Simultaneous detection of food safety hazards (*Salmonella enterica* and *Listeria monocytogenes*) in table eggs using multiplex PCR

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**Abstract**

Foods are analyzed for food safety hazards in order to comply with food safety standards. Among foodborne pathogens *Salmonella enterica* and *Listeria monocytogenes* are recognized as major foodborne pathogens of public health significance worldwide. In the present study, multiplex polymerase chain reaction (mPCR) was used to screen for *S. enterica* and *L. monocytogenes* in table eggs collected from different markets (840 eggs). Pooled egg samples categorized as commercial and backyard eggs based on the source of collection. Collected eggs were screened for *S. enterica* and *L. monocytogenes* using mPCR assays. Conserved regions *viz.* *invA* and *prfA* genes were targeted for the specific detection of *S. enterica* and *L. monocytogenes*, respectively. mPCR and conventional method showed same results, prevalence of *S. enterica* at 12.5 and 33.3 per cent in commercial and backyard eggs, respectively. While, *L. monocytogenes* was undetectable in commercial eggs; but, detected only in backyard table egg sample (8.3%). Present study indicated complete concordance between specific pre-enrichment mPCR and conventional cultural methods. Results of the study underscored mPCR as steadfast rapid tool for the screening of table eggs for listed food safety hazards *S. enterica* and *L. monocytogenes* in table eggs.

**Keywords:** Eggs; Listeria; mPCR; Public Health; Salmonella
Introduction

*Salmonella enterica* and *Listeria monocytogenes* are the major food safety hazards of public health concern often implicated in foodborne disease outbreaks (Singh *et al*., 2012). Salmonellosis, caused by *Salmonella enterica*, a disease of economic significance having implications on the food industry and it is primarily associated with poultry and poultry products. Genus *Salmonella* comprises of over 2,600 serovars and it is the second largest cause of food poisoning in the world (Gal-Mor *et al*., 2014; Salmonellae can also be transmitted from animals to humans through food; occasionally, person to person transmission also occurs through the faeco-oral route in addition to prevalent sporadic cases and it also cause severe extra-intestinal infections such as bacteremia and meningitis (Borges *et al*., 2018). Salmonellae gets transmitted vertically among poultry, has longer persistence in the environment and poultry products are often contaminated with Salmonellae (Afshari *et al*., 2018). Likewise, *L. monocytogenes* is a ubiquitous gram-positive bacterium widespread in the environment such as soil, water, animal gut, etc. *L. monocytogenes* grows over wide pH (4.39 to 9.40) range, refrigeration temperatures and is associated with listeriosis in human and animals (Singh *et al*., 2012). Among 17 *Listeria* species, *L. monocytogenes* has been identified as the most significant member owing to its pathogenic nature to humans and animals responsible for food-borne infections, meningitis, encephalitis and febrile gastroenteritis (Barbuddhe *et al*., 2002).

A rapid and sensitive method for detection of these pathogens will be absolutely useful for taking decision at field level about acceptance or the rejection of table egg lots. In the last decade, molecular techniques have appeared as most promising alternatives than conventional cultural methods in food microbiology. Conventional culture methods require series of procedure which consumes more time and are laborious. In such cases, use of molecular-based techniques thwart the delay and permits to detect the pathogens with greater sensitivity and reliability than conventional culture methods (Germini *et al*., 2009; Rattanachaikunsopon and Phumkhachorn, 2012). A rapid and sensitive assay like polymerase chain reaction (PCR) based method for identification of the food borne pathogens is proven and even several pathogen can be detected in a single reaction by multiplex PCR or mPCR (Soumet *et al*., 1999).

Therefore, present study was designed with the objectives of simultaneous detection of *S. enterica* and *L. monocytogenes* using mPCR assay targeting *invA* and *prfA* genes respectively and comparison of mPCR with that of conventional method.

Materials and Methods

**Sample collection:** Table egg (360) were collected from different markets comprising of commercial layer farms (240) and backyard rearing (120) systems. Egg samples were collected from retail markets into sterile polybags and transported to the laboratory immediately. Samples were collected on four occasions at an interval of 15 days and analyzed for *S. enterica* and *L. monocytogenes* using the multiplex PCR.

**Sample preparation:** Pool of ten eggs selected from each batch were surface sterilized with 70% ethanol, broken under aseptic conditions and whole egg contents were mixed in bag for 3 minutes using stomacher (Bagmixer®, Interscience). Ten milliliters of resultant egg homogenates were then transferred into two separate sterile polybags containing 90 milliliters of buffered peptone water (BPW) or half Fraser Broth (hFB) for the primary enrichment (1:9) of *S. enterica* or *L. monocytogenes*, respectively. Bags containing BPW or hFB were mixed well in stomacher for 4 minutes and incubated at 37 °C and 30 °C for 24 hours for *Salmonella* and *Listeria*, respectively.

**DNA isolation from primary enrichment:** DNA was extracted from pre-enriched samples using snap chill method (Manoj *et al*., 2014). Briefly, 2 mL pre-enriched broth (BPW or hFB) were centrifuged at 6,000 rpm for 10 minutes; resultant pellet containing bacterial cells was washed with sterile phosphate buffered saline and re-suspended in 50 μL nuclease free water. After boiling for 100 °C for 10 minutes, putative suspensions were subjected for one freeze-thaw cycle (-20 °C for 15 min.). Thawed suspension was centrifuged at 6,000 rpm for 10 minutes and supernatant collected into a fresh tube was used as template DNA for PCR.

**Detection of *S. enterica* and *L. monocytogenes* in primary enrichment broth by PCR:** For the detection of *S. enterica* and *L. monocytogenes* in the primary enrichment broth (BPW/hFB) multiplex PCR assay performed targeting...

Multiplex PCR was performed in 25 µL volume comprised of 12.5 µL of 2Xmaster mix (Himedia®), 0.5 µL of each primers [Salmonella genus specific invA gene primers 5’-AAT TAT CGC CAC GTT CGG GCA A-3’ and 5’-TCG CAC CGT CAA AGG AAC C-3’; L. monocytogenes specific prfA primers 5’-TCA TCG ACG GCA ACC TCG G -3’ and 5’-TGA GCA ACG TAT CCTCCA GAG T-3’], 3μl template DNA (1.5 µl each) and 7.5 µl of DNase free water. The mPCR amplification program consisted of initial denaturation for 5 min at 95°C, 40 cycles of denaturation (95°C, 60s), annealing (54°C, 40s) and elongation (72°C, 50s); followed by the final elongation at 72°C for 4 min. Amplified products were resolved in agarose gel (1.5%) and ethidium bromide stained amplicons were visualized under short-wavelength UV light source and photographed. Standard cultures of S. Enteritidis (ATCC 13076) and L. monocytogenes (MTCC 1143) were used for the standardization of mPCR.

Isolation and identification of S. enterica and L. monocytogenes: Samples showing positive by mPCR amplicons from the primary enrichment were subjected for conventional cultural isolation of S. enterica species and L. monocytogenes as per standard protocols (ISO 6579:2002 and ISO 11290:1998). Identification of S. enterica species and L. monocytogenes was carried out using a battery of biochemical tests (Blodgett, 2010).

Results and Discussion

Presence of S. enterica and L. monocytogenes was detected based on the amplification of the 284 and 217 base pairs PCR products in Salmonella and Listeria species, respectively (Figure 1). Among the table egg samples screened using the mPCR, Salmonella and Listeria were detected in 19.4 and 2.7 per cent, respectively. Distribution of these food safety hazards among different categories of table egg samples is given in Table 1. Occurrence of S. enterica was highest in backyard eggs followed commercial table eggs. Primary enrichment samples of commercial eggs were found negative for L. monocytogenes using prfA gene based mPCR. However, one sample of backyard eggs showed amplification for prfA gene.

Use of cultural media and methodology for detection and isolation of pathogens will vary and even sensitivity is also low and difficult to detect non-viable cells or injured cells of pathogens by cultural method, but they may have potential to recover and grow when the food is consumed, therefore molecular assay may be more appropriate. Several simplex or multiplex PCR assays have been developed for the rapid detection of Salmonella and Listeria species using specific primers (Germini et al., 2009; Manning et al., 2015; Liu et al., 2015; Afshari et al., 2018; Heymans et al., 2018; Dzieciol et al., 2016). Of the several amplification targets used for detection of Salmonella such as agfA, fimA, viaB, fliC-d, virulence-associated plasmids, etc the invA gene has been one of the most widely gene targets for the specific detection of Salmonellae (Phumkhachorn and Rattanachaikunsopon, 2017; Afshari et al., 2018; Heymans et al., 2018; Borges et al., 2018). Likewise, L. monocytogenes could be specifically detected using virulence factors such as actA polymerization protein (actA), phosphatidylinositol phospholipase C (plcA), hemolysin (hlyA) and invasive associated protein (iap) genes that are involved in the pathogenesis (Liu et al., 2015; Dzieciol et al., 2016); of these, prfA has been the accepted target for the specific detection of L.

Table 1: Detection of Salmonella enterica and Listeria monocytogenes in eggs using mPCR

<table>
<thead>
<tr>
<th>Egg source</th>
<th>Egg samples analyzed*</th>
<th>S. enterica</th>
<th>L. monocytogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial layer farms</td>
<td>24</td>
<td>3 (12.5 %)</td>
<td>ND</td>
</tr>
<tr>
<td>Backyard</td>
<td>12</td>
<td>4 (33.3 %)</td>
<td>1 (8.3 %)</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>7 (19.4 %)</td>
<td>1 (2.7 %)</td>
</tr>
</tbody>
</table>

*Each sample consists pool of 10 eggs; ND- Not detected.
monocytogenes (Wernars et al., 1992).

The mPCR adopted in the present study was found to detect both pathogens simultaneously and the results of the mPCR were in complete concordance with the conventional cultural methods. However, separate primary enrichment broth for Salmonella and Listeria monocytogenes is preferred over universal primary enrichment medium while screening for pathogens in food samples using mPCR, where Salmonella will predominant over the Listeria monocytogenes multiplication irrespective of initial counts (Jofre et al., 2005).

Similar studies by other investigators could detect few pathogen (Nagappa et al., 2007; Gole et al., 2013; Vinay et al., 2016; Rajashekhara et al., 2017) or have shown discrepancies (Al-Obaidi et al., 2011; Safaei et al., 2011; Paul et al., 2016). The mPCR employed in the present study with minor modification was found reliable for the simultaneously detection of two major food safety hazards in table eggs with an anticipation of food safety compliance. Keeping in view, the limitations of laborious and time consuming conventional cultural methods, results of the present study support to propose application of mPCR is for the rapid and specific detection of S. enterica and L. monocytogenes in table eggs. Nevertheless, multi-centric validations involving large samples are warranted for the wide scale acceptance of results.

Conclusions

The applications of PCR have revolutionized the molecular diagnostics including detection of food safety hazards. In the present study, mPCR assay adopted for the rapid and simultaneous detection of S. enterica and L. monocytogenes in table egg contents. The results of the mPCR were in complete concordance with the conventional cultural methods. Therefore, the mPCR assay has been proposed as alternative tool for the rapid and simultaneous detection of S. enterica and L. monocytogenes in table eggs for the purpose of food safety compliance testing of samples.

Authorship contribution statement

Vinayananda C.O.: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft
Nadeem Fairoze: Conceptualization, Writing - review & editing, Visualization, Supervision, Funding acquisition.
C. B. Madhavaprasad: Conceptualization, Writing - original draft, Writing - review & editing.
Nagappa S. K: Methodology, Data curation, Writing - original draft, Writing - review & editing.

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Declaration of Competing Interest
All authors declare that there exist no commercial or financial relationships that could, in any way, lead to a potential conflict of interest.

Reference
Paul, N. F., C. Kennedy, S. V. O. and Shoyinka. 2016. Molecular Detection of Salmonella Isolated...


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