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Comparison of Two Pre-Enrichment Broths for Recovering *Listeria monocytogenes* from Various Foods

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Abstract: The purpose of the present study was to compare the abilities of University of Vermont Broth (UVM) and 0.1% (w/v) peptone water for recovering-*Listeria monocytogenes* from various foods. A total of 210 food samples (raw fish-100, mutton-60, paneer-50) was taken and pre-enrichment was carried out in UVM broth and 0.1% (w/v) peptone water. A total of five isolates were recovered, three from mutton and two from fish. However, no isolate of *L. monocytogenes* was recovered from paneer samples. The UVM broth detected all the five isolates, while as 0.1% (w/v) peptone water detected only three isolates showing a higher efficacy of UVM broth for isolation of *L. monocytogenes* as compared to 0.1% (w/v) peptone water.

Key words: *Listeria monocytogenes* · Peptone Water · UVM

INTRODUCTION

Listeria monocytogenes in foods can pose a significant health risk, with a relatively high mortality of specific section of population, such as pregnant women, fetuses, newborn babies, cancer patients and immune-compromised people [1-3]. *Listeria* is one of most virulent food-borne pathogens with case fatality rate of 20-30% [4], which can even go up to 50% in neonatal population [5, 6]. *L. monocytogenes* is responsible for causing abortion, septicemia, meningitis, infertility, gastroenteritis and conjunctivitis in both humans and animals [7, 8]. Food acts as a vehicle for 99% of human listeriosis cases [9]. Foods most frequently implicated include salads, sea-foods, meat and dairy products [10]. *L. monocytogenes* is also problematic due to its resistance to various antibiotics posing a severe threat to human health [11].

As reported by several authors, examining food for the presence of *Listeria* spp. require a pre-enrichment step because of their susceptibility to injury and consequent ability to grow in selective media [12]. Taking this thing into account we decided to compare the UVM broth (University of Vermont broth) and 0.1% (w/v) peptone water as pre-enrichment media for recovery of *L. monocytogenes* from various foods. The secondary enrichment in both the above cases was then carried out in Fraser broth as per the USDA protocol [13].

MATERIALS AND METHODS

Sampling Procedure: A total of 210 food samples comprising of fish (100), mutton (60) and paneer (50) was collected aseptically from different retail outlets in UV sterilized polyethylene zipped sachets from their respective places. They were transported to the laboratory immediately in ice packs and kept at 4°C until processing.

Isolation Procedure: Briefly 25g of each food sample were taken in duplicate, thoroughly minced and inoculated into test tubes containing 225 ml of UVM broth and 0.1% (w/v) peptone water respectively. The samples were then incubated at 30°C for 24 h. About 0.5 ml of the primary

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enrichment broth was transferred to 10 ml of Fraser broth and incubated at 37°C for 36-48 h. A loop full of culture from the Fraser broth was streaked on polymyxin acriflavin lithium chloride ceftidizime Aesculin mannitol (PALCAM) agar and incubated at 37°C for 24-36 h. *L. monocytogenes* produced typical greenish-yellow colonies with blackening of surrounding media representing aesculun hydrolysis - characteristic of *L. monocytogenes*.

Confirmation and Identification Procedure: The isolates were subjected to standard biochemical tests as per Cowan and Steel [14]. *L. monocytogenes* isolates identified biochemically were tested for hemolytic activity by blood agar plate method and CAMP test with *S. aureus* [15]. The isolates were further subjected to PCR assay for determining their virulent and non-virulent status by verifying the presence or absence of listeriolysin O (*hly*-A) gene. The amplification of *hly*-A gene was carried out by following the protocol of Noterman *et al.* [16].

RESULTS AND DISCUSSION

A total of 210 food samples was analyzed for the presence of *L. monocytogenes*. The organism was recovered from five food samples, three from mutton and two from fish. However, no isolate was recovered from paneer. It was seen that UVM broth could recover all the five isolates, where as the peptone water recovered only three isolates of *L. monocytogenes*. Similar results were reported by Curiale and Lewus [17]. The recovery of *L. monocytogenes* from food and environmental samples requires the use of enrichment cultures followed by selective plating because the organism is a poor competitor when present with other microbial flora [18]. While comparing the efficacy of peptone water and UVM for isolation of *L. monocytogenes* it was found that pre-enrichment of samples with UVM broth yielded a positive percentage of 14.9 while as with peptone water the positive percentage was 13.1 [19].

It has been reported earlier that enrichment broths favour the growth of *L. monocytogenes* even if they are suffering from sub-lethal injury and thus increase the chances of recovering this organism from foods or other environmental samples [20]. UVM broth allows better recovery of heat-injured cells of *L. monocytogenes* [21]. UVM broth used as pre-enrichment broth in USDA protocol contains selective agents such as nalidixic acid and acriflavin with no lithium chloride. It has been seen that lithium chloride results in lower recoveries of heat injured *L. monocytogenes* [22].

In this experiment, the use of selective pre-enrichment broth increased the recovery of *L. monocytogenes* as compared to when non-selective medium was used. So the use of various selective broths or enrichment agents can be recommended when we have to isolate *L. monocytogenes* from various foods or environmental samples.

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