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Isolation and detection of *Listeria monocytogenes* in chicken meat marketed in retail outlets by using simplex PCR

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Abstract
A study was undertaken to screen chicken meat samples collected in retail outlets of Chennai city for the prevalence of *Listeria monocytogenes.* Microbiological tests for preliminary screening along with simplex PCR technique by using specific primer was performed to detect *L. monocytogenes* in the samples. The samples were collected from different corporation regions of Chennai and were tested for the presence of *L. monocytogenes* by targeting prfA gene with 290bp length by simplex PCR. *Listeria spp.* was isolated from 15 (25%) out of 60 samples. 14 isolates of *Listeria spp.* were identified as *L. monocytogenes* through developed PCR technique. This PCR technique has demonstrated to be a rapid, sensitive, reliable and appropriate method for the routine analysis of *L. monocytogenes*. From this study it can be concluded that it is imperative to control the contamination of poultry meat by every means following hygienic practices during processing, storing and transport of chicken meat.

Keywords: *Listeria monocytogenes*, Chicken meat, prf A, Simplex PCR

Introduction
Listeria monocytogenes which cause Listeriosis in humans is an important food borne pathogen having paramount importance in public health. This organism is ubiquitously found in environments such as intestines of healthy humans or animals, household environment, food products, soil, water and silage or other decaying vegetation [1]. Unprocessed animal foods like raw milk, meat, poultry, and fish are common source to humans [2, 3]. Unhygienically processed meat can be an important source of contamination, because this bacterium is often present in these foods and has the potential to survive and multiply under cold or abusive storage conditions [4]. The growth of L. monocytogenes has been reported at very low temperature (–1.5 ºC to 1 ºC) [5, 6].

L. monocytogenes is reported to cause clinical manifestations like gastroenteritis, abortion, stillbirth and neonatal meningitis or sepsis in human beings[7]. Listeriolysin O (LLO) is a hemolysin toxin responsible for *L. monocytogenes* pathogenesis[8]. In an outbreak caused by consuming hot dogs in the United States, illness was attributed to contamination levels of 0.3 cfu/g of the product [9].

Currently, the United States has a zero tolerance policy regarding the presence of *L. monocytogenes* in different foods [10]. Hence, the optimal method for Listeria detection should be sensitive enough to detect pathogens at levels as low as 1 cell/g of foods [11]. The conventional methods for the isolation and detection of *L. monocytogenes* in meat foods involves homogenization of the meat sample in a sterile stomacher bag along with some pre enrichment media which allows washing of the bacteria of target into the enrichment media and then the resultant homogenate containing target organism is incubated for 24 hours for further multiplication of the organism. After incubation, an aliquot of broth culture is taken for further selective culturing of organisms [12, 13].

Simplex PCR is a rapid alternative method which allows an accurate and unambiguous identification of microorganisms and their toxins. PCR-based detection of *L. monocytogenes* is more sensitive than culture-based methods for detecting the pathogen in contaminated food samples [14]. Occurrence of Listeria species has been reported in Egypt in chicken meat products by PCR targeting prfA gene which is a transcriptional activator of the virulence factor of the organisms [15]. Some other authors are also reported the prevalence of Listeria targeting

Listeriolysin O gene and 16S rRNA gene of *Listeria* spp. in chicken meat in Poland [16], by targeting 16S rRNA gene and hlyA genes in chicken meat in Malaysia [17], based on prs and hly A gene sequences in chicken meat in Hatay province [18] respectively.

Keeping in view of the above facts a study has been taken up to screen chicken meat samples for the presence of *Listeria monocytogenes* in raw chicken meat samples collected from different retails outlets in Chennai.

Materials and Methods

Collection of samples

Fourty raw chicken meat samples are obtained from various retail outlets and supermarkets in different corporation zones in Chennai, India from March to June 2017. Samples were collected in separate sterile zip lock covers to prevent cross contamination. The samples were brought to the laboratory in ice packed containers and were kept in a refrigerator at 4 °C until testing within three hours. These chicken meat samples were further analyzed for the presence of *Listeria monocytogenes.*

Pre-enrichment and selective enrichment

25 gram of meat sample was taken along with 225 ml of buffered peptone water in a sterile stomacher bag and homogenized to obtain a 1:10 sample dilution. Resultant homogenate was incubated at 30 ºC for 24 hours. Then 1 ml of the homogenate was taken into 9 ml of Listeria identification broth base (PALCAM broth, Himedia) in tubes and incubated at 37°C for 48± 2hr. After incubation a loopful of culture was taken from the tubes and streaked onto selective Polymixin Acriflavin Lithium Chloride Ceftazidime Asculin Mannitol (PALCAM) agar and incubated at 37 °C for 24 hours. Formation of grey-green and shiny colonies with diffuse black shadow on PALCAM agar was suspected as Listeria species (Plate 1). Motility, Gram's staining, oxidase and catalase tests were performed for preliminary conformation of Organism.

Plate 1: *Listeria monocytogenes* colonies on PALCAM agar

DNA extraction

The DNA from Listeria was extracted from colonies grown on PALCAM Listeria agar plates by boiling method as per the procedure outlined by earlier researchers with slight modifications [19]. One loopful culture of suspected *Listeria monocytogenes* colonies from agar plate was suspended in 100 microlitre of sterile nuclease free water in a 2 ml micro centrifuge tube. Resultant bacterial suspension was vortexed for 5 minutes. The bacterial suspension was boiled at 95-100 ºC for 10 minutes followed by immediate centrifugation at 15000 rpm for 10 minutes. The supernatant was collected and purified using a genomic DNA purification kit (Qiagen, Germany) which was used as a DNA template for PCR amplification. The concentration of DNA was measured by using Nanodrop® (Thermo Scientific) Spectrophotometer. The extracted DNA samples were stored at 4 ºC till further analysis.

PCR Amplification and Gel electrophoresis

Polymerase chain reaction assay targeting positive regulatory factor A (prfA) gene of *Listeria monocytogenes* has been carried out. Primers used in this study (L-Forward: 5' GAGCTATGTGCGATGCCACTT 3', L-Reverse: 5' ATTAGCGAGCAGGCTACCGCAT 3') for targeting *prf* A gene were custom synthesized (Eurofins Genomics India Pvt Ltd. Bangalore, India). A 20 µl of the PCR reaction mixture was made up in 0.2 ml PCR tube with components such as master mix - 10 µl, 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 cycling conditions of initial denaturation at 94 ºC for 5 minutes, followed by 30 cycles of denaturation at 94 ºC for 30 seconds, annealing at 52 ºC for 30 seconds, extension at 72 ºC for 30 seconds and subsequently a final extension at 72 ºC for 7 minutes. The PCR product obtained was subjected to electrophoresis in 1.5% agarose gel. Ethidium bromide with concentration of 10 mg/ml was added at the rate of 3 µl for 50 ml of agarose. Electrophoresis is carried out using 1X Tris Acetate EDTA buffer at 100 volts for 60 minutes. After running, the gel was viewed and captured with Gel doc system® (Thermo scientific).

Results and Discussion

A total of fourty different raw chicken samples were collected from retail outlets of three regions of Chennai city viz., north, south and central and subjected to screening for the presence of *Listeria* targeting prfA gene with 290 bp length by simplex PCR. In this study *Listeria spp.* was isolated from 15 (25%) out of 60 collected retail outlet samples. Out of 15 Listeria isolates, 14 isolates were identified as *L. monocytogenes* through developed PCR assay (Figure 1). The minimum level of DNA concentration for detection of *L. monocytogenes* by this assay was found to be 1 ng/µl in this study which was conclusive by taking into consideration of measured concentration of all positive samples. Identification of pathogens or spoilage organisms by nucleic acid identification methods in meat and meat products is gaining importance nowadays due to reliability, sensitivity of the method and rapidity in getting the result. The prevalence of *L. monocytogenes* as per results obtained in this study are less when compared to the prevalence reported by earlier workers [16] in which the *L. monocytogenes* was prevalent in 13 (52%) of 25 frozen chicken leg and 14 (56%) of 25 frozen chicken fillet samples. Another researcher[20] reported the distribution of *L. monocytogenes* as 8.33% in chicken, 16.66% in beef and 8.33% in chevon samples collected in retail markets of Mymensingh city, Bangladesh. Studies conducted in Thailand for prevalence of *Listeria monocytogenes* in raw beef, pork and chicken samples revealed more prevalence in chicken samples collected from open markets than samples from supermarkets[21]. Normally higher incidence of Listeria in chicken meat products have been mainly attributed to contamination caused by chopping board, knives, wash water, meat product processing equipment, other working surfaces

and more human contact [14]. Storage of such contaminated products under such low temperature conditions may further allow the growth of significant numbers of these organisms leading to food-borne illnesses among consumers [15, 16]. The persistence of the bacterium in the chicken meat outlets is the result of the ability of *L. monocytogenes* to form biofilms which is an important survival strategy. It forms biofilm on different equipment and other working surfaces where sanitary measures are poor which makes the organism difficult to eliminate with the consequent possibility of cross-contamination of meat [17]. Moreover in large commercial poultry processing plants chances of cross-contamination may take place during different stages of chicken meat processing especially during the pre-chilling and storage of carcasses which necessitates routine screening of meat samples for *Listeria spp*. as a surveillance strategy which is very important in safeguarding public health.

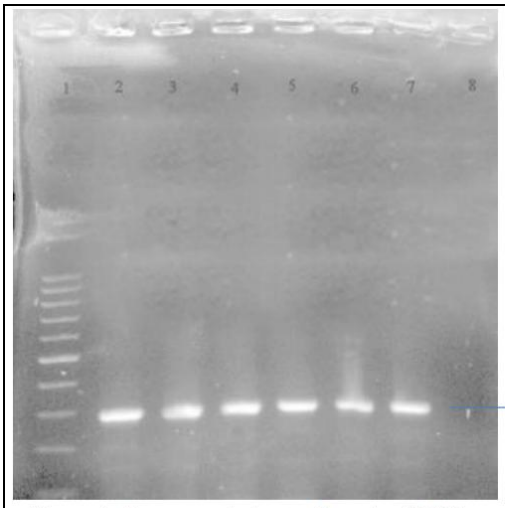

Figure 1: Agarose gel electrophoresis of PCR amplified products, Lane 2-7-Prf A gene *Listeria monocytogenes,* Lane 8-Negative control. DNA marker-100bp

Conclusion

This study has demonstrated the presence and distribution of *L. monocytogenes* in commercial chicken meat sold in different retail outlets of Chennai city. It is further recommended that it is imperative to control the contamination of *L. monocytogenes* in poultry meat by every means following hygienic practices during processing, storing and transport of chicken meat. It is also to emphasize that faulty handling of raw meat and consequent cross contamination in household practices is also an important criterion of concern.

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References

1. Ryser ET, Marth EH. Occurrence and survival of *Listeria monocytogenes* in natural environments. Listeria, listeriosis and food safety. Marcel Dekker, Inc., New *York, NY*, 1991, 22-33.
2. Doyle M, Donelly C, Wiedmann M. *Listeria monocytogenes* fact sheet. American Meat Institute. 2003. www.meatami.com.
3. Sofos J, Skandamis P, Stopforth J, Bacon T. Current issues related to meat borne pathogenic bacteria. Proceedings of the 56th American Meat Science Association Reciprocal Meat Conference. Columbia, Missouri. 2003, 33-37.
4. Buchanan RL, Stahl HG, Whiting RC. Effects and interactions of temperature, pH, atmosphere, sodium chloride, and sodium nitrite on the growth of *Listeria monocytogenes*. *Journal of Food Protection.* 1989; 52(12):844-851.
5. Grau FH, Vanderlinde PB. Occurrence, numbers, and growth of Listeria monocytogenes on some vacuum-packaged processed meats. *Journal of Food Protection.* 1992; 55(1):4-7.
6. Hudson JA, Mott SJ, Penney N. Growth of Listeria monocytogenes, Aeromonas hydrophila, and Yersinia enterocolitica on vacuum and saturated carbon dioxide controlled atmosphere-packaged sliced roast beef. Journal of Food Protection. 1994; 57(3):204-208.
7. Churchill RL, Lee H, Hall JC. Detection of *Listeria monocytogenes* and the toxin listeriolysin O in food. *Journal of Microbiological Methods.* 2006; 64(2):141-170.
8. Vazquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Dom-ı´nguez-Bernal G, Goebel W *et al*. Listeria pathogenesis and molecular virulence determinants. *Clin. Microbiol. Rev*. 2001; 14:584-640.
9. Donnelly CW. *Listeria monocytogenes*: a continuing challenge. *Nutrition Reviews.* 2001; 59(6):183-194.
10. McLauchlin J, Mitchell RT, Smerdon WJ, Jewell K. *Listeria monocytogenes* and listeriosis: a review of hazard characterization for use in microbiological assessment of foods. *Int. Jr. Food Microbiol*. 2004; 92:15-33.
11. Jantzen MM, Navas J, Corujo A, Moreno R, López V, Martínez-Suárez JV. Specific detection of Listeria monocytogenes in foods using commercial methods: from chromogenic media to real-time PCR. Spanish Journal of Agricultural Research. 2006; 4(3):235-247.
12. Hayes PS, Graves LM, Swaminathan B, Ajello GW, Malcolm GB, Weaver RE *et al*. Comparison of three selective enrichment methods for the isolation of *Listeria monocytogenes* from naturally contaminated foods. Journal of food protection. 1992; 55(12):952-959.
13. Ryser ET, Arimi SM, Bunduki MM, Donnelly CW. Recovery of different Listeria ribotypes from naturally contaminated, raw refrigerated meat and poultry products with two primary enrichment media. Applied and Environmental Microbiology. 1996; 62(5):1781-1787.
14. Aznar R, Alarcón B. PCR detection of *Listeria monocytogenes*: a study of multiple factors affecting sensitivity. Journal of applied microbiology. 2003; *95*(5):958-966.
15. El-Malek AMA, Ali SFH, Hassanein R, Mohamed MA, Elsayh KI. Occurrence of Listeria species in meat, chicken products and human stools in Assiut city, Egypt with PCR use for rapid identification of Listeria monocytogenes. Veterinary World. 2010; 3(8):353-359.
16. Kosek-Paszkowska, Katarzyna, Bania Jacek, Bystron J, Molenda Jerzy, Czerw Małgorzata. Occurrence of Listeria sp. in raw poultry meat and poultry meat products. Bulletin of the Veterinary Institute in Pulawy. 2005; 49:219-222.
17. Goh SG, Kuan CH, Loo YY, Chang WS, Lye YL, Soopna P *et al*. *Listeria monocytogenes* in retailed raw

chicken meat in Malaysia. Poultry science. 2012; 91(10), 2686-2690.
18. Elmali M, Can HY, Yaman H. Prevalence of *Listeria monocytogenes* in poultry meat. *Food* Science and Technology *(Campinas).* 2015; 35(4):672-675.
19. Ahmed SS, Tayeb BA, Ameen AM, Merza SM, Sharif YHM. Isolation and Detection of Listeria monocytogenes in Minced Meat, Frozen Chicken and Cheese in Duhok Province, Kurdistan Region by using RT-PCR. J Food Ind Microbiol. 2016; (2):109.
20. Islam MS, Husna AA, Islam MA, Khatun MM. Prevalence of *Listeria monocytogenes* in Beef, Chevon and Chicken in Bangladesh. American Journal of Food Science and Health. 2016; 2(4):39-44.
21. Indrawattana N, Nibaddhasobon T, Sookrung N, Chongsa-nguan, M, Tungtrongchitr A, Makino SI *et al*. Prevalence of Listeria monocytogenes in raw meats marketed in Bangkok and characterization of the isolates by phenotypic and molecular methods. Journal of health, population, and nutrition. 2011; 29(1):26.
22. Lowry PD, Tiong. The incidence of *Listeria monocytogenes* in meat and meat products factors affecting distribution. In Proc. 34th Int. Congress Meat Science and Technology. Part. B. 1988, 528-530.
23. Beumer RR, Te Giffel MC, De Boer E, Rombouts FM. Growth of *Listeria monocytogenes* on sliced cooked meat products. Food Microbiology. 1996; 13(4):333-340.
24. Walker SJ, Archer P, Banks JG. Growth of *Listeria monocytogenes* at refrigeration temperatures. Journal of applied microbiology. 1990; 68(2):157-162.
25. Gião MS, Keevil CW. *Listeria monocytogenes* can form biofilms in tap water and enter into the viable but non-cultivable state. *Microbial ecology* 2014; 67(3):603-611.