Animal Health Research Institute,
Assiut Regional Laboratory.

PREVALENCE OF *LISTERIA MONOCYTOGENES* IN RAW AND COOKED POULTRY WITH RAPID CONFIRMATION BY MULTIPLEX PCR.

(With 3 Tables and One Figure)

By
GHADA M. MOHAMED and LUBNA M. EBRAHEEM

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مدى تواجد ميكروب الليستيريا مونوسيتوجين فى الدجاج الطازج والمطهى مع إجراء اختبار تفاعل البلمرة المتسلسل التاكيدى له

غادة محمد محمد ، لبنى محمد ابراهيم

لقد تم تحديد مدى تواجد طائفة الليستيريا وميكروب الليستيريا مونوسيتوجين في كل من الدجاج الطازج والمشوى والمقلي ولذلك تم جمع ٤٠ عينة من كل نوع من أماكن مختلفة في محافظة أسيوط. ولقد أشارت النتائج إلى أنه قد تم عزل طائفة الليستريا من ٥٠%، ١٥%، ٧.٥% من كلا من الدجاج الطازج والمشوى والمقلي على التوالي بينما لوحظ وجود ميكروب الليستيريا مونوسيتوجين في ١٤ عينة من الدجاج الطازج بنسبة ٣٥% وفي ٥ عينات من الدجاج المشوي بنسبة ١٢.٥%وفي عينة واحدة من الدجاج المقلي بنسبة ٢.٥%. ولقد تم التاكد من عترات الليستيريا مونوسيتوجين بواسطة التجارب الكيميائية وبتفاعل البلمرة المتسلسل حيث أظهر هذا التفاعل وجود الليستيريا مونوسيتوجين في ٦ عينات من الدجاج الطازج وفي عينة واحدة فى كل من الدجاج المشوي والمقلي. وأوضح هذا البحث أن وجود الليستريا مونوسيتوجين في كلاً الدجاج المشوي والمقلي يمثل خطرا على الصحة العامة وذلك للاقبال الكبير والاستهلاك اليومي لهذه المنتجات.

SUMMARY

The incidence of *Listeria* spp. and *Listeria monocytogemes* in raw, grilled and fried poultry was determined, 40 samples of each product wich were collected from different localities in Assiut City. The obtained results pointed out that *Listeria* spp. could be isolated from 50, 15 and 7.5% of the examined raw, grilled and fried poultry respectively, while(20)of *Listeria momocytogenes* was detected in 14 (35%) of raw

poultry, 5 (12.5%) of grilled samples and 1 (2.5%) of fried samples. Strains of *Listria monocytogenes* was characterized by several biochemical tests and confirmed by polymerase chain reaction (PCR). Results showed that *Listeria moncytogenes* was confirmed by PCR in 6 isolates of raw poultry and one from each of fried and grilled samples. This study revealed that presence of *Listeria monocytogenes* in both grilled and fried poultry is an important public risk because of the great demand for daily consumption of these products.

Key words: *Listeria monocytogenes, raw poultry, cooked poultry, PCR.*

INTRODUCTION

The bacterium *Listeria monocytogenes* is gram-positive, motile organism capable of growth between – 0.4 and 50ºC and due to its ubiquitous character, *Listeria monocytogenes* easily enters the human food chain and may multiply rapidly (Farber and Peterkin, 1991).

Recent studies have confirmed the presence of *Listeria monocytogenes* in a wide variety of food stuffs. Milk (mainly unpasteurised), dairy products (especially soft ripened cheeses), poultry meat and their products and raw vegetables are considered to be the most frequently contaminated with *Listeria* (FAO, 2000).

The association between the consumption of cooked poultry products and several cases of listeriosis in England and the United States, together with the finding that *Listeria monocytogenes* was present in 12-27% of such products, indicates that ready to-eat food may constitute a public health risk (Kaczmarski and Jones, 1989). The presence of microorganisms in ready to eat food is a result of lack of hygiene because *Listeria monocytogenes* was noticed in slaughter animals and human faeces (Furowicz, 1992). The heating processes as cooking and pasteurization should eliminate *Listeria*.

Most healthy humans are not significantly affected by the intake of small numbers of *Listeria monocytogenes* in foods. However, certain sections of the population are predisposed to the development of listeriosis due to the presence of existing chronic illness, suppression of the immune system, pregnancy, or extreme youth or age under 1 year or over 60 years (Lorber, 1990). This presents a significant public health problem because in such section of the population, listeriosis is fatal in up to 30% of cases (Farber and Peterkin, 1991; Jones and MacGowan

1995).

Because of the public health significance of *Listeria monocytogemes*, the present study was undertaken to determine the prevalence of the organism in both raw and cooked poultry with using multiplex PCR as a rapid, specific and reliable means for identification of the organism.

MATERIALS and METHODS

Collection of samples:

One hundred and twenty samples of poultry were examined: 40 raw, 40 grilled and 40fried poultry samples were collected from different slaughter establishment and different restaurants in Assiut City. The samples were analyzed on the day they were collected for isolation of *Listeria monocytogenes* according to FDA (2011).

Isolation

25 g of each sample were aseptically added to 225 ml. Listeria Selective Enrichment broth (LSEB) and mixed thoroughly then incubated at 30ºC for 24-48h. and next the broth was cultured on Oxford Agar. After 24-48h of incubation at 35ºC the colonies morphologically resembling *Listeria* were submitted to confirmatory examination.

Identification

Suspected *Listeria* colonies (black with black halo on esculin-containing media and blue on Aloa agar media) were examined by Gram stain, for shape, arrangement of bacteria and its staining reaction. The organism was cultured onto semisoled media to observe umbrella-shaped motility, haemolysis on sheep blood agar and CAMP test**,** (Quinn *et al.,* 1994)

For further confirmation of *Listeria monocytogenes*, the isolates were inoculated into 0.5%carbohydrate broth fermentation media of Mannitol, L-Rhamnose, D-xylose and dextrose.

Nine strains for which expressing these standard features were selected (7 strains from raw poultry and one strain from each of grilled and fried poultry) and examined according to PCR technique.

DNA extraction:

Total DNA was obtained from the nine selected strains of confirmed isolates. Each strain was incubated overnight in Tryptose Soya Broth and the bacteria from 1ml. of this culture were centrifuged

and the pellet was resuspended in 100ml of distilled water. Then 100ml of 2% triton X-100 were added. The contents were incubated at room temperature for 10 min and the tubes were boiled for next 10 min. following incubation, the tubes were centrifuged for 5 min at 13.000xg. DNA containing supernatant was used in PCR. (Agresborg *et al.,* 1997).

Oligonuceoltide:

For identification of *Listeria monocytogenes* using RCR, two oligonuceoltide primers were selected based on the PrFA (transcriptional activator of the virulence factor) gene for *Listeria monocytogenes*, as described by Germini *et al.* (2009).

Table 1: Oligonucleotide sequences used for identification of *Listeria monocytogenes* by PCR.

Target gene	Primer sequence (5´-3´)	Amplified fragment length	Reference
PRFA gene	LIS-F:TCA TCG ACG GCA ACC TCG G LIS-R:TGA GCA ACG TAT CCT CCA GAG T	217 bp	Germini *et al*. (2009)

PCR reaction conditions:

The PCR was performed in total volume of 25µl, using 2 µl of extracted DNA as template. Each reaction mixture contained 12.5 µl. GoTag Green Master Mixture (Promega, M 7122), 1µl of 500 Pmol Forward primer (LIS-F), 1µl of 500 Pmol reverse primer (LIS-R) and 8µl of ultra-Pure DNase/ RNase –Free Distilled water (Gibco, Grand Island, Ny, USA). All tubes were overlaid with liquid parafin (2 drops) to avoid evaporation during thermal cycling, (Ependrof thermal cycler, Germany)

Amplification:

DNA was amplified by temperatue cycling through the following temperature profile: preincubation at 95ºC for 5 min, 40 cycles of 95ºC for 30s (denaturation), 54ºC for 30 s (annealing) and 72º for 30s (amplification), with a final cycle extending amplification conditions to 72ºC for 5 min.

Amplified products were kept at 4ºC and resolved by horizontal

agarose gel electrophoresis 1%, gels were stained by immersion in 2µl ethidium bromide solution for 30 min, washed briefly in running tap water, then detected under a short-wavelength UV light and photographed with EDVOTEK Gel documentation system (Germini *et al.*, 2009). The 1-KB plus DNA ladder (Invitrogen) was used as molecular size marker.

RESULTS

The obtained results were recorded in Tables 2 and 3.

Table 2: Prevalence of *Listeria* spp. and *Listeria monocytogenes* in raw, grilled and fried poultry samples.

Type of samples	No. of examined samples	+ve samples of Listeria.spp		+v samples of L.monocytogenes	
		No	%	No	%
Raw poultry	40	20	50	14	35
Grilled poultry	40	6	15	5	12.5
Fried poultry	40	3	7.5	1	2.5
Total	120	29	24.2	20	16.6

Tables 3: Results obtained in multiplex PCR.

Type of samples	No.of isolated strains of L.monocytogenes	No.of strains confirmed byPCR	No.of +ve strains out byPCR
Raw poultry	20	7	6
Grilled poultry	6	1	1
Fried poultry	3	1	1
Total	29	9	8

Identification by PCR:

Fig. 1: agarose gel electrophoresis of amplication products obtained from genomic DNA of *Listeria monocytogenes* isolated from the examined samples (217 bP PCR product).
Lane l: Molecular size marker.
Lane 2-10: +ve positive for *Listeria monocytogenes* except lane six with is negative for *Listeria monocytogenes*.
Lane 11: Positive control. (Obtained from Department of Medicine Microbiology, College of medicine-Assiut)
Lane 12: Negative control.

DISCUSSION

Cooking is the process of producing safe and edible foods.It is clear that cooking has been around for a long time and continues today to play a fundamental role in daily life across the globe. Cooking was first used for preservation but it has evolved and now it is a form of entertainment and creativity for many people. The fundamental types of

cooking are grilling and frying. Grilling is cooking of food using a direct dry heat while frying is the cooking of food in oil or fat. Common types of food that are grilled and fired include fish, meat and chicken (EUFIC 2010).

Fried chicken is an important food served at almost all fast foods restaurant chains. Surface appearance and texture are the most significant factors for consumer acceptability. Most foods cook rapidly and develop golden colour, crisp texture and good flavour at the frying temperatures between 160 and 90º (EUFIC 2010).

As shown in Table 2, 20 of 40 raw poultry meat (50%) were found to contain *Listeria* spp. This trend is higher than that recorded by Mahmood *et al.* (2003) (12.5%) and Lihan (2007) (34.8) while Lorna and Arthur (1994) and Katarzyna *et al.* (2004) recorded higher percent of *Listeria* spp. than our study (91 and 61.4%, respectively).

From the same table the incidence of *Listeria monocytogens* in raw poultry (35%) was higher than that obtained by Marinsek and Grebenc (2002) (15.78%), Mahmoood *et al.* (2003) (5%), Gudbjornsdottir (2004) (22.2%), Katarzyan *et al.* (2005) (13.9%), Hindy (2006) (8%), lihan (2007) (24.1%) while Ashraf *et al.* (2010) couldn`t isolate *Listeria monocytogenes* from any of 25 samples of frozen breast fillet examined.

On the other hand, higher result are recorded by Rama *et al.* (1994) (60%), Lorna and Arthur (1994) (59%), Miettinen *et al.* (2001) (62%) and Cristina *et al.* (2004) (60%). While other investigations of poultry meat obtained by some authors confirmed our results, they recorded nearly similar percentage as Uyttendele *et al.* (1999) (38.2%), Capita *et al.* (2001) (32%) and Vitas *et al.* (2004) (36.1%).

Several studies showed that rates of *Listeria monocytogenes* in raw chicken varied between 23% and 60% (Pini and Gilbert, 1988; Skovgaard and Morgan, 1988).

The presence of *Listeria monocytogenes* in raw poultry cannot be considered as important as in grilled and fried one since raw poultry are normally cooked or pasteurized before consumption. It has been demonstrated that normal pasteurization processes are effective in the destruction of this pathogen so conventional cooking would also be expected to eliminate this organism (Norrung, 2000).

From the tabulated data in Table 2, the percentage of *Listeria* spp. in grilled poultry was 15%, which is somewhat in agreement with that of Wilson (1995) who recorded an incidence of 11% in ready to eat chicken. As for fried poultry 7.5% contained *Listeria* spp. which is

nearly similar to the results detected by Lorna and Arthur (1994) who noticed the organism in 8% of cooked poultry.

Listeria monocytogenes as presented in Table 2 was recovered from 12.5% of grilled poultry, while Diaz-lopez *et al.* (2011), could not detect the same organism in the same product. Also this table revealed that 2.5% of *Listeria monocytogens* were isolated from fried poultry, whereas Meldrum *et al.* (2010) reported the isolation of the organism from 0.19% of chicken sandwiches (fried chicken).

On the other side, the obtained results were in disagreement with Lorna and Arthur (1994) and Katarzyna *et al.* (2005) who could not detect the organism in cooked poultry.

It is worth to mention that previous surveys on cooked and ready to eat poultry, showed that 12 to 27% of the samples were found to contain *Listeria monocytogenes* (Gilbert *et al.,* 1989; Kerr *et al.,* 1990; Ribeiro and Burge, 1992). In addition Rama *et al.* (1994) and Tareq *et al.* (2010) could detect the organisms in 22% of ready to eat chicken and in 7.8% in chicken shawirma (fried chicken) respectively.

In this study presence of *Listeria monocytogenes* in both grilled and fried poultry products may be due to spreading the organism by contact with infected surface or product during food preparation or may be due to insufficient cooking. Various researchers clamed that, poor hygiene practice and cross-contamination between raw and cooked products during food handling is one of the major factors for the outbreak of food borne illness (Schuchat *et al.,* 1992; Salvat *et al.,* 1995; Speirs *et al.,* 1995; Scott, 1996).

It is recommended that, application of good hygienic measures during preparation and good handling is essential to safe the quality of cooked poultry. In addition poultry should be cooked to an internal temperature of 70ºC for more than 20 minutes to ensure destruction of *Listeria monocytogenes* confirmed by PCR.

Amplification products obtained when the genomic DNA from these strains were subjected to PCR by using two primer sequences, 8 out 9 tested strains samples were displayed the characteristic PCR product at (217pb), that *Listeria monocytogenes* were confirmed in 6 raw poultry, 1 grilled sample and 1 fried sample (Table 3).

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