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Incidence of *Listeria monocytogenes* in Broiler Chicken Meat Sold in Ellapuram Block, Thiruvallur District of Tamil Nadu, India

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A B S T R A C T

In the present study, a total of thirty broiler chicken meat samples from different meat stalls at Ellapuram block, Thiruvallur district were screened for the presence of *Listeria monocytogenes* using conventional culture procedure and confirmed by Polymerase Chain Reaction (PCR). Of the thirty chicken meat samples screened, 11 isolates were presumptively identified as *Listeria* spp. and further confirmation by PCR revealed that none of the isolates were recognized as *L. moncoytogenes*.

Introduction

Tiruvallur is an administrative district in the South Indian state of Tamil Nadu. The district has a mixture of urban and rural characteristics. The Eastern part of Tiruvallur district is dominated by urban characteristics while the Southern and Northern part of the district has influence of Andhra culture due to its position. Approximately more than 70% of the population in the district is non-vegetarian where consumption of chicken meat predominates. With respect to poultry processing, 'wet market' system is largely being followed in which the birds, especially broiler chickens, are slaughter and dressed in small processing units adopting ritual slaughter procedures such as jatka/halal method and, sold to the consumer as hot meat. The later procedure usually lacks meat inspection to be carried out by qualified veterinarians. Further, lack of basic facilities, training and personnel hygiene of the butchers' involved etc., adds up to the microbial load of chicken meat. Bhaisare *et al.,* (2014) reported that poultry meats are often found contaminated with potentially pathogenic microorganisms such as Salmonella spp., Campylobacter spp, *S. aureus, E. coli etc., Listeria monocytogenes* is one such psychrotrophic pathogen and is widely distributed in the natural environment

and some strains can persist in processing plants with opportunities to contaminate and survive in RTE food (Todd and Notermans, 2011). WHO (2004) also revealed that *L. monocytogenes* strongly influences risk, though the extent to which growth occurs is dependent on the characteristics of the food and the conditions and duration of refrigerated storage. Further, stated that physical handling of foods may lead to contamination of food products due to the ubiquitous nature of *L. monocytogenes* and the packaged, long shelf-life food which is not heat-treated in the final package, represents the most critical food commodity group for *Listeria* contamination. Jalali and Abedi (2008) also stated that consumption of ready to eat food or raw and undercooked food is more potential risk for *L. monocytogenes* contamination. Several studies have also indicated the prevalence of *L. monocytogenes* in poultry meat, its products and processing environment (Elmali *et al.,* 2015; Goh *et al.,* 2012; Lawrence and Gilmour, 1994; Siriken *et al.,* 2014; Mahmood *et al.,* 2003; Crespo *et al.,* 2013). However, very scarce studies have been carried out in Tamil Nadu to assess the incidence of *L. monocytogenes* in broiler chicken meat.

Hence, the present study has been proposed with the objective to assess the prevalence of *L. monocytogenes* in broiler chicken meat sold in local markets of Thiruvallur District.

Materials and Methods

This study was performed at Department of Food and Industrial Microbiology, College of Food and Dairy Technology, Koduvalli to assess the incidence of *L.monocytogenes* in broiler chicken meat.

Collection of samples

The broiler chicken meat samples were collected from local markets at Ellapuram block, Thiruvallur District in clean polythene bags and were brought to the laboratory under refrigerated conditions (4±1°C).

Detection of *L.monocytogenes*

A total of 30 broiler chicken meat samples were collected and screened for the presence of *L.monocytogenes* using the standard procedure described in Bacteriological Analytical Manual, 2011 with modifications.

Pre-enrichment

25g of each sample was aseptically suspended in the stomacher bag containing 225ml of sterile Buffered listeria enrichment broth base (BLEB), so as to attain a final dilution of 1:10 (w/v). The inoculated medium was incubated at 30°C for 4 hours.

Enrichment

After the pre-enrichment, 2.5ml of rehydrated contents of the selective agent Listeria selective supplement II was added and incubated for another 44 hours at 30 ^{o} c. this supplement contains antifungal agents for selective cultivation of *Listeria* species.

For the preparation of Listeria selective supplement II, 5ml of sterile distilled water was aseptically injected into a single vial of Listeria selective supplement II for rehydrating the contents and then 2.5ml of the diluted supplement was aseptically added to the pre-enriched broth.

Selective plating

A loopful of inoculum from the enriched BLEB culture was streaked onto an esculin containing media, Listeria Oxford agar base w/v 1.2% agar. The plates were incubated at 35°C/48 hours. After incubation, the presumptive colonies of *Listeria* spp. (colonies

that appeared black with a black halo) were observed.

The Oxford agar was sterilised and the media was cooled to 45-50°C. at this temperature rehydrated content of 1 vial of Listeria Moxalactam supplement (modified) was added and mixed well.

Purification of cultures

Five or more typical colonies from Listeria oxford agar were picked and streaked on yeast extract added Tryptone Soya Agar plates in order to achieve purity and obtain typical isolated colonies. Purification is a mandatory step in the conventional analysis because isolated colonies on selective agar media may be in contact with an invisible weak background of partially inhibited competitors. Then the streaked plates were incubated at 35°C/48 hours.

Selection of suspected *Listeria* spp. colonies and identification

Characteristic *Listeria* colonies i.e. colonies on yeast extract added tryptone soya agar that appeared small, regular, smooth and dense/iridescent white coloured were picked up and stored in glycerol stock for PCR analysis.

Preparation of 50% glycerol stock

50% glycerol is prepared and required quantity of BLEB base (50ml) is prepared and sterilised. Take 2ml sterile Eppendorf tube and transfer 800µl of BLEB base and 200µl of 50% glycerol into it.

Storing of purified culture in glycerol stock

Typical colonies that were observed as characteristic *Listeria* colonies are identified and a loopful of isolated colony was inoculated into 50% glycerol stock solution and the Eppendorf tubes were sealed with parafilm tapes and stored at -18°C.

Revival of culture

Purified culture stored in 50% glycerol stock is revived in Listeria Oxford agar base w/v 1.2% agar added with 1 vial of rehydrated content of *Listeria moxalactam* supplement (modified) and was incubated at 35°C/48 hours. The plates which have characteristic *Listeria* colonies were selected for confirmation using PCR.

Polymerase chain reaction

A set of primers described by Ueda *et al.*, 2005 were used in this study. The details of the primers used are shown in the table below.

Standardization of PCR protocol

PCR amplification of hlyA gene fragment was setup in 25µl reactions. The PCR protocol was initially standardized by using standard culture of *L.monocytogenes* by optimizing the concentration of the components of the reaction mixture in the PCR assay and by varying the annealing temperature and cyclic conditions as shown in Table 2 below.

A 25µl of reaction volume which contain 12.5µl of PCR master mix, 1µl of each forward primer and reverse primers, 2µl of Template DNA and 8.5µl of Nuclease free water. The cyclic conditions comprise the following steps:

Agarose gel electrophoresis

On completion, the PCR amplified products were electrophoresed on agarose gel (1%). This is prepared by boiling agarose in an appropriate volume of 1X borate buffer (SBB). After cooling for about 5min, 5µl of

ethidium bromide (10mg/ml conc.) was added to the agarose solution to a final concentration of 10mg/ml. then molten agar was poured into the tray. The comb was filled into the slots on the tray. The tray was kept undisturbed till the gel had solidified. The comb was then taken-out carefully and the tray containing the gel was then placed in a submarine horizontal electrophoresis unit filled with 1X SBB buffer up to a level of 1mm above the gel surface.

About 10µl of each PCR product was mixed with 2µl of Bromophenol blue (6X) loading dye and loaded into each well. Electrophoresis was performed at 5 V/cm and the mobility was observed under UV trasnsilluminator to visualise the bands. The PCR product size was determined by comparing with a standard molecular weight marker and was photographed by the gel documentation System.

Results and Discussion

A study to assess the incidence of *L.monocytogenes* in broiler chicken meat. Samples was collected from different meat stalls at Thiruvallur district. From 30 samples examined, the incidence of *L.monocytogenes* and the results are presented below:

Isolation and Characterisation of *L.monocytogenes* from Broiler chicken meat

A total of 30 samples were collected from market in and around Thiruvallur district. The samples were screened for the presence of *L.monocytogenes* using conventional culture protocol mentioned in BAM, 2011 with modifications and confirmation was done using PCR Assay where species specific primer (hlyA gene) for *L. monocytogenes* was used. As per the protocol, the samples were enriched in BLEB and then streaked on Listeria oxford agar plates added with Modified Listeria Moxalactam supplement. The plates were incubated at 35 ^{o} C/48 hours. Characteristic colonies were confirmated using PCR.

Colony morphology

11 colonies that appeared black with a black halo were observed after incubation of streaked, supplement added Listeria oxford agar plates at 35 ^{o} C/48 hours under aerobic conditions (Fig 1). The colonies were circular and convex. Typical colonies were picked and streaked on yeast extract added Tryptone soya agar plates for purification and incubated at 35 ^{o} C/48 hours and all the 11 isolates showed typical Listeria colony morphology i.e. the colonies were small, regular, smooth, dense/iridescent white in colour (Fig 2).

PCR assay for the detection of *L.monocytogenes*

For the confirmation of *L.monocytogenes*, polymerase chain reaction assay was standardized employing a set of primer targeting species specific hlyA gene. Glycerol stocks of 11 isolates were enriched in Buffered Listeria Enrichment Broth using standard protocol. Then, the isolates were streaked on TSA agar and incubated at 35 ^{o} C/48 hours. The details of the primer set used for assay is mentioned in table 1.

Optimization of PCR reaction condition was done as per standardization protocol mentioned. On completion, amplified products were subjected to electrophoresis on Agarose gel (1%). After sufficient migration, the gels were observed under UV transilluminator to visualize the bands.

Table.1 Details of the primers used in the PCR

PCR target species	Primer	Tm (°C)	Sequence (5'to 3')	Size (bp)	reference
L. monocytogenes	hly A F	59.4	F-AATCTAGCACCACTCTCGGG	733 bp	**Ueda *et al.*, 2005**
	hly A R	57.3	R- TGTGACCTTCTTTTACGGGC		

Table.2 Different temperature and cycling conditions for the PCR assay

S. no	Step	Temperature	time
1.	Initial denaturation	95° C	5 mins
2.	Cyclic denaturation	95° C	1 min
3.	Annealing	58° C	1 min
4.	Extension	72° C	1 min
5.	Go to cycle 2-4 repeat 30 cycles		
6.	Final extension	72° C	10 mins
7.	Hold at 4°C		

Fig.1 Listeria oxford agar base before incubation

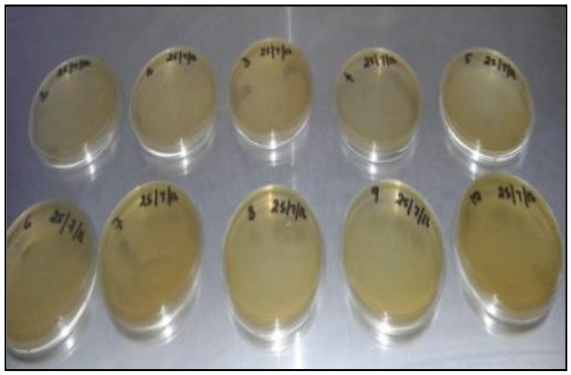

Fig.2 Black halo colonies of *Listeria* spp. on Listeria oxford agar base after incubation

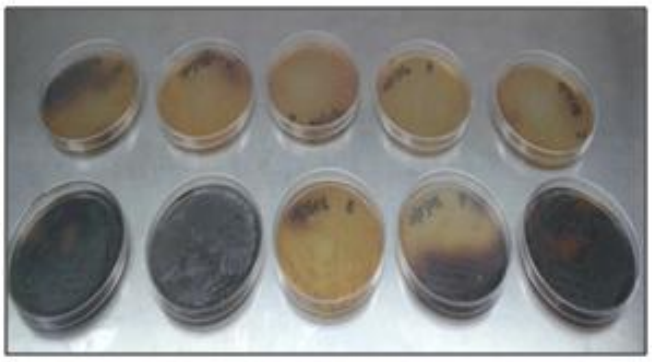

Fig.3 Purified colonies of *Listeria* spp. isolates on Tryptone soya agar

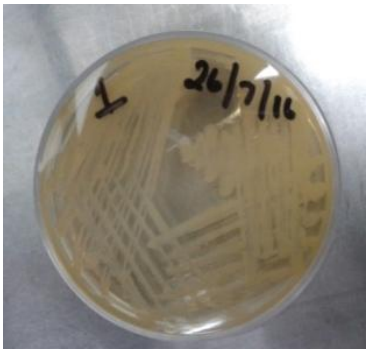

Fig.4 Isolated colonies stored in 50% glycerol stock

Fig.5 Electrophoresis of PCR amplified products on Agarose gel

Positive culture always yield a specific product size of 733bp which is specific for hlyA gene of *L.monocytogenes* whereas none of 11 isolates examined have shown any amplification which in turn indicates that all of the samples were negative for *L.monocytogenes*.

The result obtained in this study is in concordance with the results of Suriya Priya *et al.,* (2015) who reported that out of 150 traditional milk products screened none were positive for *L. monocytogenes*. Similarly, several authors (Kalorey *et al.,* 2005; Gudbjornsdottir *et al.,* 2004) have reported the low prevalence of *L. monocytogenes* in poultry meat and its products. As described by Curiale and Lewus (1994), the no incidence of *L. monocytogenes* in the present study might be an underestimation and the enrichment procedure used in the present study would have favored the growth of other non-pathogenic *Listeria* species.

References

Bhaisare, B. D., Thyagarajan, D., Richard Churchil, R., and Punniamurthy, N., 2014. Bacterial Pathogens in Chicken Meat: Review. International Journal of Life Sciences Research. 2(3):1-7.

Crespo R, Garner M. M., Hopkins S. G. and Shah D. H. 2013. Outbreak of *Listeria monocytogenes* in an urban poultry flock. BMC Veterinary Research. 9:204.

Curiale M. S. and Lewus, C. 1994. Detection of *Listeria monocytogenes* in Samples Containing *Listeria innocua*. Journal of Food Protection. 57(12):1048-1051.

Elmali, M., Can, H. Y. and Yaman, H. 2015. Prevalence of *Listeria monocytogenes* in poultry meat. Food Science and Technology Campinas, 35(4): 672-675.

Goh, S. G., Kuan, C. H., Loo, Y. Y., Chang, W. S., Lye, Y. L., Soopna, P., Tang, J. Y. H., Nakaguchi, Y., Nishibuchi, M., Afsah-Hejri, L. and Son, R. 2012. *Listeria monocytogenes* in retailed raw chicken meat in Malaysia. 91(10): 2686-2690.

Gudbjörnsdóttir, B., Suihko, M.-L., Gustavsson, P, Thorkelsson, G., Salo, S., Sjöberg, A.-M., Niclasen, O., and Bredholt, S. 2004. The incidence of *Listeria monocytogenes* in meat, poultry and seafood plants in the Nordic Countries.Food Microbiology. 21: 217-225.

Jalali, M. and Abedi, D., 2008. Prevalence of Listeria spp. in food products in Isfahan, Iran. International Journal of Food Microbiology, 122(3):336-40.

Kalorey, D. R. Barbuddhe, S. B. Kurkure, N. V. and Gunjal, P. S. 2005. XVII th European Symposium on the Quality of Poultry Meat Doorwerth, The

Netherlands. 172-173.

Lawrence, L. M. and Gilmour A. 1994. Incidence of Listeria spp. and *Listeria monocytogenes* in a poultry processing environment and in poultry products and their rapid confirmation by multiplex PCR. Applied and Environmental Microbiology. 60(12): 4600-4604.

Mahmood, M. S., Ahmed, A. N. and Hussain, I. 2003. Prevalence of *Listeria monocytogenes* in poultry meat, poultry meat products and other related in animates at Faisalabad.Pakistan Journal of Nutrition. 2(6): 346-349.

Siriken, B., Ayaz, N. D. and Erol, I. 2014. *Listeria monocytogenes* in retailed raw chicken meat in Turkey. Berliner und Munchener Tierarztliche Wochenschrift 127(1-2):43-49.

Todd, E. C. D. and Noterman, S. 2011. Surveillance of Listeriosis and its causative pathogen, *Listeria monocytogenes.* Food Control, 22: 1484-1490.

Ueda F, Anahara R, Yamada F, Mochizuki M, Ochiai Y, Hon do R (2005): Discrimination of *Listeria monocytogenes* contaminated commercial Japanese meats. International Journal of Food Microbiology 15, 455–462.

WHO, 2004. Microbiological risk assessment series5. Risk assessment of *Listeria monocytogenes* in RTE foods technical report.

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