International Journal of Science, Environment and Technology, Vol. 5, No 4, 2016, 2432 – 2437

ISSN 2278-3687 (O)
2277-663X (P)

# SCREENING OF CHICKEN MEAT FOR *LISTERIA MONOCYTOGENES* BY POLYMERASE CHAIN REACTION IN CHENNAI

**Satheesh Raja S[1*], V. Apparao[2], R. Narendra Babu[3], A. Aravinth[4] and M. Thamizhannal[5]**

[1]Postgraduate Student, Dept of Meat Science and Technology
[2]Professor, Dept of Meat Science and Technology
[3]Professor, Dept of Meat Science and Technology
[4]Postgraduate Student, Dept of Poultry Science
[5]Postgraduate Student, Dept of Meat Science and Technology
Madras Veterinary College, TANUVAS, Chennai (Tamil Nadu), India
E-mails: drsatheeshtyke@gmail.com (**Corresponding Author*); [2]varao1966@yahoo.com; [3]nbabu.ravindran@gmail.com; [4]draravinthvet10@gmail.com; [5]thamizhlpt@gmail.com;

**Abstract:** A study was conducted to identify the presence of *L.monocytogenes* in chicken meat by polymerase chain reaction. 40 samples were collected from different areas of chennai and were tested for the presence of *L.monocytogenes* by targeting *prfA* gene with 290bp by PCR. None of the samples were shown to be positive for *L.monocytogenes*. The test reveals that the processing can be performed hygienically.
**Keywords:** Chicken meat, *L.monocytogenes*, Polymerase Chain Reaction.

## Introduction

Microbial quality of meat is an important aspect for industries engaged in production, processing and distribution. Meat can be contaminated at various stages of production by most of the microorganisms. *Listeria monocytogenes* is widely distributed and found in many food commodities. *Listeria monocytogenes* has also been found in raw or processed foods like meat and seafood (Gugnani, 1999; Meng and Doyle, 1997). Listeriosis is a food borne infection with flu-like symptoms in healthy people and severe complications in immune-compromised children, pregnant women and elderly person (Pesavento *et al.* 2009). It is very persistent microorganism that survives on surfaces and equipment of food processing units in conditions of insufficient cleaning. Post processing contamination is the major source and cross contamination may also occur at the retail shop and also in products due to improper hygienic practices. Ingestion of uncooked meat contaminated during processing can produce infection. *L. monocytogenes* was found prevalently high in poultry meat (24.5%), intermediate in beef meat (24.4%) and less prevalent in pork meat (21.4%). Hence a study is

*Received July 15, 2016 * Published Aug 2, 2016 * www.ijset.net*

planned to conduct a screening of chicken meat for the presence of *L.monocytogenes* in different zones of Chennai with PCR.

**Material and Methods**

Around 40 chicken meat samples were collected from different retail outlets of Chennai city. The samples placed in sterile polythene bags and transported hygienically to the Department of Meat Science and Technology, Madras Veterinary College, Chennai – 7 in clean insulated box with ice packs. Before screening, 25 gram of meat sample was homogenized in 225 ml of BPW and incubated at 37°C for 18 hours. The meat homogenate obtained was then subjected to DNA extraction using Bacterial DNA extraction kit and PCR analysis for the presence of *L.monocytogenes* by targeting *prfA* gene with 290bp. A 20 µl of reaction mixture was set up in 0.2 ml PCR tube with following components such as master mix - 10µl, forward primer-1 µl, reverse primer-1 µl, template DNA-1 µl and nuclease free water-7 µl. The PCR amplification was carried out in Master Cycler Gradient Thermo cycler (M/s. Eppendorf, Germany) with the following cycling conditions of initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation (94°C for 30 seconds), annealing (52°C for 30 seconds) and extension (72°C for 30 seconds) and subsequently a final extension at 72°C for 7 minutes. The PCR product obtained was subjected to electrophoresis in 2% Agarose gel. Ethidium bromide with concentration of 10mg/ml was added at the rate of 5µl / 100 ml of Agarose. Electrophoresis is carried out using 1X TAE buffer at 100 volts for 30 minutes. The gel was viewed under UV illuminator and documented using gel documentation system.

**Result**

A total of 40 chicken meat samples were collected from different retail outlets of Chennai. The meat homogenate obtained was subjected to DNA extraction using Bacterial DNA extraction kit and the developed PCR was used to detect *Listeria monocytogenes*. None of the sample showed positive for the presence of *Listeria monocytogenes* in the retail chicken meat by PCR (Figure. 1&2). Screening of chicken meat from different zone wise details were given below (Table. 1).

**Table 1**

**Screening of chicken meat samples collected from different retail outlets of Chennai**

| Zone | Name | No. of samples | No. of positive samples by m-PCR |
|---|---|---|---|
| | | | *Listeria monocytogenes* |
| 1 | Thiruvottiyur | 6 | - |
| 2 | Madhavaram | 6 | - |
| 3 | Royapuram | 6 | - |
| 4 | Ambattur | 6 | - |
| 5 | Anna nagar | 6 | - |
| 6 | Teynapet | 5 | - |
| 7 | Kodambakkam | 5 | - |
| **Total** | | **40** | 0 |

**Figure- 1**

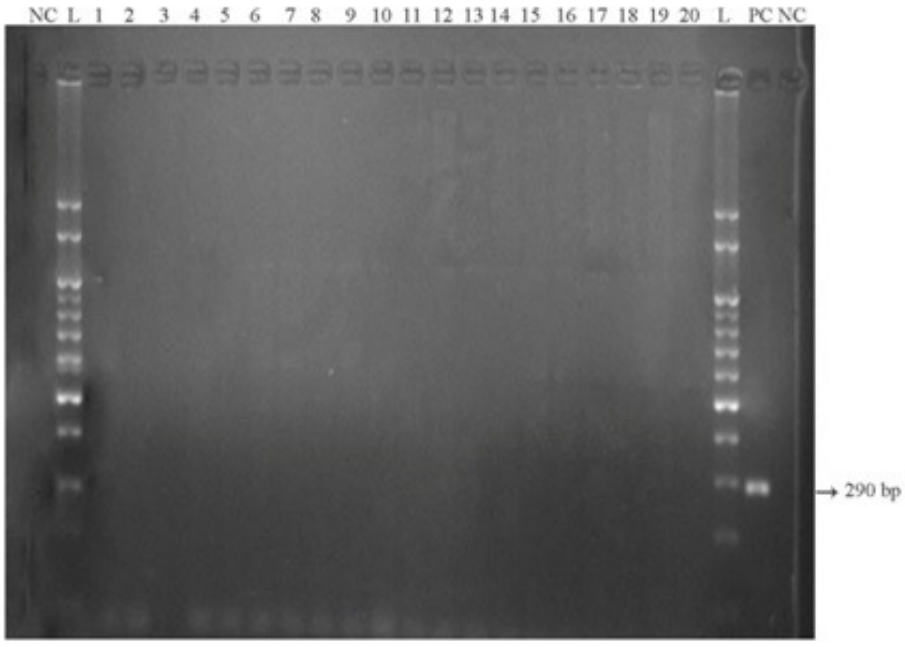
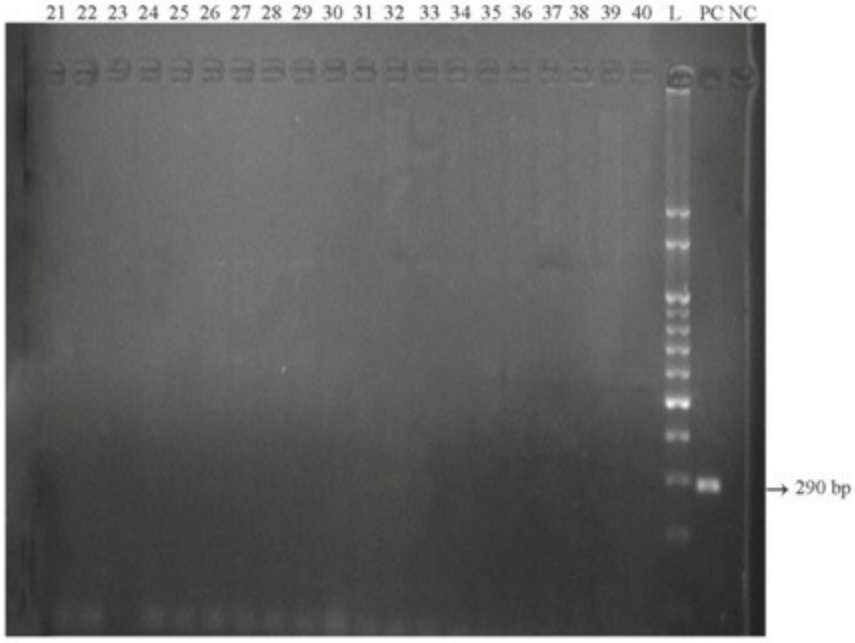


**Figure-2**




**Note:** L: 100 bp DNA Ladder, 1-20, 20-40: Sample result showing absence of *L. monocytogenes* in chicken meat, PC: Positive control of *L. monocytogenes* with 290bp, NC: Negative control

## Discussion

In this study, screening of chicken meat samples from retail outlets were carried out to assess the usefulness of the PCR technique and the level of processing of retail chicken meat. The 40 chicken meat samples collected from different areas of Chennai city were not positive for *Listeria monocytogens*. Similarly He (Kozacinski *et al.,* 2006) conducted a study to assess the microbial quality of chicken meat in Croatian market and reported *L.monocytogenes* in chicken breast and skin (4.76%). The existence and control of Listeria species and *Listeria monocytogenes* in broiler's ceca, meat and skin at the retail outlets of Ismailia city were studied in Egypt. The results of the study revealed that 92, 42 and 70 percent of broiler's ceca, meat and skin respectively were contaminated with *Listeria spp.* From the *Listeria spp.* positive samples, *L. monocytogenes* were highest in ceca (60%, 30 of 50 samples), followed by skin (34%, 17 of 50 samples), and meat (16%, 8 of 50 samples) (Ahmed and Nashwa, 2010). Among the 443 samples from equipment and meat processing area of Brazil, the occurrence of *Listeria spp*., in the samples was 38.1% of which 51.4% were from equipment, 35.4% from installations and 30.2% from products. The identified species were: *L. monocytogenes* (12.6%), *L. innocua* (78.4%), *L. seeligeri* (1.2%), *L. welshimeri* (7.2%) and

*L. grayi* (0.6%). The identified serotypes of *L. monocytogenes* were 1/2a and 4b. Finally they concluded that the equipment and installations are sources of contamination for *Listeria spp*. especially *L.monocytogenes* in the processing of beef and meat products (Barros *et al.,* 2007). Screening of food samples like ground meat, beef and pork revealed the presence of 10 copies /µl of *Listeria monocytogenes* (Zhang *et al.* 2009). He detected $10^5$ CFU/ml of *Listeria monocytogenes* in 500 µl of pre-enrichment broth without incubation and 3 x $10^1$ CFU/ml after incubation (Barocci *et al.,* 2008). *Listeria monocytogenes* was detected in pork sausage and mozzarella cheese at a contamination level of 1 CFU/g before culture enrichment (Amagliani *et al.,* 2007). This study reveals that PCR is one of the effective and time saving methods to detect micro organism. Hence the process is carried out in a hygienic manner or there may be some other reason for the absence of microorganism in chicken meat. A study was conducted to detect the prevalence of Listeria spp. in raw chicken and ready-to-eat (RTE) chicken products in Amman, Jordan and they found that *L. monocytogenes* was present in 9.4% of fresh dressed broiler chickens, 13.3% of RTE chicken-shawirma, 76.7% of RTE chickenburger, and 30% of RTE chicken-sausages whereas mortadella samples were free of *L. monocytogenes.* (Osaili *et al.,* 2011).

**References**

[1] Ahmed, M.A. and Nashwa, M.A. (2010). Existence of *Listeria* species in broiler carcasses with an attempt to control *Listeria monocytogenes* using trisodium phosphate. *African Journal of Food Science*, 4: 46-51.

[2] Amagliani, G., Giammarini, C., Omiccioli, E., Brandi, G., Magnani, M. (2007). Detection of *Listeria monocytogenes* using a commercial PCR kit and different DNA extraction methods. *Food Control*, 18: 1137–1142.

[3] Barocci, S., Calza, L., Blasi, G., Briscolini, S., Curtis,D.M., Palombo, B., Cucco, L., Postacchini, M., Sabbatini, M., Graziosi, T., Nardi, S. and Pezzotti, G. (2008). Evaluation of a rapid molecular method for detection of *Listeria monocytogenes* directly from enrichment broth media. *Food Control*, 19: 750–756.

[4] Barros, M.A.F., Nero, L.A., Silva, C.L., Loredana, O., Monteiro, F.A., Tamanini, R., Fagnani, R., Hofer, E. and Beloti, V. (2007). *Listeria monocytogenes*: Occurrence in beef and identification of the main contamination points in processing plants. *Meat Science*, 76: 591–596.

[5] Gugnani, H.C. (1999). Some emerging food and water borne pathogens. *Journal of Communicable Disease*, 31: 65– 72.

[6] Kozacinski, L., Hadziosmanovic, M. and Zdolec, N. (2006). Microbiological quality of poultry meat on the Croatian market. *Veterinarski arhiv,* 76: 305-313.

[7] Meng, J. and Doyle, M.P. (1997). Emerging issues in microbiological food safety. *Annual Review of Nutrition,* 17: 255– 275.

[8] Osaili M.T., Alaboudi A.R. and Nesiar 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: 586-590.

[9] Pesavento, G., Ducci, B., Nieri, D., Comodo, N. and Nostro, A.L. (2010). Prevalence and antibiotic susceptibility of *Listeria spp*. isolated from raw meat and retail foods. *Food Control*, 21: 708–713.

[10] Zhang D., Zhang, H., Yang, L., Guo, J., X.L and Feng, Y. (2009). Simultaneous detection of *Listeria monocytogenes, Staphylococcus aureus, Salmonella enterica* and *Escherichia coli O157:H7* in food samples using multiplex PCR method. *Journal of Food Safety*, 29: 348–363.