pregnant women**(Yücel *et al* 2005).** All strains of *L. monocytogenes* appear to be pathogenic and infections can be life threatening, with fatality rates of 20-30% **(WHO/ FAO, 2004)**. The infective dose of *L.monocytogenes* is considered to be about 100 to 1000 cells particularly for the sensitive groups (**Smerdon*et al*., 2001).**Several groups of virulence factors which are important in the pathogenicity of *Listeria monocytogenes* strains have recently been characterized such as the internaline genes and listeriolysin O gene, which take a part in the invasion of human epithelial cells **(Dramsi*et al*., 1997).** Plant extracts and spices, in addition to contributing to taste and flavor, can act against Gram-positive pathogens such as *L. monocytogenes*.

2. Materials and methods:

2.1.Sampling and isolation of *Listeria monocytogenes*:

A total of 200 fresh chicken samples comprising comprising thigh , breast muscles, gizzrads and liver samples (50 for each)), were randomly collected from different localities and poultry shops of different sanitation levels at Mansoura, Dakahlia,Egypt. The samples were analyzed according to ISO 11290 method whereby pre-enrichment of 25 g sample was done in 225 ml half strength Fraser broth containing selective supplements (HiMedia) for 24 h at 30°C, which was followed by second enrichment of 0.1 ml of pre-enriched Fraser broth content in 10 ml full strength Fraser broth containing selective supplements (HiMedia) for 48 h at 37°C incubation temperature. After the enrichment procedure, the inoculum was plated on OXFORD agar (HiMedia) and ALOA agar incubated for 48 h at 37°C .

The gray-green colonies surrounded by diffuse black zone on OXFORD agar were picked up and further purified on Tryptone Soya Yeast Extract agar (TSYEA). Subsequently, pinpoint colonies of TSYEA were subjected to identification procedures which included Gram's staining followed by a microscopic examination, catalase test, and oxidase test. The characteristic Gram-positive, coccobacillary or short rod-shaped organisms which were catalase positive and oxidase negative, were sub-cultured in Brain heart infusion (BHI) broth at 25°C for 12-18 h. Subsequently, the cultures showing typical tumbling motility were considered as "presumptive" listeria isolates, which were in turn subjected to detailed biochemical tests viz.; methyl red, Voges-Proskauer, nitrate, and sugar fermentation tests with xylose, rhamnose, mannitol, and α-methyl D-mannopyranoside

2.2. Molecular amplification: The DNA extraction was performed according to the manufacture guide line using a Bacterial DNA Extraction Kit (QIA amp) (BioshopR, Canada).The Oligonucleotide primers targeting the internalin genes and Lysteriolysin O gene of *L.monocytogenes* were synthesized commercially by AlphaDNA, Canada.

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
hlyA (F)	5′ GCAGTTGCAAGCGCTTGGAGTGAA ′3	456	Swethaet al. (2013)
hlyA (R)	5′ GCAACGTATCCTCCAGAGTGATCG ′3		
inlA (F)	5′ ACGAGTAACGGGACAAATGC ′3	800	Pournajafet al. (2016)
inlA (R)	5′CCCGACAGTGGTGCTAGTTT ′3		
inlC (F)	5′ AATTCCCACAGGACACAACC ′3	517	
inlC (R)	5′ CGGGAATGCAATTTTTCACTA ′3		
inlJ (F)	5′ TGTAACCCCGCTTACACAGTT ′3	237	
inlJ (R)	5′ AGCGGCTTGGCAGTCTAATA ′3		

Table (1) shows the inlA, inlC,inlJand hlyAprimers.

The amplification reaction of internaline gene and *hylA* gene were performed according to **Liu *et al.*, (2007) and Kaur *et al.*, (2007)**

Results and discussion:

Result achieved in table (2) Pointed out that *Listeria* spp. were isolated from 42(21%) out of 200 raw fresh chicken samples. The obtained results were similar to the results reported by **Nancy *et al*. (1997)**

who revealed that *listeria* was isolated from 11.1% of examined poultry samples. However, lower prevalence rate was reported by **Sakaridis*et al*. (2011)** who isolated *Listeria* species from 8% from the examined chicken samples and **El-Bayomi. (2013)** who isolated *Listeria* species from9.3% of the examined chicken samples. Meanwhile, higher isolation rate of 32.2% was approved by **AbdEl-azi*et al*. (2001)** and 33.3% by **Barbalho*et al*. (2005)** in the examined chicken meat samples. In addition **Ahmed and Abd El-Atti (2010), Osaili*et al*. (2011), Fallah*et al*. (2012), Dahshan*et al*. (2016)and Zeinali*et al*. (2017)** cited also higher incidence of *Listeria* species in chicken meat (42%, 50%, 33.3%, 47.5% and 40%, respectively).

Table (2): Prevalence of *Listeria* spp in the examined fresh chicken meat samples.

Type of examined samples	L.monocytogen	L . innocua	L . welshimeri	L . murrayi
Thigh	2 (4 %)	3(6%)	3(6%)	1(2 %)
Breast	4(8 %)	3(6%)	2(4%)	1(2 %)
Liver	3(6%)	7(14 %)	3(6%)	2(4%)
Gizzard	1(2 %)	3(7%)	3(6%)	1(2 %)
total	10 (5 %)	16 (8%)	11 (5.5%)	5 (2.5%)

It is evident from the data recorded in table (2) that the prevalence of *Listeria* species was nearly equal in the examined thigh and breast muscle (4.5% and5%). The percentage of *Listeria* positive from thigh and breast muscles were nearly similar to 4(8%) and 2(4%), respectively (**El-Bayomi, 2013)** and lower than **Erol*et al*. (1999)** who detected *Listeria* spp. in 16.6% from the examined breast samples. The overall incidence of *L.monocytogenes* was 10(5%). *L.monocytogenes* was isolated from 2(4%) of thigh muscles and 4(8%)of breast muscles (Table 2). These results were nearly similar to **Wang *et al*. (1992)** who found *L.monocytogenes* in 4.76% of the examined chicken samples and **Fatma and Omnia, (2002)** who isolated *L.monocytogenes* from 3.3% of the whole chicken carcass and **Mohamed (2005)** who isolated *L.monocytogenes* from 5.87% of the examined raw chicken samples. However, lower percentages of *L.monocytogenes*(2.1%)(**Varabioff, 1990)** who failed to detect *L.monocytogenes* in examined liver and gizzard sample and **Yoshimasa*et al*. (2013)**who cited lower prevalence of 2.3% in liver samples . On the other hand, higher percentage of 36.45% **(Villari, 1991)** and15.5% **(Nancy *et al*., 1997).** In the same line, in Nordic Countries, **Gudbjornsdottir*et al*. (2004)** reported that the average incidence of *L.monocytogenes* was 22.2% for poultry meat In Egypt, *L. monocytogenes* reported in 8.9% (**Abd El-azi*et al*., 2001)** and 17% (**Sameer and El-shennawy, 2008)** in the examined poultry meat samples. *L.innocua* was isolated from overall 3(6%) of the examined thigh , breast and gizzards while 7(14%) in liver samples. These results were lower than 10.4% and 17.5 of fresh and frozen chicken samples, respectively (**Varabioff, 1990),** 16.6, 10, 13.3% of whole carcass, gizzard and liver, respectively (**Fatma and Omnia, 2002)** and 46.3% (**Fallah*et al*. 2012)**, 28.55(**Dahshan*et al*., 2016)** and28.75%**(Zeinali*et al*., 2017)**of the examined samples. The overall prevalence of *L.welshimeri* was 2(4%) in breast and 3(6%) in thigh samples, this investigation was nearly similar to the results recorded

by **Zeinali*et al*. (2017)** who isolate *L. welshimeri* from 2% of the examined chicken samples. These achieved results were lower than 7% (**Osaili*et al*., 2011)** in raw chicken meat and chicken products samples and **Dahshan*et al*. (2016)** who detected this bacteria in 4.5% of the examined poultry farm samples.In this research neither *L.seelegri nor L.ivanovii* was detected, and less of L. murrayi (2.5%)from all samples this finding may be similar to 1.8% (**Osaili *et al*., 2011)** and 1% (**Dahshan*et al*., 2016)** of the examined poultry samples. However, higher incidence of 5.22% (**Fallah*et al*., 2012)** in the examined raw chicken samples.

Polymerase chain reaction had proved to be an efficient method for the detection of virulent *L. monocytogenes* by the amplification of different virulence genes (**Jaradat*et al*., 2002**). Among a panel of *L. monocytogenes* virulence-specific genes, internalin gene (*inl*J) was identified as a useful target for a rapid differentiation of virulent from a virulent *L. monocytogenes* strains (**Liu*et al*., 2003, Jaradat*et al*., 2002 and Liu, 2007**). The internalin gene (*inl*J) involvement in *L. monocytogenes* passage through the intestinal barrier as well as involvement in the subsequent stages of infection and virulence support for the validity of using this gene as virulence indicator (**Jaradat*et al*., 200**2). Internalin A and *InlC* was virulence-associated gene that contributed to the post intestinal stages of *L. monocytogenes* infection (**Jaradat*et al*., 2002**). The combined application of *inl*A which is species-specific, *inl*C and *inl*J gene primers in a multiplex PCR confirm *L. monocytogenes* species identity and its potential virulence (**Jaradat*et al*., 2002**).In the present study, a multiplex PCR assay was carried out for detection of *L. monocytogenes* and presence of internalin genes. Most of tested strains were positive for the *inl*A, *inl*C, and *inl*J genes (Fig.2). Similar results were documented by **Mammina*et al*. (2009)**, **Ahmed *et al*. (2013), El Bayomi. (2013) and Dahshan*et al*. (2016)** who identified the three virulent genes in the isolated *L. monocytogenes* from different sources.

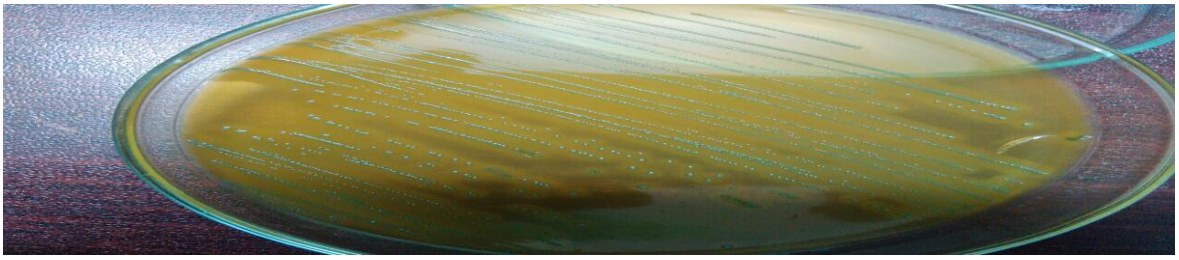

Fig (1) Listeria monocytogene on ALOA agar

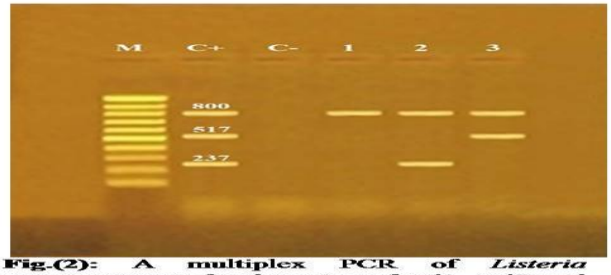

Fig.(2): A multiplex PCR of *Listeria monocytogenes* for detection of *inl*A, *inl*C and *inl*J genes in 1.5% agarose gel (M: 100 bp ladder; C+: positive control; C-: negative control; 1 virulent *L. monocytogenes* for *inl*A; 2 virulent *L. monocytogenes* for *inl*A and *inl*J;3 virulent *L. monocytogenes* for *inl*A and *inl*C genes).

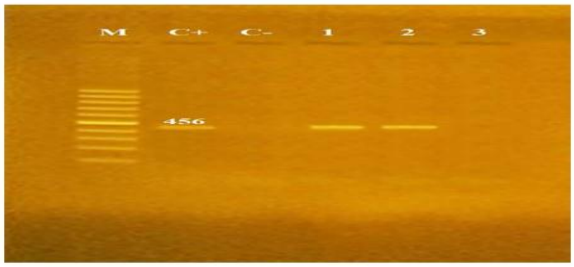

Fig.(3): Listeriolysin O PCR of *Listeria monocytogenes* suspected isolates. M:100bp ladder; C+: positive control; C-: negative control; 1-2 positive *L. monocytogenes* for Listeriolysin O; 3 negative L. *monocytogenes* for Listeriolysin O.

For *L.monocytogenes* serotypes to cause infection to human through ingestion, it desired involvement of other virulence genes such as listeriolysin O. In this regard, in this study a PCR amplification of LLO has been used to perfectly explore the potency of the isolated strains to cause human listeriosis (**Ahmed *et al*., 2013).** The results declared in fig. (3) Indicated that LLO was detected in 2 out of 3 isolated strains of *L.monocytogenes*, these results illustrated the importance of LLO as a virulent index for *L.monocytogenes* causing human listeriosis. The presence of virulence gene *hyl*Ain the isolated strains, suggesting that they are potentially pathogenic as recorded by **Jaradat*et al*., (2002).**

In conclusion, this study highlighted that chicken meat can act as vehicles for transmission of L. monocytogenes. Polymerase chain reaction is an efficient method for the detection of virulent L. monocytogenes in food.

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الملخص العربي

مدى تواجد الليستيريا فى ذبائح الدواجن

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أوضحت النتائج أن ميكروب الليستيريا تم عزله من عينات الدجاج الطازجة التى تم فحصها بنسبة 21% من اجمالي العينات. ايضا تم عزل ميكروب الليستيريا بنسبة متقاربة وهى (18&20%) من الأوراك والصدور وبنسبة 16 % من القوانص وبنسبة 30% من الاكباد. تم تصنيف معزولات الليستيريا سيرولوجيا كالآتى: الليستيريامونوسيتوجين 10(5%)، الليستيريا انوكوا 16(8%)، الليستيرياولشيميري11(5.5%) والليستريا ميوراي 5(2.5%) .تم إجراء تفاعل البلمرة المتسلسل لمعزولات الليستيريامونوسيتوجين التي تم تصنيفها وذلك للتأكيد علي ما تم التوصل إليه من نتائج والبحث عن(internaline genes) و (listeriolysin O) عالي الضراوة. تم مناقشة الأهمية الصحية للميكروب الذى تم عزله وكذلك الشروط الصحية الواجب توافرها لتجنب خطر هذا الميكروب.